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# Analysis of Genetic Diversity in Hybrid Solanum lycopersicum L Lines with the RAPD Method

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## Abstract

The five hybrid tomato genotypes were investigated. Samples were obtained from disease- and insect-free young leaves; DNA was extracted with a Genomic DNA Mini Kit. The amount of DNA was determined by measuring the absorbency at 260 and 280 nm. The purity of the samples was 1.8–1.9, indicating isolation efficiency. Ten primers of RAPD from Operon Technology were initially applied among which five primers (A10, I14, A9, C13 and K01) produced good discriminative bands. Long distance polymerase chain reactions (PCR) were conducted with AccuPower® TLA PCR PreMix tubes, StCyclesY PCR was repeated 40 times here. The electrophoresis gel on 1.2% agarose was then run and the ethidium bromide-stained gel viewed under UV light the bands being noted. The results revealed that Different numbers of amplified bands were generated under the influence of primers. It presented primer K01, with the highest total number of bands (20 bands) and primer I14 with the lowest number of them (8 bands). In line with that, Prime C13 presented the highest amplification efficiency (100), and discriminatory power values of 10.4 which make it suitable to de- tect genetic variation among the genotypes studied.

The efficiencies of primers were between 21.43 and 100%. Results showed that certain primers have a stronger affinity with repetitive sites in the genome to affect

the detection of genetic variation. Genetic similarities showed a notable diversity among studied cultivars (0.24 to 0.51). This presented variability was of a genetic nature between the genotypes and indicated a medium to high genetic diversity. The varieties were grouped into two main clusters based on their genetic similarity and dependence from a common genetic background using the UPGMA analysis to generate a dendrogram.

**Keywords:** Molecular Markers, RAPD, Primer, Genetic Variations.

## Introduction

Solanum lycopersicum L. in the Solanaceae family, is an economically important plant worldwide as it has both nutritional and industrial value, as well as serving as a main object material in the breeding program for plants and its adaptation to climate change and resistance to environmental stress (1). Accordingly, this has found extensive use in illustrating how plants interact with pathogens and with defense against environmental pressures such as drought, salinity, and extreme temperatures that are of high relevance to these climate changes (2). Solanum lycopersicum L is the most important crop in terms of nutritional value altogether, because it contains an abundance of potassium, phosphorus, iron and B vitamins as well as being a good source of dietary fiber. It is recognized by red color it has, which comes from the pigment lycopene, also present in tomatoes, as it is a natural antioxidant (3). This fruit contains other compounds such as carotenes, anthocyaninom and vitamins like the ascorbic acid (vitamin C), vitamin E but also of phenols and flavonoids. It has antioxidant, anti-inflammatory, antiallergic and anticoagulant activity and is helpful in attenuation of the development of cardiovascular diseases, cancer and neurological diseases (4 – 9).

The flowers of Solanum lycopersicum L. are small and yellow in colour. They grow in clusters containing mixed reproductive organs, i.e. male and female, which helps them to self-pollinate (5). The stems of the plant are long, green, ribbed and covered

with fine hairs its fruits are berry-shaped and vary in size, shape and colour. The most common colour is red, but there are other varieties that contain yellow, orange and black colours (6).

Solanum lycopersicum L. varieties differ in their growth. Some are determinate, growing in a specific period of time, while others are indeterminate, continuing to grow throughout the year and producing fruits and flowers. Very high and very low temperatures lead to a negative impact on plant growth (7). The need to study genetic diversity among foreign and local varieties and evaluate their genetic efficiency has emerged as an essential step in developing genetic improvement programs and identifying genetic indicators associated with the expression of desired traits such as disease resistance, fruit quality and productivity (8).

