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

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## Abstract

The study performed a genetic fingerprinting investigation of ten eggplant varieties (*Solanum melongena* L.) using random amplified polymorphic DNA (RAPD) markers based on polymerase chain reaction (PCR) and calculated the percentage of genetic distance. Using five RAPD primers in the fall of 2024 in the Seed Inspection and Certification Department's biotechnology labs.

The maximum discriminatory ability was 25.53% and 31.42% with primer OPC-05, while polymorphisms of 74.75% were found across all genotypes. The efficiency percentage of each primer was 25.53%. The findings demonstrated that the lowest genetic dimension (0.679) between Varieties S6 and S3 and the highest percentage of similarity (0.666) between Varieties S10 and S3 are correlated. The ten varieties were found to be divided into A and B, the two primary groups. Cluster A was further subdivided into two subgroups, A1 and A2, and branch A1 contained two secondary groups, the first of which contained two genotypes: S7 and S4, and the second was S1 and S9. Group A2 contained both types, S5 and S2, while cluster (B) was divided into two clusters, with both strains present in the first cluster, S3, S6, and S10, while the variety (S8) remained isolated from the other varieties.

***Key Word: Eggplant , RAPD***

## Introduction

One of the most significant members of the Solanaceae family is the eggplant (*Solanum melongena* L.). It is one of the economically significant vegetable plants, particularly in the world's hot and temperate zones. Since it was growing wild in both China and India, its native home countries, it has long been known (Matlub et al., 1989). Following its cultivation there, it started to spread to other areas, including North Africa and the Caribbean (Daunay et al., 2001). The plant is cultivated for its fruits, which are eaten after cooking. It is also used in the manufacture and preservation of pickles. It is frozen or canned for export (DGCIS, 2008). The country's local plant varieties are considered a national treasure and are part of its identity and heritage,they must be preserved. Therefore, because of their high tolerance to environmental conditions, their nature, and their suitability for those conditions, as well as their resistance to diseases and insects, countries especially developed ones are interested in protecting their natural plant genetic resources (NCRCAV 2022).

Genetic diversity can be assessed using a number of criteria, the oldest of which are morphological and later genetic criteria, such as by examining proteins or deoxynucleic acids and the

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The latter belong to the field of biotechnology and are referred to as molecular markers. There are some differences between technologies that are based on the analysis of molecular markers. The polymerase chain reaction-based Randomly Amplified Polymorphic DNA (RAPD) technology is one of the most important genetic variations it reveals. (Hussein, 2010).

In contrast to SSR markers, which detect a few, if not a single, sites in the genome (Goldstein et al., 1995, Powell et al., 1996), RAPD markers are distinguished by a number of characteristics that make them the most prevalent among PCR-based markers. The most significant of these characteristics is their capacity to detect multiple sites in the genome because primers bind to multiple sites, as well as the uniform distribution of these sites throughout the genome (Edwards, 1998). In a study by Hussein (2010), the genetic analysis revealed the presence of genetic variations between ten eggplant (*Solanum melongena* L) varieties, and the chosen eggplant varieties gave different phenotypic and genetic variations. The study also used markers of random polymorphic duplication of the RAPD (DNA) chain to determine the percentage of genetic distance, the percentage of genetic dimension that emerged from the study of the RAPD marker results—the highest of which was 77.77% between the two kinds (Moussalli X Abu jethea)—confirmed this.

In an experiment on 12 Eggplant varieties to study their genetic and morphological diversity, Amplification with five primers utilizing RAPD technique yielded 54 bands, 49 of which were polymorphic. It was discovered that the maximum 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).

Therefore, by identifying the unique genetic fingerprint of the investigated eggplant varieties, which acts as the identity used to diagnose those varieties, the research aims to investigate the variety of genes among the eggplant varieties included in the research that were collected from various regions of Iraq using RAPD technology. In order to help plant breeders select the right parents for breeding and improvement operations, the genetic link between the investigated varieties is thus determined based on the degree of genetic similarity between those types.

# 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 conducted in the Ministry of Agriculture's Seed Inspection and Certification Department's biotechnology labs. 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**

The immature leaves of the types under study were used to harvest DNA. Each eggplant type yielded an estimated 50–150 µg of DNA per 1.5 g of leaves, with purity ranging from 1.7–2 according to a Nanodrop device. The concentration of 50 ng $\mu L^{-1}$, the right concentration for carrying out PCR reactions, was obtained by adjusting the dilution of the DNA samples.

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randomly amplified polymorphic DNA (RAPD) technology, which is based on PCR technology, was utilized to extract DNA from plants, this is because various plants include varying amounts of vegetal components, including proteins and complex carbohydrates, in addition to DNA. Among the most efficient techniques for separating plant DNA is the approach used to extract the DNA from the eggplant plant (Weigand et al., 1993). Sahgi-Maroof et al. (1984) use eggplant as an example. In addition to the fact that some plants contain high levels of phenolic substances and polysaccharides, which are regarded as pollutants because they can create a very viscous liquid by precipitating with the DNA, the thick wall surrounding the cell membrane makes it more difficult to extract DNA from plants than from other organisms. inhibits the PCR process. Dilution of the isolated DNA was done to lower the amount of inhibitory sugars in order to eliminate these compounds.

