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STUDYING THE GENETIC DIVERSITY OF SOME *SOLANUM MELONGENA* L. VARIETIES USING MOLECULAR TECHNOLOGY

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Abstract

Using polymerase chain reaction (PCR)- based random amplified polymorphic DNA (RAPD) markers, the study conducted a genetic fingerprinting analysis of ten Eggplant Varieties Solanum melongena L. and measured the percentage of genetic distance. In the biotechnology laboratories of the Seed Inspection and Certification Department, during the fall season of 2024, using 5 primers from RAPD.

Polymorphisms were of 74.75% detected among all genotypes, while the percentage of efficiency of each primer was 25.53% for the primer OPC-05, while the maximum discriminatory ability was 25.53% and 31.42% with primer OPC-05. The results showed that the highest percentage of similarity corresponds to the lowest genetic dimension (0.679) between Varieties S6 and S3, then the dimension (0.666) between Varieties S10 and S3. It was noted that the ten Varieties were distributed into two main groups A) and (B, as cluster A split into two subgroups (A1 and A2), and branch A1 included two secondary groups, the first of which included two genotypes: (S7, and S4) and the second was(S1 and S9), While group A2 included both types (S5 and S2). As for cluster (B), it was divided into two clusters, as the first cluster included both strains S3, S6, and S10, while the variety (S8) remained isolated from the other Varieties.

Key Word: Eggplant , RAPD

Introduction

Eggplant (*Solanum melongena* L.) is one of the most important of the Solanaceae family. It is one of the vegetable plants of economic importance, especially in the hot and temperate regions of the world. It has been known for a long time since it was growing wild in both India and China, which are its original homelands (Matlub et al., 1989). After its cultivation there, it began to spread to other regions, such as the Caribbean, and North Africa (Daunay et al., 2001). The crop is grown for its fruits, which are eaten after cooking. It is also used in making and preserving pickles. Frozen or canned for export (DGCIS, 2008). The country's local plant varieties are considered a national treasure and part of its identity and heritage. They must be preserved. Therefore, countries, especially developed ones, are interested in preserving their natural plant genetic resources due to their high tolerance to environmental conditions, their nature, and their suitability to those conditions, in addition to their resistance to diseases and insects (NCRCAV 2022).

There are several criteria through which genetic diversity can be evaluated, the most ancient of which are morphological and then genetic criteria, whether by analyzing proteins or deoxynucleic acids and the

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latter are known as molecular markers, which are part of the science of biotechnology. Technologies based on studying molecular markers differ from each other in a way. The genetic variations it reveals are among the most important ones used in this study, which is the technology of Randomly Amplified Polymorphic DNA (RAPD), which relies on the polymerase chain Reaction (Hussein, 2010).

RAPD markers are characterized by several features that make them the most common among PCR-based markers, the most important of which is the ability of these markers to detect multiple sites in the genome due to the binding of primers to more than one site, as well as the uniform distribution of these sites along the genome (Edwards (1998), compared to SSR markers, which detect a few if not a single site (Goldstein et al., 1995, Powell et al., 1996). In a study by (Hussein, 2010) to analyze the DNA profiling of ten varieties of eggplant (*Solanum melongena* L) and find the percentage of genetic distance using markers of random polymorphic duplication of the RAPD (DNA) chain, the results of the genetic analysis showed the presence of genetic variations between them, and the selected Eggplant varieties gave different phenotypic and genetic variations. This was confirmed by the percentage of genetic dimension resulting from the analysis of the results of the RAPD markers, the highest of which reached 77.77% between the two varieties (Moussalli X Abu jethea).

In an experiment on 12 Eggplant varieties to study their genetic and morphological diversity using RAPD technology, amplification using 5 primers produced 54 bands, of which 49 were polymorphic. It was noted that the highest genetic distance was (0.9445) for the SM59 Variety and the SM11 Variety, and the lowest distance was (0.3889) between SM 29 and SM 22. (Shetul et al., 2022).

Accordingly, the aim of this research is to study the genetic diversity among the eggplant varieties included in the study that were collected from different regions of Iraq using RAPD technology by finding the distinctive genetic fingerprint of the studied varieties, which serves as the identity used to diagnose those varieties. Thus, determining the genetic relationship between the studied varieties based on the degree of genetic similarity between those varieties to guide plant breeders to choose the appropriate parents to conduct breeding and improvement operations.

