



Genetic Diversity of Some Cotton Genotypes Using RAPD Markers

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Abstract

Molecular analysis techniques such as RAPD represent modern tools that have brought a significant advancement in studying genetic diversity in plants. They provide high accuracy in distinguishing genetic structures without the need for prior knowledge of genome sequences. In this study, RAPD markers were employed to analyze genetic variation and assess the genetic diversity among ten Iraqi cotton genotypes. The RAPD profiles revealed substantial differences in the number and size of amplified fragments, confirming the presence of genetic diversity among the tested genotypes. A total of 258 DNA bands were detected, including 213 polymorphic and 45 monomorphic bands. Primer OPN-07 generated the highest number of bands (31), whereas OPQ-17 yielded the fewest (10). Primer OPA-11 exhibited the highest discriminatory power (13.17), while OPQ-20 showed the lowest (2.71). Genetic distance analysis revealed maximum divergence between 'Nazly 87' and 'Awar 310' (0.9677), and the minimum between 'Ashour' and 'Marsoumi' (0.1002). Distinctive DNA fragments were identified as molecular fingerprints for each genotype, such as a 780 bp band for 'Nazly 87', a 460 bp band for 'Awar 310', a 920 bp band for 'Marsoumi', and a 650 bp band for 'Ashour'. Overall, the findings demonstrate the effectiveness of RAPD markers in detecting genetic diversity and establishing genetic relationships among Iraqi cotton genotypes. This study provides a molecular foundation for breeding programs aiming to enhance cotton productivity and develop superior, resistant cultivars in Iraq. This study represents a valuable foundation for future research aimed at enhancing cotton productivity in Iraq through the use of molecular techniques.

Keywords: Genetic Relatedness Genetic Divergence, Genetic distance, genetic variation, DNA

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Introduction

Cotton crop belonging to the genus *Gossypium* within the Malvaceae family, encompasses approximately twenty species globally, with four cultivated commercially. The Old World contributed *G. herbaceum* and *G. arboreum*, while the New World originated *G. hirsutum* and *G. barbadense*. Most global commercial cotton varieties are classified under *G. hirsutum* (1). Despite synthetic fiber competition and diverse applications of cotton by products, cotton fibers maintain a distinguished global position among textile fibers. Within the Arab agricultural scope, cotton cultivation holds significant importance, particularly in Egypt, Sudan, and Syria. A primary objective in cotton breeding is the synergistic integration of high productivity with superior fiber traits attributes. Plant breeders strive to

develop ideal genetic genotypes through conventional breeding methodologies and marker-assisted selection. The demand for specific fiber qualities, driven by end-product manufacturing requirements, necessitates a deep understanding of the genetic diversity within cotton germplasm. Modern cotton breeding programs require precise knowledge of the genetic relationships among different genotypes to develop superior hybrids with desirable traits such as improved length, fineness, and durability. This study utilizes Random Amplified Polymorphic DNA (RAPD) markers as a powerful and cost-effective tool to assess the genetic diversity and relationships among Iraqi cotton genotypes (2,5,6). The RAPD technique is instrumental in identifying unique genetic fingerprints that can serve to distinguish between different cultivars and provide insights into their genetic backgrounds. By revealing the extent of genetic

polymorphism, this research provides a crucial foundation for future breeding programs aimed at enhancing cotton productivity and resistance to various environmental stresses. The genotype of cotton represents its complete genetic makeup, which dictates all its characteristics, from fiber quality and yield to its resistance to diseases and environmental stresses. Understanding these genetic variations is essential for effective breeding programs (11).

