**Original Article**

# DNA Fingerprinting for Determination of Phylogenetic Tree: A Genomic Study on Samples of Egyptian and Iraqi Populations

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## ABSTRACT

**Background:** Phylogenetic analysis is widely used to determine the evolutionary relationships, and it is expressed by the phylogenetic tree. The current work aimed to identify the phylogenetic tree of Egyptian and Iraqi population samples, and to compare between the used deoxyribonucleic acid (DNA) fingerprinting techniques [Inter-Simple Sequence Repeat (ISSR)- polymerase chain reaction (PCR) and Random Amplified Polymorphic DNA (RAPD)-PCR]. **Methodology:** This comparative cross-sectional study was carried out on 48 unrelated healthy volunteers from different geographic areas [Northern Egypt "NE", Southern Egypt "SE", Northern Iraq "NI" and Southern Iraq "SI"]; twelve volunteers from each area. Seven primers (OP-A3, OP-A9, OP-B3, OP-C3, OP-D1, OP-C15 and OP-K2) were used in RAPD-PCR. Seven primers (14A, 44B, HB-9, HB-10, HB-11, HB-12, and HB-13) were used in ISSR-PCR. **Results:** Similarity coefficients and phylogenetic trees revealed the genetic distance and relationship of the studied groups as follows: the highest genetic overlap "similarity" was between SE and both NI and NE, respectively. The biggest genetic distance (variation) was between SI and both "NI and NE". **Conclusion:** The study concluded that: (1) RAPD-PCR method was more valuable in detecting genetic polymorphism as compared to ISSR-PCR method, (2) Combined analysis using both RAPD and ISSR-PCR methods gave a clear indication of genetic variations than either RAPD or ISSR-PCR techniques.

**Keywords:** DNA fingerprinting, phylogenetic tree, Egyptian, Iraqi, RAPD-ISSR, PCR.

## I. INTRODUCTION

As a method of human identification; DNA fingerprinting is a rapid investigation with high-quality and certainty (probability of an identification is ≥ 99.95%), and with less costs in large-scale samples as compared to other identifiers, e.g., dactylography or dental records (Parsons et al., 2019). DNA is nearly present in all cells, so it is a valuable target for identification. It can be extracted from several tissues (body fluids, soft, degraded tissues, bones, teeth, and hairs) with the most common being whole blood and saliva (Ibrahim et al., 2019 and Zgonjanin et al., 2019).

Phylogenetic analysis is a procedure used to predict the evolutionary history and genetic relationships between different organisms or species, through analysis of certain DNA sequences (Bienstock et al., 2014 and Mathur and Adlakha, 2016).

Many DNA regions that exhibit genetic variations between individuals were discovered, such as microsatellites which are highly repetitive polymorphic DNA loci commonly known as short tandem repeats (STR), that are considered ideal for forensic investigations due to high polymorphism and ability to be amplified by PCR even in very degraded samples (Ghaleb et al., 2019 and Romano et al., 2019).

Several molecular methods are used to determine the genetic differences among humans, most of them are PCR-based methods e.g., random amplified polymorphism DNA (RAPD) and inter-simple sequence repeats (ISSR), which are valuable techniques for detection of genetic variations, even in extremely related individuals (Song et al., 2017; Young et al., 2019 and Zare et al., 2019).

Inter-simple sequence repeats (ISSRs) are regions in the human genome flanked by microsatellite sequences, that can be amplified by PCR using a single primer giving multi locus patterns that can be used to identify genetic variations (Pérez de la Torre et al., 2012). As a molecular marker, RAPD technique does not require any specific knowledge of the DNA sequence of the target organism, in addition fewer requirements of template DNA are required (Fu et al., 3013).

Egypt is an ideal place for population genetics studies, due to its important geographic location and interactions with cultural areas in Africa, Asia, and Europe. Egypt has an increasing flow of foreigners either for living or working, especially from Arab countries like Iraq, which lead to a complicated and entangled cultural and genetic exchange (Schuenemann et al., 2017).