I. Materials and Methods

By using the seeds of 10 genotypes of eggplant *Solanum melongena* L., which were collected from different places from the research centres spread throughout the country, namely (Saica, Barcelona, Black beauty, Al-Rafidain, Pamela, Rashidiya, Ibaa 1006, Scorpio, Madrid, Thorny). Which are symbolized by (S1, S2, S3, ……S9 and S10) respectively. The research was carried out in the biotechnology laboratories of the Ministry of Agriculture - Seed Inspection and Certification Department. The seeds of the selected varieties were planted in anvils filled with peat moss and placed in the greenhouse. When the true leaves appeared, samples were taken from them for the purpose of using them in DNA extraction.

Molecular characterization

DNA extraction

DNA was extracted from the young leaves of the studied varieties. An amount of DNA estimated at 50-150 µg was obtained per 1.5 g of leaves for each eggplant variety, with purity ranging between 1.7-2 as measured with a Nanodrop device. The dilution of the DNA samples was adjusted to obtain the concentration. 50 ng µL-1, which is the appropriate concentration for performing PCR reactions.

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There are several methods for extraction of DNA from plants because plants of their diversity contain different amounts of vegetable compounds such as proteins and multiple and complex sugars as well as DNA, Randomly Amplified Polymorphic DNA (RAPD) technology based on PCR technology was used. The method (Weigand et al., 1993) was relied upon in the extraction of DNA from the eggplant plant, which is one of the most efficient methods in isolating DNA from plants. Such as eggplant (Sahgi-Maroof et al., 1984). The process of extraction DNA from plants is relatively more difficult than from other organisms due to the thick wall surrounding the cell membrane, in addition to the fact that some plants contain a large amount of phenolic substances and polysaccharides, which are considered pollutants, as they sometimes precipitate with the DNA, giving a liquid with a high viscosity. Inhibitory to PCR reactions. To get rid of these substances, dilution of the extracted DNA was performed to reduce the percentage of inhibitory sugars.

RAPD analysis

the study used 5 primers obtained by importing them from Bloneer. Table 2 shows the nucleotide sequence and fusion temperature of the primers used in the study.

The polymerase Chain Reaction (PCR) was carried out according to (Williams et al., 1990) with some modifications. The final reaction volume was (25 ul) using a Master mix of Distilled and DNA at a concentration of ul.ng40^{-1}. This reaction takes place in the thermal cycler according to the following conditions:

1-Partitioning: at a temperature of 94°C for 5 minutes to separate the two strands of DNA.

2-40cycles, each of which includes the following stages:

2-1-Partitioning takes place at a temperature of 94°C for 30 seconds.

2-2 -Coalescence: Depending on the coalescence temperature for each initiator, for a minute.

2-3-Elongation at a temperature of 72°C for a minute.

3-The reaction is completed at a temperature of 72°C for a minute.

Then the samples are stored at a temperature of 4 °C, then we migrate onto an agarose gel.

Table 1 Nucleotide sequences of primers used in RAPD technology.

Primer code	Sequences (5´-3´)	(G +C) %
OPA-04	AATCGGGCTG	60
OPC-05	GATGACCGCC	70
OPE-09	CTTCACCCGA	60
OPA-11	CAATCGCCGT	60
OPL04	GACTGCACAC	70

Electrophoresis, staining and photography:

Migration was performed on a 2% across gel in 1X TBE buffer

TBE = (10X TBE buffer = 108 g Tris borate + 55g Boric acid + 9.2 EDTA, Ph 0.8)

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1X with the addition of Ul5 from the ethidium bromide dye (10 mg.ml^{-1}). DNA samples were loaded on an agarose gel with the addition of Ul5 from the special loading fluid (Bromophenol blue 1X Loading buffer). An indicator of DNA Kpb1 from Geneaid was also injected to determine the size and molecular weight of the resulting bands. The migration was then carried out by passing an electric field of 100 volts, in order to separate the DNA bands resulting from the amplification, so that the gel could then be photographed with an agarose gel imaging device (Agle Eye. II Staratagene).Figure 1.