Recent years have witnessed significant advancements in molecular techniques for determining plant genetic distance. Random Amplified Polymorphic DNA (RAPD) analysis is a prevalent and contemporary marker system (8, 9). Its advantages include ease of use, rapid laboratory analysis, applicability to numerous genetic lines, and the utilization of random, universal primers. This technique allows for the comprehensive coverage of multiple genomic regions in the studied individuals (10). While numerous researchers have employed diverse DNA markers to investigate genetic variation and molecular characterization in cotton, studies utilizing DNA markers to assess genetic diversity in Iraqi cotton remain limited. Consequently, this research employs RAPD molecular markers to analyze genetic differences among cotton genotypes. Ten cotton genotypes were analyzed, most of which are introduced, with some being local Iraqi genotypes

Materials and Methods

DNA Extraction Protocol

Genomic DNA was isolated from fresh young leaves of ten cotton genotypes, namely Coker 310, Montana, Lachata, Back Coat, IK259, BA440, DP396, EDESSA, FLASH, and ST468, following a modified CTAB protocol based on established methods [1, 10 and 18]. Briefly, 0.5–1.0 g of leaf tissue was collected from each

genotype for DNA extraction. of fresh young leaves per sample was collected, thoroughly rinsed with sterile distilled water, and then sectioned into small fragments. The tissue was rapidly frozen in liquid nitrogen and ground to a fine powder using a pre-cooled ceramic mortar and pestle. The resulting powder was transferred into sterile glass tubes containing 4 mL of extraction buffer (100 mM Tris-HCl, pH 8.0; 20 mM EDTA; 1.4 M NaCl; and 2% CTAB supplemented with 0.5 M glucose). The tubes were incubated at 60°C for 60–90 minutes in a vibrating water bath to facilitate cell lysis and release of nucleic acids.

Following the incubation period, the samples were allowed to cool to room temperature. Subsequently, 4 mL of a chloroform-isoamyl alcohol solution (24:1, v/v) was added to each tube, and the mixtures were continuously agitated for 15 minutes. This was followed by centrifugation at 8000 rpm for 10 minutes at 4°C. The aqueous supernatant was then carefully transferred to new sterile tubes, and an equal volume of pre-cooled isopropanol was added to precipitate the DNA. Gentle mixing was performed until the DNA strands became visible as white, fibrous clumps, confirming successful precipitation (12).

The DNA threads were spooled using a sterile glass hook and transferred into tubes containing 200 µL of sterile distilled water. The DNA was dissolved by intermittent mixing until fully solubilized. DNA quality was assessed using 1% agarose gel electrophoresis, and quantification was carried out using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Korea). DNA purity was determined by evaluating the A260/A280 ratio, which ranged between 1.80 and 2.00, indicating high-quality DNA. All samples were normalized to a final concentration of 25 ng/µL using sterile distilled water for downstream applications (program PAST), as shown in **Figure 1**.

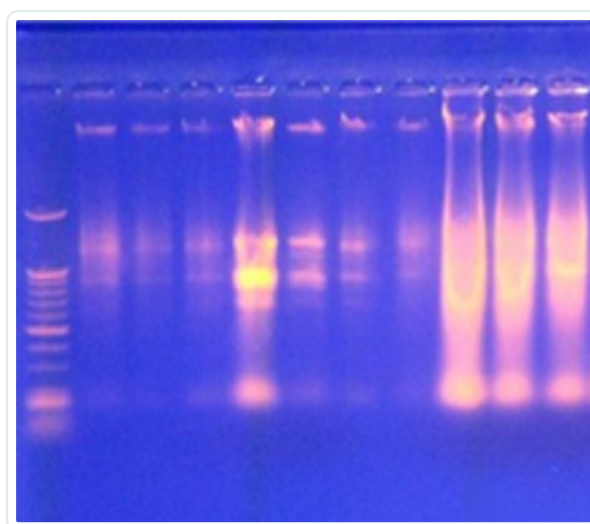


Figure 1. Agarose Gel Electrophoresis of DNA Samples

Preparation of RAPD-PCR Interactions

Random DNA amplification reactions (RAPD-PCR) were performed according to Willams ACCU Power PCR (20) (premix Kit supplied by Bioneer of Korea and according to the attached instructions (Table 1 and 2). The tubes contain the basic components of a polymerase chain reaction, which include a single unit of an enzyme, Taq DNA polymerase 250 μ M from a mixture of nitrogenous bases, dNTPs1,5 and KCL mM30, Tris-HCl (pH9) mM10.MgCl₂ mM was added to picomole 10 from the initiator 25 nanograms of DNA, and then the reaction size with sterile distilled water to μ L20per tube. The tubes were well mixed and transferred to the Thermocycler device to complete the multiplicative reaction after it was programmed according to the