The current work aimed to identify the phylogenetic tree (genetic distance and relationship) of samples from Egyptian and Iraqi populations, as well as to compare between the used DNA fingerprinting techniques [Inter-Simple Sequence Repeat (ISSR)-PCR and Random Amplified Polymorphic DNA (RAPD)-PCR].

## II. SUBJECTS AND METHODS

**Subjects:**

This comparative cross-sectional study was performed on 48 unrelated healthy volunteers from different geographic areas [Northern Egypt "NE", Southern Egypt "SE", Northern Iraq "NI" and Southern Iraq "SI"]; twelve volunteers from each area. Inclusion criteria: all participants were healthy, unrelated individuals, original habitants of the selected geographic areas and of age >18 years. Exclusion criteria: individuals of age <18 years, or those with any chronic disease.

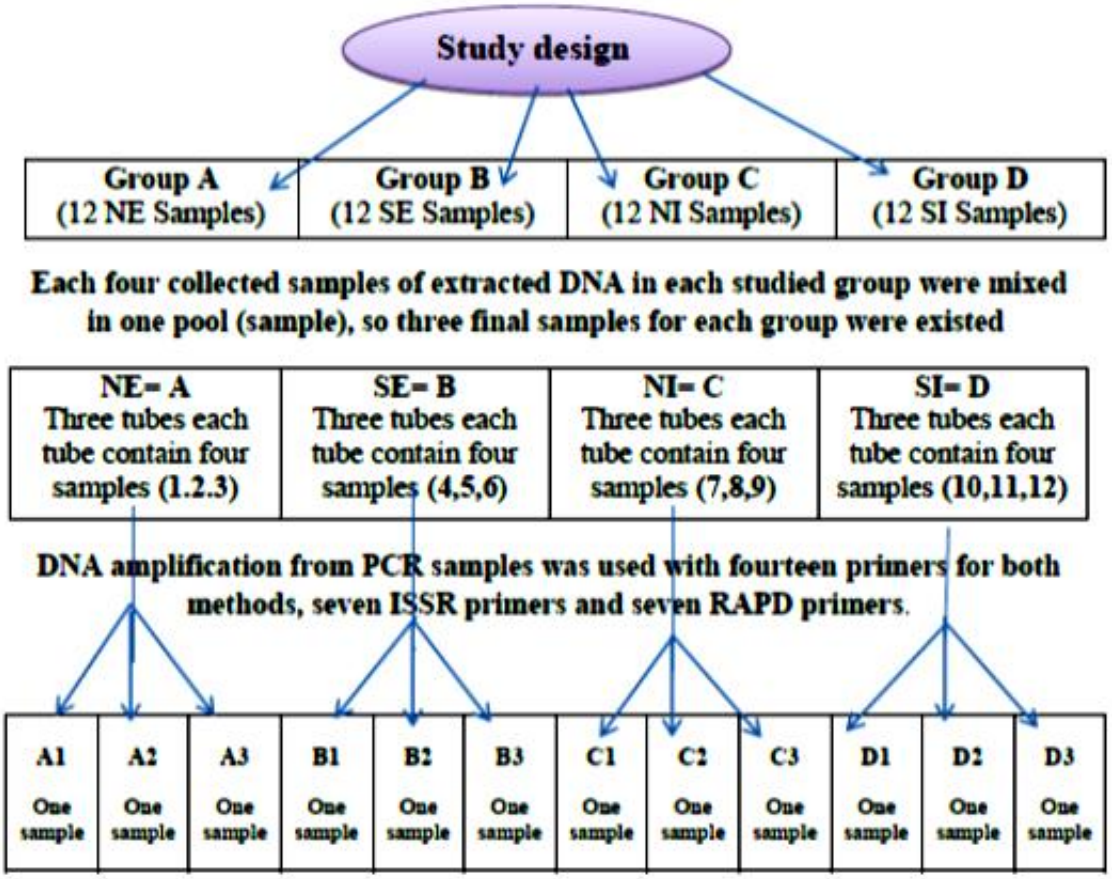
Personal interviews were conducted with volunteers in order to make sure of their ethnic origins [all participants and their families have been living in the geographic area for at least three generations]. An informed consent was gathered from each participant in this work. All results obtained were registered in special sheets, which were confidential. The study protocol was accepted by the Research Ethics Committee of Faculty of Science, Benha University.