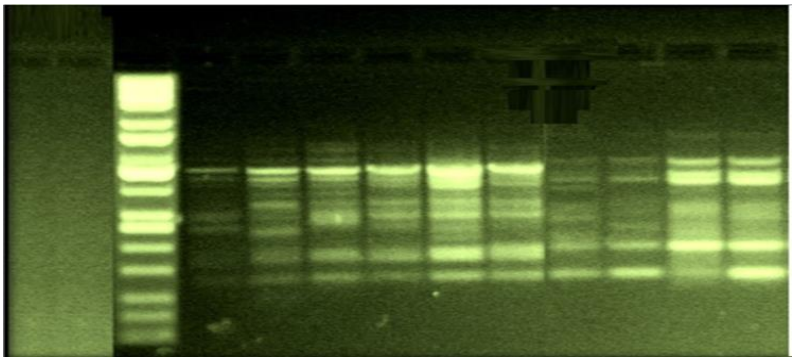

Figure 1: PCR products on an agarose gel.

Statistical analysis

The results of the amplification process were collected in a table based on the presence or absence of DNA bands in the studied samples. The number 1 indicates the presence of only a clear DNA band and the number 0 indicates the absence of the band. The tables were organized for each primer separately and the genetic kinship tree was drawn using the Unweighted Pair Group application. Method With Arithmetic (UPGMA) Averaging using Past statistical program. Based on (Nei and Lei, 1979).

The percentage of primer polymorphism was calculated through the following equation :

Percentage (%) of primer polymorphism for each primer = (number of divergent bands in the primer/total number of primer bands) x 100

The percentage of the discriminatory ability of each primer was calculated according to the following equation:

The discriminatory ability of each primer (%) = (the number of different packets for the initiator / the number of different packets for all primers) x 100

As for the percentage of the efficiency of each primer, it was calculated according to the following equation:

The efficiency of each Primer (%) = (total number of primer bands/total number of all primers bands) x 100 (Grundmann et al., 1995).

II. Results

In the study, 5 primers were used. The results of the RAPD markers were based on the differences in the number of duplicated bands depending on the primer used, resulting from the difference in the number of sites complementary to that primer in the genome of each plant in this study. The method of analyzing the results of the genetic relationship study was based on the presence or absence of bands resulting from the duplication of certain parts of the genome of the plants used, and on the molecular

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weights of those bands, which depend on the number and complementary positions of the primer sequences on the DNA strand (Nei's, 1973).

Polymorphism

Table 2 shows that the primers used produced 47 bands, of which 35 bands were polymorphic, and the percentage of bands with polymorphism was 74.75%. This indicates the presence of genetic divergence between the eggplant types used in the study. The table shows that the primer (OPC-05) gave the highest number of bands (12 bands) compared to the rest of the primers used, As we find that the primer OPE-09 showed the least number of bands (5 bands) while migrating the samples on the polyacrylate gel. The variation in the number of bands resulting from each pair of primers used depends on the extent of compatibility of the primer's binding to the plant genome, as well as the components of each primer's nitrogenous bases, and the difference in the sequence of the bases of the primers used leads to differences in their binding sites with the genome of the plant under study, and this leads to variation Numbers of bands resulting from the primers used (Vos et al., 1995), despite this discrepancy in the number of bands, the primers used succeeded in giving polymorphism among the resulting bands, as the highest percentage of polymorphism reached 91.66% in the primers (OPC-05), As we find that the primer (OPL04) did not give more than 45.45% of the polymorphism. Formally, this difference in percentages is due to the difference in the sequence of bases in the plant genome, which affects the sites of attachment of the primers. This difference results from rearrangement, linkage, genetic crossing, etc., so we see the different bands appearing clearly on the gel or not appearing in certain locations on the gel. Jelly, (Clark and Lanigan, 1993).

The results showed that the percentage of efficiency of each primer was 25.53% for primer OPC-05, while for primer OPE-09 it was 10.63%. We find the maximum discriminatory ability of 31.42% with primer OPC-05 and the lowest discriminatory ability of 11.42% with primer OPE-09. Therefore, the efficiency of the primer OPC-05 achieved the highest discriminatory ability compared to the rest of the primers, at a rate of 31.42%. This primer can be used in the future to obtain common bands and analyze them to design primers that are more specialized in distinguishing between genotypes with a large genetic dimension.