program. One cycle for two minutes at a temperature of 94C for the initial monster of the DNA tape, followed by 40 doubling cycles that include a one-minute cycle at a degree of 92C for the monster of the mold. One minute at a degree of 36C to connect the prefixes to the mold and one minute at 72C for elongation with a last cycle for 7 min and at 72C for the final elongation. The outputs of the amplification process on the acarose gel were carried out at a concentration of 1.2% with the volumetric evidence consisting of DNA to supply the section with the enzyme Hind III and Eco RI for 90 min by (5V/ cm). The gel was examined after being dyed with ethyl bromide dye for 45-30 min under ultraviolet light (UV-Light Gel Documentation System) and photographed using (1, 7 and 20).

Table 1. Materials and Reagents Used in the Study

Material / Reagent	Catalog Number	Manufacturer
Agarose	8100.11	Conda / USA
Red safe staining souluion	21141	Intron / Korea
6X Loading dye	21161	Intron / Korea
Ladder 1000 bp plus	24075	Intron / Korea
Pre mix pcr	25025	Intron / Korea
TBE buffer 10 X	IBS.BT004	Conda / USA
Primer	---	Integrated DNA technologies / USA
ZR Plant/Seed DNA MiniPrep™	D6020	Zymo / USA

Table 2. Apparatus and Equipment Used in the Study

Apparatus	Origin	Manufacturer
AURA TM PCR Cabinet	Italy	EuroClone
Microspin 12, High-speed Mini-centrifuge	Germany	Bio San
V-1 plus, Personal Vortex for tubes	Germany	Digsystem
Bio TDB-100, Dry block thermostat built	Germany	Bio San
Biopette Variable Volume Micropipettes	Germany	Labnet
Mini-Power Supply 300V, 2200V	Chain	Supplier
MultiGeneOptiMax Thermal Cycler	USA	Labnet
Electrophoreses	USA	CBS, Scientific
Document system	USA	Labnet
UV.transmission	Farance	Vilber lourmat
Microspin	Lativa	Biosan
Combi-spin	Lative	Biosan
Balance	Germany	Kernpfb
Incubation	China	Jrad
Microwave	China	Gosonic
Water distilater	China	Rovsun
Nanodrop	Korea	Nabi

Recording the results of RAPD and statistical analysis

To record the results of random multiplication of the RAPD technique, the images of the electrical separation patterns of each prefix were examined, and the bands of each prefix were recorded in a table so that they were treated as a binary trait representing the presence of the bundle (Band B (1) and the absence of the bundle (BandB) (0). From this table, the total number of RAPD bundles shown by the prefixes in the genetic genotypes

of the studied cotton was calculated, the polymorphic bundles were identified, the monomorphic bundles were matched, and the degree of variation in the bundle products was calculated. RAPD The genetic distance and similarity coefficient between the studied samples were calculated using the coefficients of (Nei and Li, Nei's 72) (7). The unweighted pair group was then analyzed using the method for the arithmetic average (UPGMA).) conducted the statistical analysis using the Numerical Taxonomy and Multivariate Analysis program. (Table 3)

Table 3. The sequence of prefixes used in the study

No.	Primers	Sequence (3-5)	GC
1	OP-T19	GTCCGTATGG	60
2	OP-T20	GACCAATGCC	60
3	OP-V02	AGTCACTCCC	60
4	OP-V09	TGTACCCGTC	60
5	OP-V19	GGGTGTGCAG	70
6	OP-M20	AGGTCTTGGG	60
7	OP-M05	GGGAACGTGT	60
8	OP-R12	ACAGGTGCGT	60
9	OP-M14	AGGGTCGTTC	60
10	OP-P04	GTGTCTCAGG	60

Results and discussion

Table 4 presents the distribution of RAPD primer-generated bands and their molecular sizes. The number of divergent bands varied among primers, with some primers, such as OP-R12, showing the highest divergence (100%), indicating strong discriminatory power. Molecular sizes ranged from 200 to 2000 bp,

reflecting a wide diversity in the targeted genomic regions. The percentages of distinguishing ability and primer efficiency indicate that certain primers are more effective in detecting genetic variation. The table highlights the importance of selecting appropriate primers to obtain accurate information on the genetic diversity among the studied cotton genotypes. PCR amplification profiles of the ten RAPD primers are illustrated in **Figure 2**.