**Sampling:**

Approximately 3ml whole peripheral venous blood sample was taken from each participant, by a well-trained nursing team and put immediately into EDTA vacutainer tube. Each sample was transferred with special cooling compartments to the Molecular Biology Laboratories at Faculty of Science, Benha University, where it was splitted into two eppendorf tubes, stored at -80°C for further handling steps.

**Bulked segregant analysis (BSA):**

Bulked segregant analysis (BSA) is a technique used to pool multiple DNA samples to make a bulked DNA sample that can be analyzed using methods such as RAPD or ISSR-PCR. BSA enables rapid recognition of markers linked to any particular gene or genomic location, as well as precise detection of genetic similarities and differences (Michelmore et al., 1991). In this study BSA technique was adopted, as in each studied group; four samples were mixed to make one pool "sample" (Fig. 1).




**NE:** Northern Egypt; **SE:** Southern Egypt; **NI:** Northern Iraq; **SI:** Southern Iraq; **DNA:** Deoxyribonucleic Acid; **PCR:** Polymerase Chain Reaction**; ISSR:** Inter-Simple Sequence Repeat; **RAPD:** Random Amplified Polymorphic DNA

**Figure (1):** The study design [bulked segregant analysis (BSA)].

## Methods:

### I- Genomic DNA extraction:

- Extraction of DNA was performed using QIAamp DNA blood mini kit (Qiagen, Germany) according to the manufacturer instructions.
- The concentration of extracted DNA was measured and confirmed by NanoDrop 2000c UV/VIS Spectrophotometer (Thermo Fisher).
- Readings were recorded at wave lengths of 260 and 280 nm, and DNA sample concentration was measured = 50 ug $mL^{-1}$×A260×, according to Alhusseini et al. (2014).
- The final concentration of DNA was adjusted to 10 ng/UL for molecular analysis.

### II- PCR amplification:

- In rapid cycler PCR (ABI-Verti Thermal cycler, Applied Biosystem Life Technology,

USA), 10 µL from each sample of extracted DNA were used for both RAPD and ISSR using PCR master mix kit (2X Easy Taq PCR super Mix, Tran China), followinhg the manufacturer's instructions.

- Amplification was carried out using seven random primer sets for RAPD and another seven specific primers for ISSR (Table 1), obtained from Operon, inc Huntsville, Alabama, Germany.

**Table (1):** List of the primer names and their nucleotide sequences used in RAPD and ISSR procedures.

| No. | Primer | Sequence Primer |
|---|---|---|
| **RAPD procedure** | | |
| **1** | **OP-A3** | **5´CAG CAC CCA C 3`** |
| **2** | **OP-A9** | **5´ CCT TGA CGC A 3`** |
| **3** | **OP-B3** | **5` CAT CCC CCT G 3`** |
| **4** | **OP-C3** | **5`GGG GGT CTT T 3`** |
| **5** | **OP-D1** | **5´ GAC GGA TCA G 3`** |
| **6** | **OP-C15** | **5´ ACC GCG AAG G 3`** |
| **7** | **OP-K2** | **5´ GTC AGG CGT C 3`** |
| **ISSR procedure** | | |
| **1** | **14A** | **5 $(CTC)_3$ $(TCT)_2$ TTG 3`** |
| **2** | **44B** | **5` $(CTC)_3$ $(TCT)_2$ CTC TGC 3`** |
| **3** | **HB-9** | **5`$(CAC)_3$ GC 3`** |
| **4** | **HB-10** | **5` $(GAG)_4$ CC 3`** |
| **5** | **HB-11** | **5` $(GAG)_4$ CC 3`** |
| **6** | **HB-12** | **5`$(CAC)_3$ GC 3`** |
| **7** | **HB-13** | **5´ $(GAG)_3$ C 3`** |