Table 2. It shows the number of bands, polymorphism, efficiency and discriminatory ability of the primers.

Primer codes	Total number of bands scored	Number of polymorphic bands	Proportion of polymorphic loci (%)	Primer efficiency(%)	Primer discriminatory ability (%)
OPA-04	10	9	90%	21.27%	25.71%
OPC-05	12	11	91.66%	25.53%	31.42%
OPE-09	5	4	80%	10.63%	11.42%
OPA-11	9	6	66.66%	19.14%	17.14%
OPL04	11	5	45.45%	23.40%	14.28%
Total	47	35			
Average	9.4	7	74.75%	19.99%	19.99%

Determination of Genetic Affinity among Studied Genotypes:

Genetic distance, among studied genotypes, was calculated according to the equation of (Powell et . al 1996). The results of Table 3 showed the extent of similarity and Variance between the selected varieties,

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as it was found that the highest percentage of similarity corresponded to the lowest genetic dimension (0.679) between the varieties S6 and S3, then the distance (0.666) between the varieties S10 and S3. The results also showed that the lowest genetic similarity corresponded to the highest genetic dimension that was between the varieties. S10 and S9 reached (0.382). They were followed by the S10 and S1 categories (0.407).

Table 5. Inter-variety similarity indices and pair-wise genetic distances in different Eggplant varieties.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
S1	1									
S2	0.6	1								
S3	0.475	0.443	1							
S4	0.614	0.525	0.619	1						
S5	0.615	0.707	0.488	0.538	1					
S6	0.506	0.451	0.679	0.6	0.463	1				
S7	0.587	0.492	0.464	0.619	0.507	0.513	1			
S8	0.448	0.487	0.6	0.560	0.558	0.542	0.419	1		
S9	0.609	0.493	0.518	0.573	0.549	0.532	0.544	0.421	1	
S10	0.407	0.430	0.666	0.544	0.443	0.506	0.415	0.506	0.382	1

Cluster Analysis

The Dendogram based on the genetic dimension values using the UPGMA method (Nei 1972) (Figure 2) and constructed based on the results of the RAPD markers is shown.

the ten varieties were distributed into two main groups (A) and B), as cluster A split into two sub-groups (A1 and A2). Branch A1 included two secondary groups, the first of which included two genetic combinations (S7 and S4), and the second (S1 and S9). While group A2 included both varieties (S5 and S2). Cluster (B), was divided into two clusters, as the first cluster included each of the two subspecies (S3, S6, and S10), As the variety (S8) remained isolated from the other varieties.

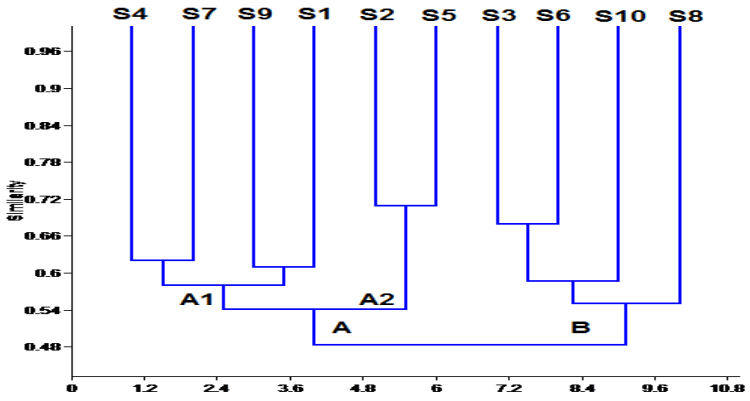

Fig 2: Dendrogram of the Studied Genotypes.

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Conclusion

Molecular study showed that the eggplant varieties under study contain a high genetic diversity that can be used as a basic material in breeding programs to improve the quantitative and qualitative traits and produce individual eggplant hybrids. The results were similar to what was found by (Nunome et al., 2001 and Demir et al.,. 2010, Islam et al, 2014, and Almiah and AL-Jaf, 2023).

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