Table 4. Products of the prefixes of the total and differential beams and their molecular sizes

Primers	Molecular weight (Chem.)	BP	Total bands	Polymorphic bands	Polymorphic %	Percentage of Distinguishing Ability %	Efficiency ratio of initiators
OP-T19	500-1500		18	12	66.67	8.70	10.23
OP-T20	400-1000		13	8	61.54	5.80	7.38
OP-V02	400-2000		7	5	71.42	3.68	11.36
OP-V09	400-1500		20	15	75.00	10.87	11.36
OP-V19	300-1100		17	14	82.35	10.15	9.66
OP-M20	200-1200		20	11	55.00	7.97	11.36
OP-M05	200-1500		21	18	85.71	13.04	11.93
OP-R12	200-1000		18	18	100.00	13.04	10.23
OP-M14	400-1300		19	17	89.47	12.32	10.80
OP-P04	200-1500		23	20	86.95	14.49	13.07
Total			176	138			
Average			17.6	13.8	78.41		

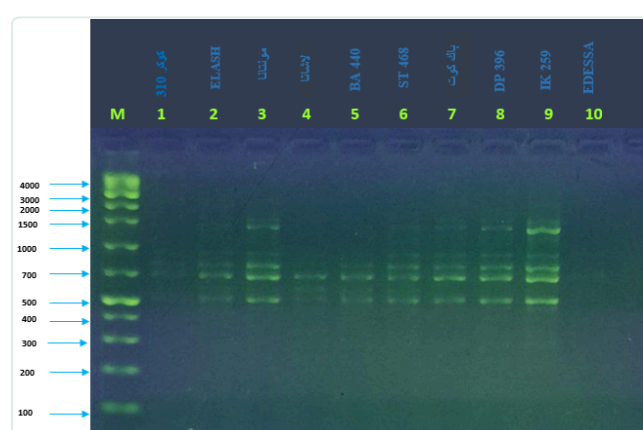


Figure (1) PCR product of primer OP-T 19 The product was electrophoresis on 2% agarose at 5 volt/cm 2 . 1x TBE buffer for 1:30 hours. N: DNA ladder 1000bp (plus).

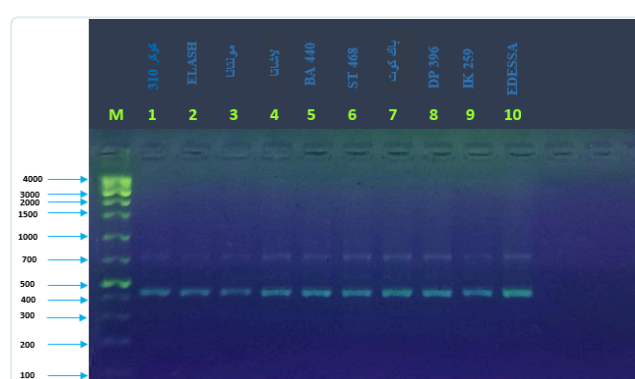


Figure (2) PCR product of primer OP-T 20 The product was electrophoresis on 2% agarose at 5 volt/cm 2 . 1x TBE buffer for 1:30 hours. N: DNA ladder 1000bp (plus).

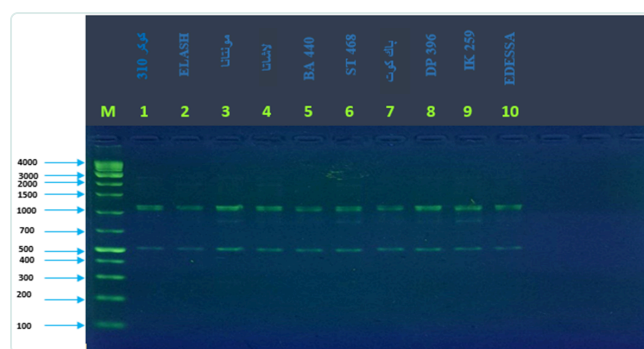


Figure (3) PCR product of primer OP-V02 The product was electrophoresis on 2% agarose at 5 volt/cm 2 . 1x TBE buffer for 1:30 hours. N: DNA ladder 1000bp (plus).