This study aimed to identify genetic diversity using precise molecular markers, namely RAPD (Random Amplified Polymorphic DNA) in a group of local and hybrid seeds of Solanum lycopersicum L., as it depends on random polymorphic amplification of DNA by polymerase chain reaction (PCR) for the corresponding regions of the genome using short primers (10 nitrogenous bases) and random sequences that are linked to scattered sites on the genome and produce distinctive banding patterns. It is used as an efficient approach for evaluation of genetic relatedness and a molecular fingerprinting method to identify genotypic variations among cultivars and lines. This technology is inexpensive, produces rapid results and requires only small quantities of DNA as it analyses an enormous number of samples in a very short time by which to create a genetic database for use in genetic improvement programmes and maintaining germplasm resources in tomatoes.

(9) Studied 64 Solanum lycopersicum L. cultivars from six northern governorates in Iraq with CDDP, SCOT and ISSR. The data indicated high genetic diversity among collected cultivars, which can help in the study of population structure and genetic heterogeneity for Solanum lycopersicum L. in Iraq. Eleven SLL cultivars were

analyzed using ISSR and RAPD, and the genetic diversity they identified greatly enriches the genetic relationship of different cultivars.

(10) 100% with polymorphism, that is the wide genetic diversity which was available for improvement programs using 6 primers of RAPD technique on a total of 5 accessions in Solanum lycopersicum L.. In Nigeria (11) 14 S. lycopersicum L. cultivars were accessed and a high genetic variation was presented that could be used in plant breeding scheme.

(12) Presented a study to evaluate the genetic diversity among Solanum lycopersicum L. cultivars grown in Iraq using RAPD molecular markers. The results showed genetic variation among the cultivars selected for the study. The results of the analysis of 8 local Solanum lycopersicum L. cultivars in Morocco showed high genetic diversity, which contributes to understanding the genetic relationships between different cultivars (13). (14) Demonstrated high genetic diversity using the RAPD marker at 100% for 28 Solanum lycopersicum L. cultivars using UPGMA analysis. In Bangladesh, two main groups of cultivars were identified based on cluster analysis of 16 Solanum lycopersicum L. cultivars using 8 RAPD primers (15). (16) Demonstrated 78.6% genetic diversity in the analysis of 35 Brazilian Solanum lycopersicum L. cultivars using 20 RAPD primers. Using genomics for comparison helped identify genetic regions responsible for traits such as fruit ripening, resistance to pests and plant diseases, and tolerance to environmental conditions. Genomic studies have enabled the identification of genes associated with increased lycopene content or improved resistance to certain diseases and viruses. Using RNA Seq technology, researchers have been able to study a specific tissue to determine which genes are activated or inhibited at a specific time point and under specific conditions (17).

CRISPR has been used to improve the quality of Solanum lycopersicum L. fruits and to modify the genes responsible for fruit ripening, which leads to preserving them

for a long period without spoilage, or improving their content of desired compounds such as lycopene or taste or flavor (18).

## Materials and Methods

### Sample Preparation:

Five hybrid species of the genus Solanum lycopersicum L. were used in local markets in Najaf Governorate. A. Nur, B. Ankon, C. Marmande, D. Imperial, E. Hend

Seeds of the hybrid species were sown on cork plates inside the wooden canopy of the Najaf Governorate Agriculture Department. After six weeks, the seedlings were relocated to clay pots contained a soil mixture. Leaves, clean of disease and insect infestations, were gathered from each species singly. The leaves are used for DNA isolation and analysis employing the RAPD technique. The searches were denoted by the letters of the sequence given in the results photographs.

### DNA Isolation:

The Genomic DNA Mini Kit with Geneaid Biotech Ltd., Taiwan, presents an easily understood and rapid procedure for obtaining DNA, contingent upon adherence to the published instructions. The level of DNA in the samples has been measured utilizing a UV Spectrophotometer (Shimadzu, Japan) under ultraviolet light at a wavelength of 260 nm. Each optical density measurements on the gadget was 1, which translates to 50 µg of DNA per 1 ml of fluid.

The purity of DNA was assessed by calculating the ratio of the optical density at 260 nm to that at 280 nm. DNA was deemed clean if the ratio of the two measurements fell between 1.8 and 2. Preparation of RAPD reactions. Ten primers from Operon Technology, each comprising ten nucleotide bases, were assessed. Five primers have been picked for their abilities for creating different changes in DNA (Table) (1).