**RAPD analysis**

The five primers used in the investigation were imported from Bloneer. The fusion temperature and nucleotide sequence of the primers employed in the investigation are displayed in Table 2.

With certain adjustments, the polymerase chain reaction (PCR) was performed in accordance with Williams et al. (1990). Utilizing a distilled and DNA master mix with an ul.ng40$^{-1}$ concentration, the final reaction volume was 25 ul. Under the following circumstances, this reaction occurs in the thermal cycler:

**1. Partitioning: to separate the two strands of DNA, heat the mixture to 94°C for five minutes. 2–40 cycles, with the following phases included in each cycle:**

**2-1-The partitioning process is conducted for 30 seconds at 94°C.**

**2-2 -Coalescence: For one minute, each initiator's coalescence temperature is varied. Two to three minutes of elongation at 72°C.**

**3. For one minute, the reaction is carried out at 72°C.**

Following that, the samples are kept at 4 °C before being transferred to an agarose gel.

**Table 1: Primers' nucleotide sequences utilized 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 = (108 g of Tris borate, 55 g of boric acid, 9.2 EDTA, and pH 0.8 make up 10X TBE buffer)

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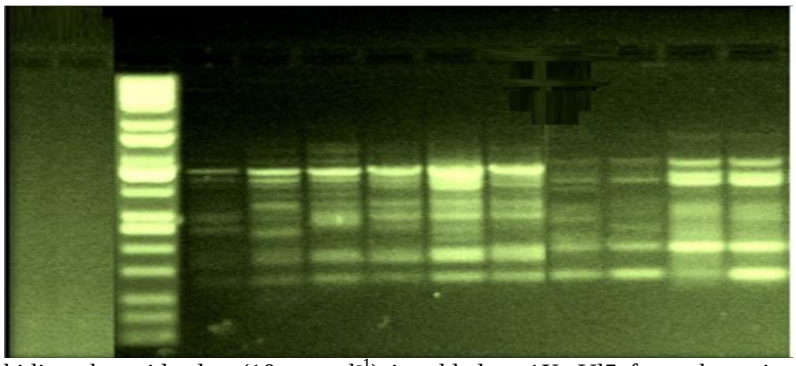

Ul5 from the ethidium bromide dye (10 mg.ml$^{-1}$) is added to 1X. Ul5 from the unique loading fluid (Bromophenol blue 1X Loading solution) was added to DNA samples before they were put onto an agarose gel. The size and molecular mass of the resultant bands were also ascertained by injecting an indication of DNA Kpb1 from Geneaid. To separate the bands of DNA that were produced by the amplification and enable the gel to be photographed using an agarose gel imaging instrument (Agle Eye II Staratagene), the migration was then completed by applying an electric field of 100 volts. Figure 1.

**Figure 1: PCR products on an agarose gel.**

**Statistical analysis**

Based on whether or not DNA bands were present in the examined samples, the amplification process's outcomes were compiled in a table. Only a visible DNA band is present when the number 1 is present, and the band is absent when the number 0 is present. The Unweighted Pair Group program was used to create the genetic kinship tree after the tables for each primer were arranged independently. Arithmetic Method With (UPGMA): Applying the statistical program from the past to average. Nei and Lei (1979) served as the basis.

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

Each primer's percentage (%) of primer polymorphism= (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 (%) = (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:

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

# II. Results

Five primers were utilized in the investigation. Each plant in this study had a different number of sites complementary to that primer in its genome, which led to variations in the number of duplicated bands depending on the primer used. These variations were the basis for the RAPD marker results. The presence or lack of bands originating from the duplication of specific regions of the plants' genomes served as the basis for evaluating the genetic relationship study's findings, and the molecular

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The number and complementary locations of the primer sequences on the DNA strand determine the weights of the bands (Nei's, 1973).

**Polymorphism**

According to Table 2, the primers employed resulted in 47 bands, 35 of which were polymorphic, meaning that 74.75% of the bands had polymorphism, this suggests that the eggplant varieties included in the study exhibit genetic divergence. When the samples were migrated on the polyacrylate gel, the primer OPE-09 produced the fewest bands (5 bands), whereas the primer OPC-05 produced the most bands (12 bands) when compared to the other primers utilized. The degree of compatibility between the primers' binding to the plant genome and the components of each primer's nitrogenous bases determines changes in the quantity of bands produced by each pair of primers used. Additionally, variations in the bases' sequences result in different binding sites between the primers and the plant genome under investigation, which causes variation. The number of bands that the primers used produced (Vos et al., 1995)Since the highest percentage of polymorphism in the primers (OPC-05) reached 91.66%, the primers employed were successful in providing polymorphism among the resultant bands despite this disparity in the number of bands. However, we discovered that the primer (OPL04) did not provide more than 45.45% of the polymorphism. Formally, differences in the plant genome's nucleotide sequence are the cause of this disparity in percentages, which influences the primers' attachment sites. Rearrangement, linkage, genetic crossover, and other factors cause this variance, which is why we see the various bands either clearly on the gel or not at specific spots. Jelly (Lanigan and Clark, 1993).