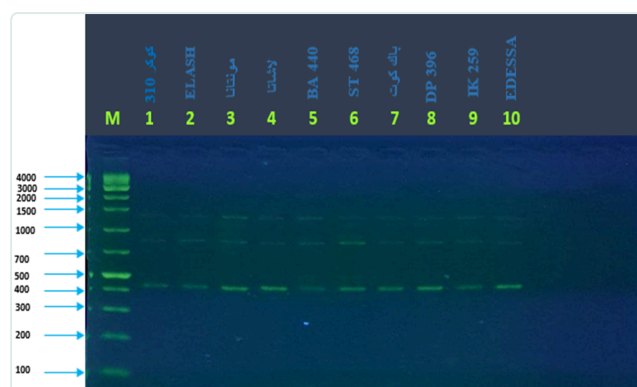


Figure (4) PCR product of primer OP-V 09 The product was electrophoresis on 2% agarose at 5 volt/cm 2 . 1x TBE buffer for 1:30 hours. N: DNA ladder 1000 bp (plus).

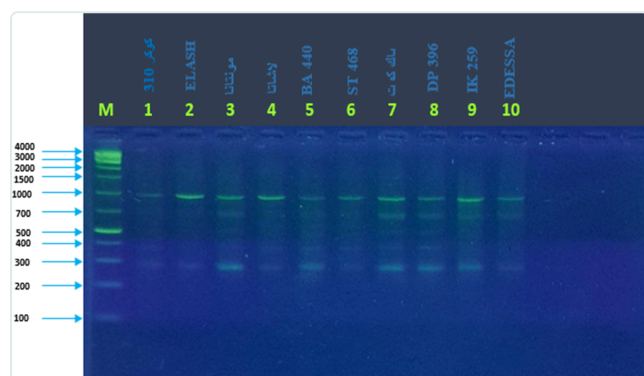


Figure (5) PCR product of primer OP-V 19 The product was electrophoresis on 2% agarose at 5 volt/cm 2 . 1x TBE buffer for 1:30 hours. N: DNA ladder 1000bp (plus) .

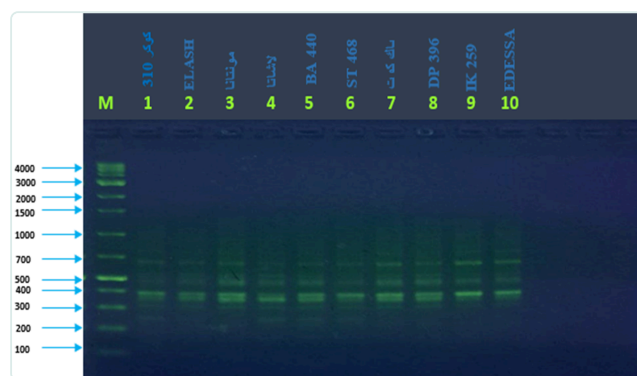


Figure (6) PCR product of primer OP-M20 The product was electrophoresis on 2% agarose at 5 volt/cm 2 . 1x TBE buffer for 1:30 hours. N: DNA ladder 1000 bp (plus).

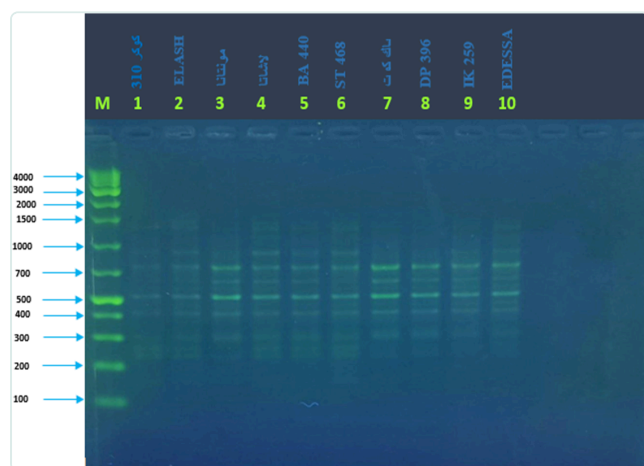


Figure (7) PCR product of primer OP-M05 The product was electrophoresis on 2% agarose at 5 volt/cm 2 . 1x TBE buffer for 1:30 hours. N: DNA ladder 1000bp (plus).