Table (1): Ten RAPD primers, base sequences and molecular weight (bp)

| No. | primer | Base sequence ( 5` - 3` ) | Molecular weight (bp) |
|---|---|---|---|
| 1 | A10 | GTGATCGCAG | 3068 |
| 3 | I14 | TGACGGCGGT | 3084 |
| 6 | A9 | GGGTAACGCC | 3053 |
| 9 | C13 | AAGCCTCGTC | 2988 |
| 10 | K01 | CATTCGAGCC | 2988 |

All random amplification reactions were performed employing pre-mixed tubes (EcoPower ® TLA PCR PreMix tubes) which contained many polymerase reaction components. 5 microliters of DNA sample, 2 microliters of primer, and the rest of the volume was modified with sterile distilled water to a total of 20 microliters. The tubes were placed in a thermocycler for starting the amplification reaction (20) following the resulting schedule:

| No. | Phase | Tm (°C) | Time | No. of cycle |
|---|---|---|---|---|
| **1-** | Initial Denaturation | 95°C | 3 min. | 40 cycle |
| **2-** | Denaturation -2 | 95°C | 1min | |
| **3-** | Annealing | 35°C | 1min | |
| **4-** | Extension-1 | 72°C | 1 min | |
| **5-** | Extension -2 | 72°C | 10 min. | |

**Data Analysis:**

The results shown in the gel were converted into tables, and the bands resulting from the replication process were calculated by using the number 1 to represent the presence of a band and the number 0 to represent the absence of a band. The molecular sizes of the replication products were estimated by comparing them with the size index. To determine the genetic relationship between the hybrid Solanum lycopersicum varieties used in this study and to draw a dendrogram representing the genetic groups to which these samples belong, the data were analyzed and the genetic distance was extracted from them by entering them into a computer using the Applied Biostatistics program (Version 1.8) (21).

## Results and Discussion

Genomic DNA has been obtained from the leaves of the hybrid plant Solanum Lycopersicon, resulting in levels of DNA ranging within 150 and 290 nanograms per microliter, with a purity that varies from 1.9 to 1.8. This demonstrates how effective of the utilized technique for extraction, isolating a sufficient pure volume of DNA from plants is significantly more difficult than from other organisms due to the solid cell wall encasing the membrane. In addition, several plants possess a high amount of phenolic compounds and polysaccharides, which may impede PCR reactions. The species have been named by letters corresponding with the sequence provided in the results images: A. Nur, B. Ankon, C. Marmande, D. Imperial, E. Hend.

Five primers generated through Operon Techniques, a world leader in high-quality primers, were evaluated to ensure reliable results. The results gathered from employing these primers in RAPD reactions demonstrated variability in the number of multiple bands and their molecular weights depends upon whichever primer utilized, attributable to the varied quantities of complementary sites for each primer within the gene sequences of the several marigold breeds researched in this study. This is consistent with all existing literature in this region, including (23).

The study attempted to determine genetic variations across the tomato hybrids tested, causing to the rejection of five primers that produced common bands. Therefore, only five primers were utilized in the analysis of the RAPD reaction products (Table 1), demonstrating that the number of primers producing separate replication products in the RAPD reactions is depending upon the type of template DNA (24). The duplicated products then get transferred to a 1.2% agarose gel, stained with ethidium bromide, and combined with a DNA ladder indicator containing 1 kilobase pair (250-10000 base pairs) for a duration of 3-4 hours at 70 volts. The gel is afterwards examined under UV light and photographed with an imaging device. Following that,

the quantity of ensuing bands is measured beside their molecular sizes employed each primer (25).