According to the data, primer OPC-05 had an efficiency percentage of 25.53%, while primer OPE-09 had an efficiency rate of 10.63%. Primers OPC-05 and OPE-09 had the highest and lowest discriminating abilities, respectively, with 31.42% and 11.42%. As a result, at a rate of 31.42%, the primer OPC-05's efficiency had the highest discriminatory ability when compared to the other primers. In the future, this primer can be used to identify common bands and analyze them to create more specialized primers that can differentiate between genotypes with a wide genetic dimension.

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

| Primer codes | Total bands scored | polymorphic bands number | 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% |

**Calculating Genetic Affinity Among the Genotypes Under Study:**

The genetic distance between the genotypes under study was determined using the formula 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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since it was discovered that the genetic dimension between variety S6 and S3 was the lowest (0.679), followed by the distance (0.666) between types S10 and S3. Additionally, the data demonstrated that the biggest genetic dimension between the kinds correlated with the lowest genetic similarity. S9 and S10 arrived at (0.382). The S10 and S1 classifications (0.407) came after them.

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

| | S 1 | S 2 | S 3 | S 4 | S 5 | S 6 | S 7 | S 8 | S 9 | S 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| S 1 | 1 | | | | | | | | | |
| S 2 | 0.600 | 1 | | | | | | | | |
| S 3 | 0.475 | 0.443 | 1 | | | | | | | |
| S 4 | 0.614 | 0.525 | 0.619 | 1 | | | | | | |
| S 5 | 0.615 | 0.707 | 0.488 | 0.538 | 1 | | | | | |
| S 6 | 0.506 | 0.451 | 0.679 | 0.600 | 0.463 | 1 | | | | |
| S 7 | 0.587 | 0.492 | 0.464 | 0.619 | 0.507 | 0.513 | 1 | | | |
| S 8 | 0.448 | 0.487 | 0.600 | 0.560 | 0.558 | 0.542 | 0.419 | 1 | | |
| S 9 | 0.609 | 0.493 | 0.518 | 0.573 | 0.549 | 0.532 | 0.544 | 0.421 | 1 | |
| S 10 | 0.407 | 0.430 | 0.666 | 0.544 | 0.443 | 0.506 | 0.415 | 0.506 | 0.382 | 1 |

**Cluster Analysis**

Figure 2, displays the dendogram that was created using the RAPD marker results and based on the genetic dimension values using the UPGMA algorithm (Nei 1972).

After cluster A divided into two subgroups (A1 and A2), the ten variations were divided into two primary groupings (A) and B). The two secondary groups in Branch A1 were S1 and S9, and the first group had two genetic combinations (S7 and S4). However, both variants (S5 and S2) were present in group A2. Because the variation (S8) remained isolated from the other varieties, Cluster (B) was divided into two groups. The first cluster contained each of the two subspecies (S3, S6, and S10).

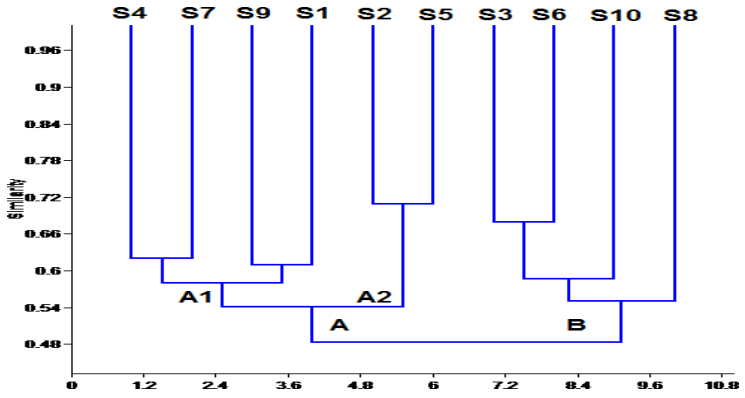


Fig 2: Dendrogram of the Studied Genotypes.

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**Conclusion**

According to a molecular analysis, the eggplant types under investigation have a high level of genetic variety, making them suitable as starting material for breeding programs aimed at enhancing both quantitative and qualitative qualities and creating individual hybrids. The findings mirrored those of Nunome et al. (2001), Demir et al. (2010), Islam et al. (2014), and Almiah and AL-Jaf (2023).

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