**RAPD:** Random Amplified Polymorphic DNA; **ISSR:** Inter-Simple Sequence Repeat; **No.:** Number

- The amplification program was 94°C for 5 minute as initial denaturation then cycling for 45 cycle 94°C 45 sec., 40°C 1 minute in RAPD and 52°C for ISSR and 72°C 1 minute). The final extension was 72°C for 5 minute.

**Analysis of the Amplified Products:**

- The amplified DNA was analyzed by gel electrophoresis. About 10 µL of each reaction mixture and 100Base Pair (BP) ladder (Molecular weight marker) was separated on 1.5 % agarose gel containing 0.3 µg $mL^{-1}$ of ethidium bromide.
- The bands of marker ranged from 100bp- 3000bp; were visualized using UV and Li coefficient system analysis (Nei and Li, 1979).

Tranilluminator (254 nm) and photographed using a digital camera 8 mega pixel. The resulted image was analyzed by computer software (Alpha Inno Tech Gel Documentation System, Germany).

**Data analysis:**

- All bands gained by either RAPD or ISSR methods within gel profiles were analyzed as appeared records (1) and absence records (0). Indistinctly stained bands (unclear bands) were not included in the data collection.
- The similarity indices between all studied samples were calculated using Nei
- Dendrograms (phylogenic trees) from the results of each method based on

unweighted pair-group procedure with arithmetic mean (UPGMA), according to Poyraz (2016) and Mei et al. (2017), were produced using NTSYS-pc Version 2.1 packages (Informer Technologies, Inc., Los Angeles, CA 90045, US), to illustrate the genetic relationships between the different studied population samples.

## III. RESULTS

### A- RAPD-PCR method:

Seven random primers were used: OP-A3, OP-A9, OP-B3, OP-C3, OP-D1, OP-C15 and OP-K2. The phylomorphic amplification band sizes of RAPD products ranged from 100bp–1000bp with number of bands ranged from 20-50 bands, average (28 band/primer).

Maximum bands (50) appeared with OP-A9 primer with 14 polymorphic bands and 36 unique bands. Minimum bands (20) appeared with OP-K2, OP-C15 and OP-C3 with 3,6,6 polymorphic bands and 17,14,14 unique bands respectively (Table 2 and Fig. 2).

**Table (2):** Total amplified fragments by arbitrary nucleotide sequence RAPD primers (%); polymorphism and frequency.

| RAPD Primers / Polymorphism | OP-K2 | OP-C15 | OP-D1 | OP-C3 | OP-B3 | OP-A9 | OP-A3 |
|---|---|---|---|---|---|---|---|
| Monomorphic | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Polymorphic | 3 | 6 | 3 | 6 | 7 | 14 | 9 |
| Unique | 17 | 14 | 22 | 14 | 13 | 36 | 31 |
| Total number of bands | 20 | 20 | 25 | 20 | 21 | 50 | 40 |
| (%) Polymorphism | 100.0 | 100.0 | 100.0 | 100.0 | 95.2 | 100.0 | 100.0 |
| Frequency | 0.100 | 0.116 | 0.093 | 0.116 | 0.170 | 0.107 | 0.104 |