The primers differ in terms of their ability of generating an adequate number of duplicated fragments (bands). The primer K01 provided the highest number of bands, in excess of 20, followed by 19 bands from primer C13, 18 bands from primer A10, 14 bands from primer A9, and 8 bands from primer I14 (Table No. (2). Screening indicated that primer C13 yielded the largest number of divergent bands, averaging 19, whereas primer K01 generated 14 bands (Table 2). The smallest number of duplicated bands was 14 and 8, respectively, with primers A9 and I14. The minimal amount of divergent bands for primers A9 and I14 was three bands (Figure 1). Some primers can recognize several binding sites, which is more favorable compared to products that discover fewer binding sites. The sheer number of duplicated bands is going to grow, hence raising the likelihood of distinguishing DNA variability amongst individuals. (26) The mere existence of a complementary site for a certain sequence in a specific DNA type, nonexistent in others, results in the regularity of certain sequences that are repeating in a given organism, though other sequences are uncommon or nonexistent (27).

The primer efficiency fluctuated from 100.00 for the C13 primer to 21.43 for the A9 primer. The results of this examination revealed that the C13 primer demonstrated the greatest discrimination ability value of 10.400, although the I14 primer exhibited the lowest value of 1.600 (Table 2). The discriminatory power can be determined by the primers' aptitude to accurately portray the genetic variation among individuals by considering the overall count of variations. Consequently, it was shown that the primer demonstrating the largest ability to bond additionally creates the highest supply of variant bands (International Union for the Protection of New Varieties of Plants, 2010).

Table (2): delineates the primer type, total band count, distinct band count, primer efficiency, and discrimination value

| Primer | TB | PB | Contrast ratio | RP % |
|---|---|---|---|---|
| A10 | 18 | 7 | 38.89 | 3.200 |
| I14 | 8 | 3 | 37.50 | 1.600 |
| A9 | 14 | 3 | 21.43 | 2.000 |
| K01 | 20 | 14 | 70.00 | 8.000 |
| C13 | 19 | 19 | 100.00 | 10.400 |