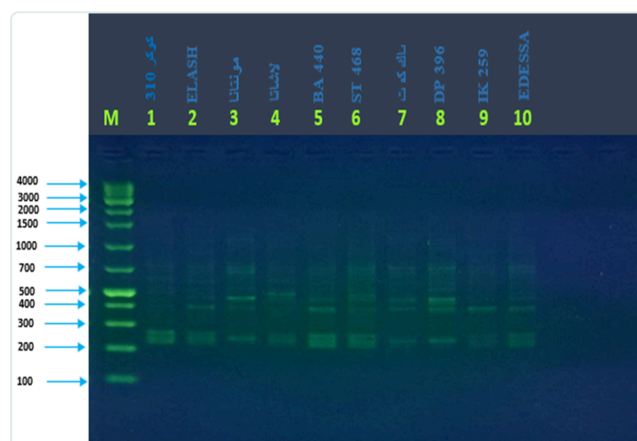


Figure (8) PCR product of primer OP-R12 The product was electrophoresis on 2% agarose at 5 volt/cm 2 . 1x TBE buffer for 1:30 hours. N: DNA ladder 1000bp (plus).

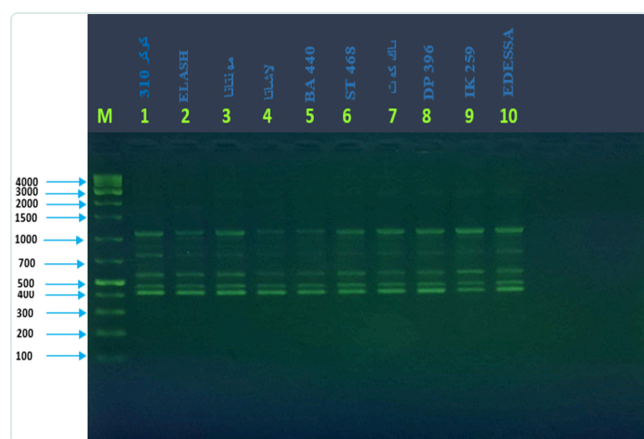


Figure (9) PCR product of primer OP-M14 The product was electrophoresis on 2% agarose at 5 volt/cm 2 . 1x TBE buffer for 1:30 hours. N: DNA ladder 1000bp (plus).

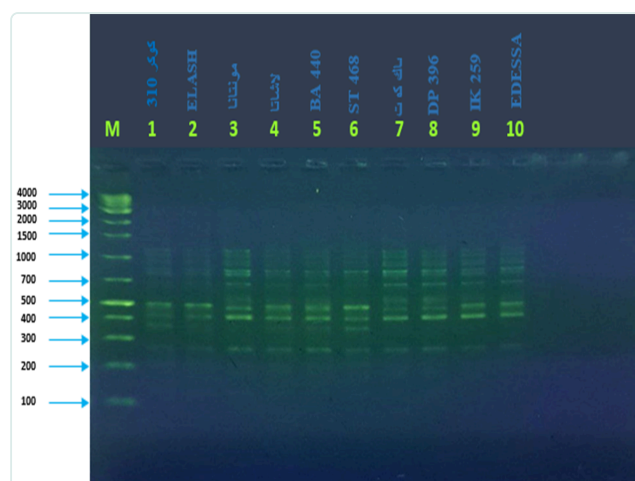


Figure (10) PCR product of primer OP-P04 The product was electrophoresis on 2% agarose at 5 volt/cm 2 . 1x TBE buffer for 1:30 hours. N: DNA ladder 1000bp (plus).