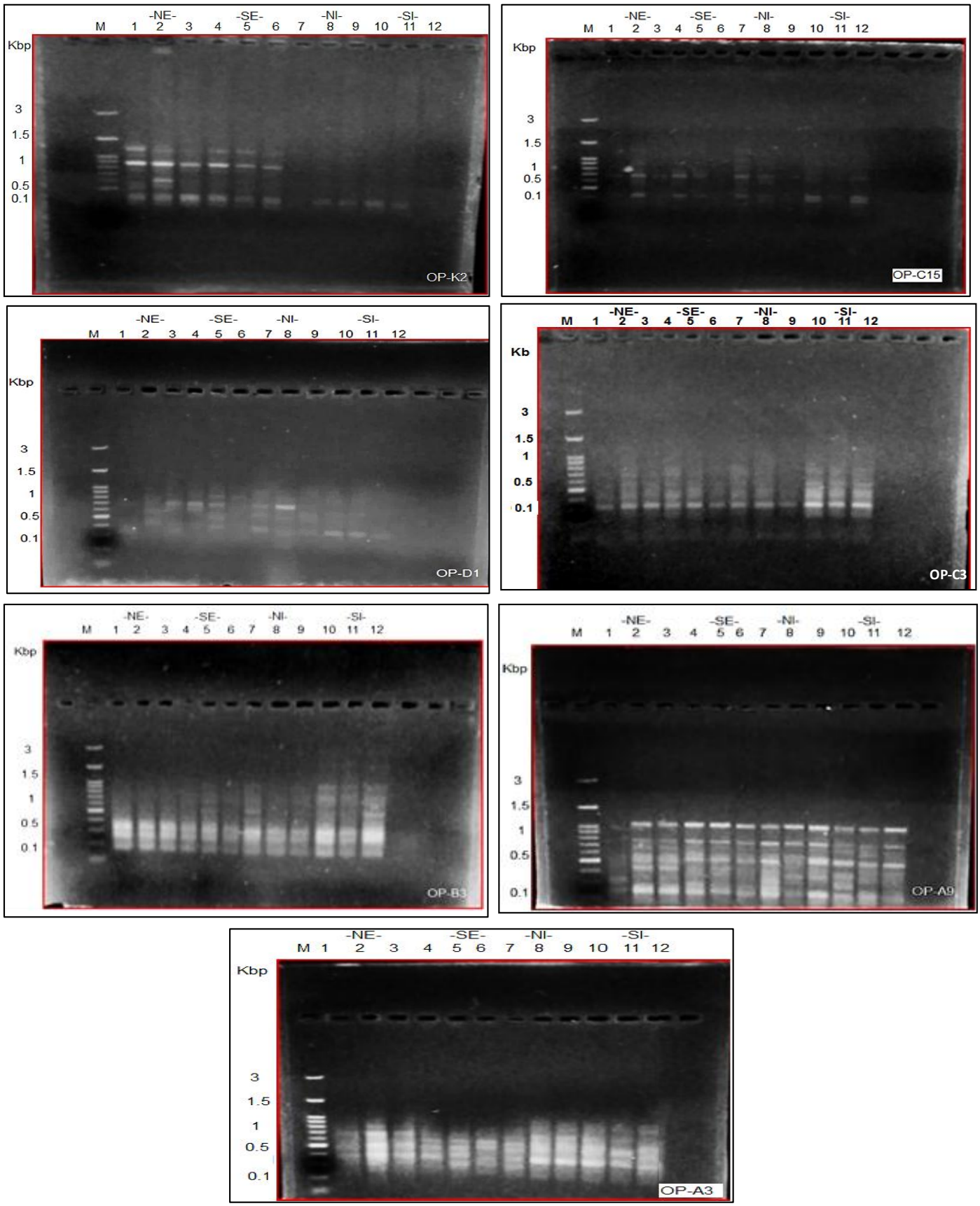
**RAPD:** Random Amplified Polymorphic DNA

**Similarity coefficient [index]:**

Similarity index (Nei and Li coefficient) was done between different groups; Northern Egypt (A1, A2, A3), Southern Egypt (B1, B2, B3), Northern Iraq (C1, C2, C3) and Southern Iraq (D1, D2, D3), using RAPD-PCR method to find out phylogeny. The highest value was 0.353, which fell within the groups D1 and D2, the lowest value was 0.087, which fell within the group A1 and D1, and showed the biggest genetic distance (Table 3).