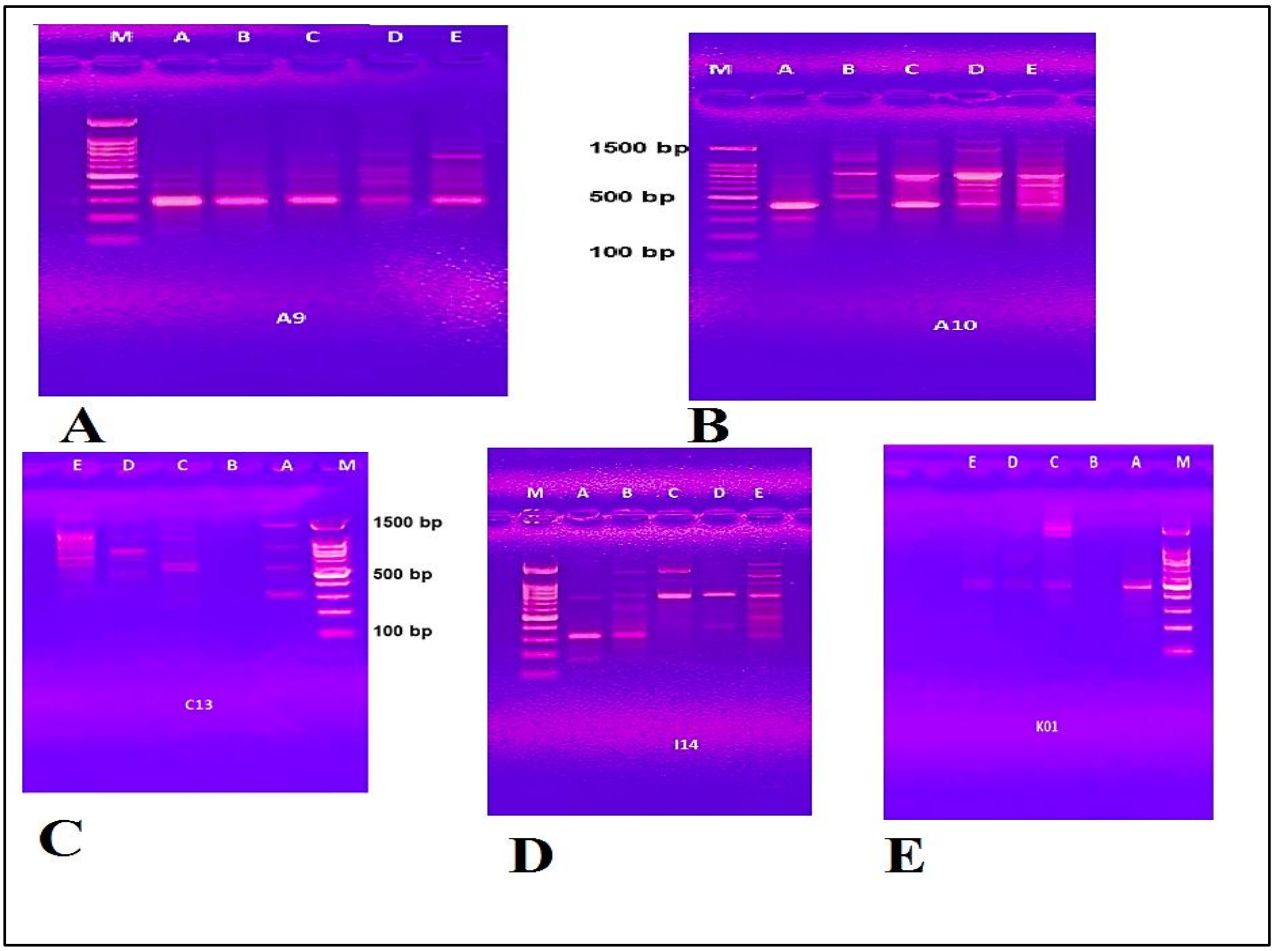


Figure (1): represents the multiplication primers staged on 1.2% agarose gel A10, I14, A9, K01 and C13 with the standard size indicator (M) for the plant varieties Solanum lycopersicum L. A. Nur B. Ankon C. Marmande D. Imperial E. Hend

Numerous studies have highlighted the significance of assessing the effectiveness and discriminative capacity (high correlation ability) of any primer to evaluate the genetic variability of the species in question (12). The genetic relationship tree (dendrogram) depicted in Figure 2 illustrates a distinct variation in genetic similarity among the five examined *Solanum lycopersicum* L. variants.

They are Nur, Ankon, Marmande, Imperial, and Hend, and are indicated by the symbols A, B, C, D, and E, respectively, using the similarity coefficients (Jaccard) and UPGMA analysis, as the similarity values ranged between 0.24 and 0.51. They were characterized by the formation of two main groups:

1. **The First Group:**

   It is distinguished from the cultivars Imperial by higher genetic similarity (about 0.80), which suggest her closeness relation or a common using of genetic structures/many equal bands in RAPD/ISSR-markers). Cultivars D and E may share the same genetic background, which is distinct from that of other cultivar

2. **The Second Group:**

   Includes cultivars A, B, and C (Nur, Ankon, and Marmande), respectively. It is noted that A and B are more similar to each other than cultivar C, which recorded a lower value. This indicates that they possess traits that distinguish them from cultivars A and B. The difference between the first and second groups may be related to similarities in morphology (morphological traits) or productive performance. They often have similar genetic structures that contributed to the formation of identical bands in molecular analysis. While A and B are close but different from C, which may reflect the influence of hybridization or the introduction of different genes in genetic improvement programs.