Figure 2. 1–10 show the PCR amplification profiles of the selected RAPD primers

Similarity and distance indices

Figure 3 showed clear genetic differences among the studied cotton genotypes according to RAPD marker analysis. The dendrogram separated the ten genotypes into three main clusters based on their genetic similarity. The first cluster included ST_468 and ELASH, which appeared genetically closer to each other than to the remaining genotypes. The second cluster contained Montana, BA_440, Back_coat, IK_259, and EDESSA, indicating moderate genetic relatedness among these genotypes. Meanwhile, Coker_310 and DP_396 formed a separate cluster, reflecting noticeable genetic divergence from the other genotypes. Lachata showed an intermediate position between some groups, suggesting partial similarity with several genotypes.

The RAPD profiles revealed considerable polymorphism among the studied cotton genotypes, indicating the presence of genetic variation within the tested materials. The highest genetic distance was detected between Coker_310 and ST_468 (0.949), which indicates wide genetic divergence between these two genotypes. Such divergence could be useful in future breeding programs to increase genetic variability and exploit heterosis. The selected RAPD primers were effective in differentiating the cotton genotypes and produced distinct molecular patterns for most of the studied materials., suggesting they are genetically distinct. (13, 14, 15, 16, 21)

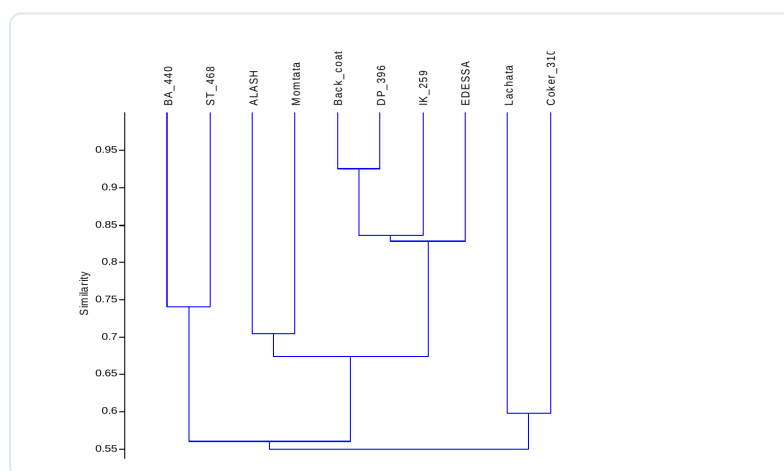


Figure 3. Dendrogram tree of ten cotton genotypes based on results from 176 clusters.

On the other hand, the lowest genetic distance was found between the Coker_310 and Lachata genotypes, with a ratio of 0.255, which reflects a closer genetic relationship. These findings highlight the extent of genetic variation within the cotton genotypes, which can provide valuable insights for breeding programs aiming to enhance desirable traits such as yield, disease resistance, and environmental adaptability (3, 7, and 19). Furthermore, applying the RAPD technique, coupled with the analysis of genetic distances, provides a robust framework for understanding the genetic structure of cotton plants. This method has proven to be an efficient and effective tool for genetic analysis, particularly when dealing with large numbers of genotypes. The ability to discern genetic differences with minimal primer usage underscores the utility of RAPD as a cost-effective approach in genetic research and cotton breeding efforts. The findings of this study contribute to the growing body of knowledge on cotton genetics and may serve as a

valuable resource for future research and development in crop improvement, particularly in optimizing genetic diversity for agricultural sustainability.

The results underscore the importance of molecular techniques like RAPD in identifying genetically diverse and agriculturally significant cotton cultivars. The analysis reveals a clear genetic relationship and differentiation among the studied genotypes, where each value in the **table 5** represents the degree of relatedness between the different genetic variants. This distinction was further confirmed by the genetic fingerprints generated by the ten primers used, which allowed for the differentiation of cotton genotypes without the need for an extensive number of primers. The findings show a maximum genetic distance of 0.949 between the Coker_310 and ST_468 cultivars, indicating significant genetic divergence, while the minimum genetic distance of 0.255 was found between Coker_310 and Lachata, suggesting a higher degree of genetic similarity. This information is crucial for selecting the most suitable parents for hybrid breeding programs. (9, 14, and 15).