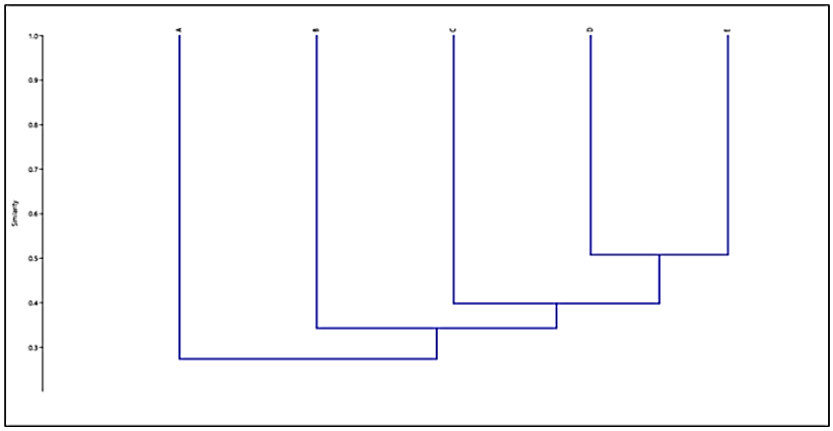

Figure (2): represents the genetic relationship tree Dendrogram and genetic similarity between the Solanum lycopersicum L. A. Nur B. Ankon C. Marmande D. Imperial E. Hend varieties studied using RAPD analysis.

The results of the genetic tree showed a clear genetic divergence, which may be due to differences in genetic origins or to different natural and agricultural selection that led to the differentiation of traits. This difference can be practically exploited in breeding or genetic improvement programs, as a variety can be selected from each group, such as A from the first group and E from the second, to generate hybrids characterized by increased genetic diversity and the emergence of new improved traits (Heterosis) (Figure 3)

The studied cultivars recorded the highest similarity value between cultivars D and E, i.e. hend, imperial, which is (0.5077), indicating that these two cultivars have a high degree of common characteristics, both at the level of phenotypic and genetic characteristics. This similarity may be attributed to their descent from a close genetic origin or to their exposure to similar environmental conditions that helped in the convergence of their characteristics. This is followed by the convergence of Marmande, imperial, symbolized by the letters D, C, with a value estimated between (0.4219), which suggests that the cultivar Imperial (D) forms a link between cultivars C and E, Figure 3.

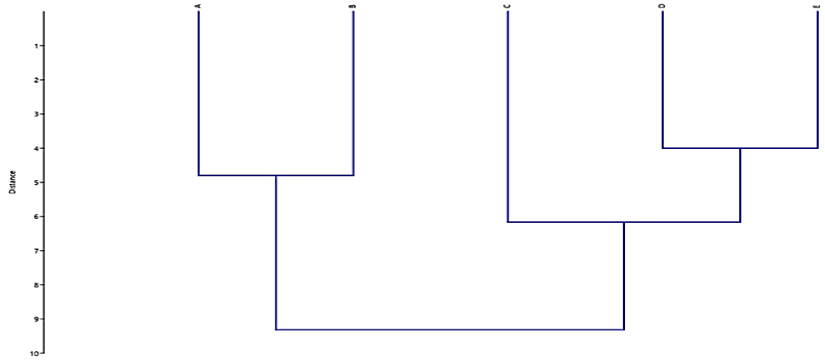

Figure (3): represents the genetic relationship tree Dendrogram and genetic variation among the studied Solanum lycopersicum L. varieties A. Nur B. Ankon C. Marmande D. Imperial E. Hend using RAPD analysis.

The two cultivars, nur and ankon, indicated by the symbols A and B, showed the lowest similarity values with the rest of the cultivars, as the highest similarity value for cultivar A with cultivar E was 0.348, and 0.377 for cultivar B with cultivar E. Both are less homogeneous compared to the D–E–C group, and may represent distinct genetic structures or distant from the rest of the group. The results proved that the cultivar (Hend), indicated by the symbol E, was closest to most of the other cultivars, as it appeared as a pivotal element linking the rest of the cultivars, making it the most homogeneous and diverse in common traits. Table No. (3).

Table No. (3): Genetic distance between the studied Solanum lycopersicum L varieties A, B, C, D, E using RAPD analysis

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| A | 1 | 0.258065 | 0.242424 | 0.246154 | 0.348485 |
| B | 0.258065 | 1 | 0.313433 | 0.338462 | 0.376812 |
| C | 0.242424 | 0.313433 | 1 | 0.421875 | 0.375000 |
| D | 0.246154 | 0.338462 | 0.421875 | 1 | 0.507692 |
| E | 0.348485 | 0.376812 | 0.375000 | 0.507692 | 1 |

These results prove that RAPD markers are a reliable and efficient molecular tool with low cost, fast reaction, minimum DNA amount requirement as well as capability

for the analysis of high population samples within short working period. The research also establishes a key genetic resource in order to be employed for future breeding and conservation of indigenous tomato genetic resources.