Table 5. Shows the values of the genetic dimensions of the varieties and their crosses for cotton using the RAPD technique

	Coker_310	ELASH	Momtata	Lachata	BA_440	ST_468	Back_coat	DP_396	IK_259	EDESSA
Coker_310	1.000									
ELASH	0.791	1.000								
Momtata	0.901	0.569	1.000							
Lachata	0.255	0.899	0.833	1.000						
BA_440	0.927	0.306	0.793	0.884	1.000					
ST_468	0.949	0.791	0.792	0.939	0.410	1.000				
Back_coat	0.618	0.561	0.603	0.726	0.714	0.720	1.000			
DP_396	0.561	0.494	0.695	0.665	0.719	0.801	0.368	1.000		
IK_259	0.730	0.501	0.714	0.517	0.584	0.742	0.329	0.381	1.000	
EDESSA	0.709	0.670	0.714	0.548	0.826	0.841	0.495	0.278	0.317	1.000

Conclusion

RAPD markers successfully detected genetic differences among the studied cotton genotypes. The amplification patterns revealed noticeable variation between the genotypes, indicating the presence of genetic diversity within the tested materials. Cluster analysis grouped the genotypes according to their genetic similarity, where the greatest genetic distance was observed between Coker_310 and ST_468, suggesting substantial genetic divergence between these two genotypes.

The detected variation could be useful in future cotton breeding programs, especially for selecting genetically diverse parents in hybridization programs. In general, the RAPD technique proved to be a suitable method for studying genetic relationships among cotton genotypes grown under Iraqi conditions.

Supplementary Materials: No Supplementary Materials.

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مقالة أصيلة

التنوع الوراثي لبعض تراكيب القطن الوراثية باستخدام مؤشرات RAPD

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الخلاصة

تمثل تقنيات التحليل الجيني مثل RAPD أدوات حديثة أحدثت تقدماً كبيراً في دراسة التنوع الوراثي في النباتات، إذ توفر دقة عالية في التمييز بين التراكيب الوراثية دون الحاجة إلى معرفة مسبقة بتسلسل الجينوم. في هذه الدراسة، تم استخدام مؤشرات RAPD لتحليل التباين الوراثي وتقييم التنوع الوراثي بين عشرة تراكيب وراثية من القطن العراقي. أظهرت أنماط RAPD اختلافات واضحة في عدد وحجم الأجزاء المضخمة، مما يؤكد وجود تنوع وراثي بين التراكيب المدروسة. تم الكشف عن ما مجموعه 258 حزمة DNA، منها 213 متعددة الأشكال و45 أحادية الشكل. أعطى البادئ OPN-07 أعلى عدد من الحزم 31، في حين أعطى OPQ-17 أقل عدد (10). أظهر البادئ OPA-11 أعلى قوة تمييز 13.17، بينما سجل OPQ-20 أدنى قيمة 2.71. أظهر تحليل المسافة الوراثية أكبر تباعد بين الصنفين Nazly 87 وAwar 310 (0.9677)، وأقل تباعد بين Ashour وMarsoumi (0.1002). تم تحديد أجزاء DNA مميزة تُعد بصمات وراثية لكل تركيب، مثل الحزمة 780 زوج قاعدة (bp) للصنف Nazly 87، و460 bp للصنف Awar 310، و920 bp للصنف Marsoumi، و650 bp للصنف Ashour. بشكل عام، توضح النتائج كفاءة مؤشرات RAPD في الكشف عن التنوع الوراثي وتحديد العلاقات الوراثية بين تراكيب القطن العراقي، وتوفر أساساً جزيئياً لبرامج التربية الهادفة إلى تعزيز إنتاجية القطن وتطوير تراكيب متميزة ومقاومة في العراق. تمثل هذه الدراسة أساساً قيماً لدراسات مستقبلية تهدف إلى تعزيز إنتاجية القطن في العراق باستخدام التقنيات الجزيئية.

الكلمات المفتاحية: التباين الوراثي، المسافة الجينية، التباين الجيني، القرابة الوراثية