The findings in this study are in agreement with a number of previous works that have shown a widespread existence of genetic variation among S. lycopersicum L. cultivars around the world. Nasrin et al. (15) analyzed 16 Solanum lycopersicum L. cultivars with eight RAPD primers to determine genetic variation between the former ones.

Results revealed a wide genetic diversity among the sequenced cultivars. Cultivars were split into two primary genetic clusters, based on UPGMA analysis. The genetic similarity estimates varied from 0.35 to 0.80, showing a medium- to high-divergence between the genotype.

The authors stressed that these findings are reflection of the broad genetic resources that exist in the tomato germplasm in the region under study and therefore this can be utilized for further enhancement and selection of hybrids. Fourteen tomato varieties based on the researcher's description and morphological parameters (Ezekiel, 2011) were used in this study from markets of three southwestern states in Nigeria. DNAs extracted from apical leaves were examined by RAPD-PCR and 74 amplified products (bands) observed with an estimated 62.2% polymorphism, thereby indicating a strong genetic diversity among the cultivars. Heterogeneity degree of the primers tested among OPB-18 was higher (83.3%) and lowest was in OPU-14 (44.4%).

The results show that there is genetic divergence between the groups (A–B–C) and group (D–E), suggesting that such variation may be used by genetic improvement programs to broaden the genetic base and improving productive characteristics, resistance to diseases, and quality of fruits. The E-Hend type is the most homogenous and has very close relation to previous varieties, so it can be an important variety for

a genetic resource in further new hybridization works (27, 15, 20) and others. This also suggests that RAPD markers are important for studying the genetics of varieties, and to ascertain exactly whether different varieties such as grown together at IBADAN fields in Nigeria will not be mixed up due to their genetic relationship.

(28, 29) A total of eight local tomato types have been sampled from four regions in the Kingdom of Morocco and analysed using RAPD and SSR markers. The majority of RAPD markers were present at monomorphic band profiles indicating that many primers did not distinguish among genotypes. Only primer OPU03A yielded four heterozygous amplification products out of eight obtained in FIGUIG2, while the level of polymorphism using SSR markers was higher between cultivars compared to RAPD, with alleles number varying for each marker from 2 to 5 between cultivars(30, 31). Similar results using UPGMA clustering analysis were found and it is possible to discriminate four groups of cultivars, the one walking away from other FIGUIG2, as a tentative to select for such genetic distance. And it also proved that this is a traditional cultivar with very high level of homozygocity (99%).

## Conclusion

The present study successfully extracted high-quality genomic DNA from five Solanum lycopersicum L. hybrids, confirming the efficiency of the applied isolation technique. The RAPD-PCR analysis using five primers revealed substantial genetic variation among the tested cultivars, as indicated by the differences in the number of amplified bands, primer efficiency, and discrimination values. Primer C13 demonstrated the highest discriminatory power and polymorphism percentage, highlighting its effectiveness in differentiating closely related genotypes.The dendrogram analysis divided the cultivars into two main genetic groups: the first comprising Imperial and Hend with the highest genetic similarity (0.5077), and the second including Nur, Ankon, and Marmande, which showed lower similarity values. These variations suggest distinct genetic backgrounds likely shaped by

diverse breeding histories and environmental adaptations. The cultivar Hend appeared as a central genotype linking most of the others, suggesting its genetic richness and potential as a valuable parent in future hybridization programs. Overall, the findings confirm that RAPD markers are a rapid, reliable, and cost-effective molecular tool for assessing genetic diversity in tomato germplasm. The detected polymorphism levels demonstrate a wide genetic base among the tested cultivars, which can be strategically utilized in breeding programs to enhance productivity, disease resistance, and fruit quality. Furthermore, the genetic information obtained here can serve as a reference for conserving and managing local tomato genetic resources and for guiding future genetic improvement efforts.

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