**Phylogenic (Kinship) tree:**

Phylogenetic tree obtained from the results of RAPD-PCR method showed the genetic relationships (similarities and differences) between groups, in which, each group appeared from the original branch of the tree, however, some groups had a basic overlap with other groups, for example; B3 overlapped with group C. Also, group C3 overlapped with group D (Fig. 3).



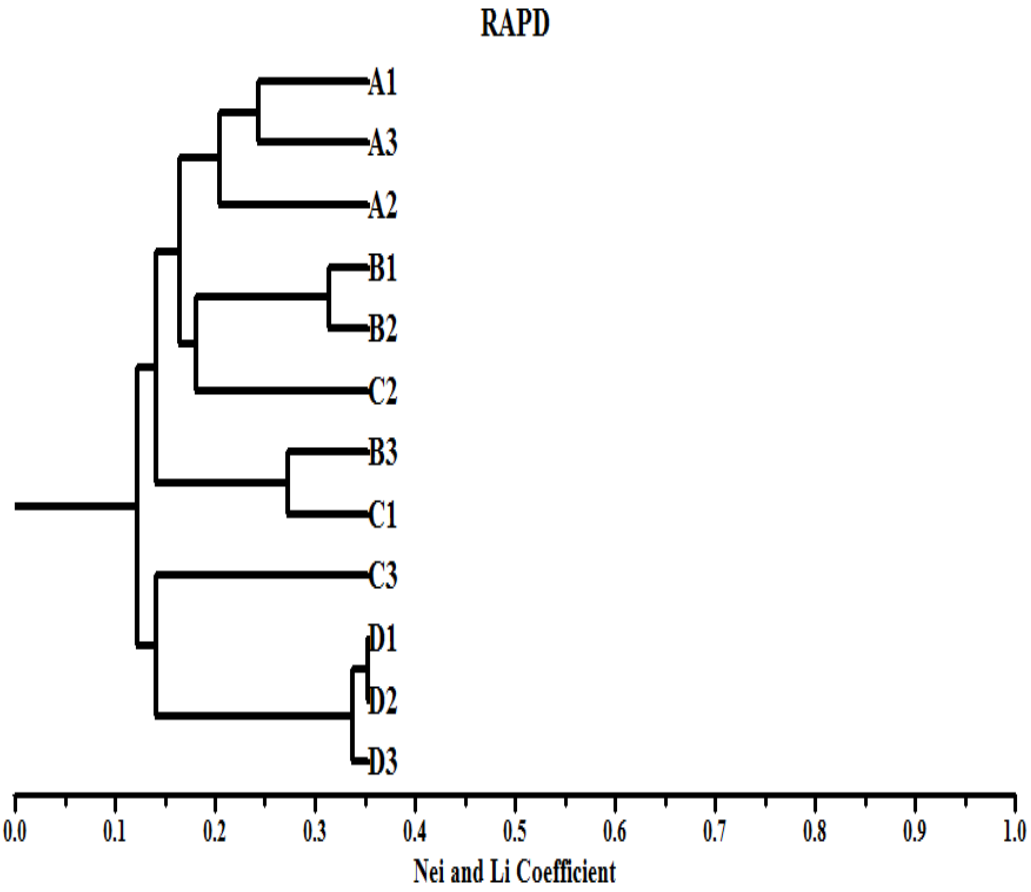

**NE:** Northern Egypt; **SE:** Southern Egypt; **NI:** Northern Iraq; **SI:** Southern Iraq; **RAPD:** Random Amplified Polymorphic DNA; **PCR:** Polymerase Chain Reaction

**Figure (2):** Agarose gel electrophoresis of representative results (banding patterns) of RAPD-PCR finger-printing method using the seven random primers [OP-K2; OP-C15; OP-D1; OP-C3; OP-B3; OP-A9 and OP-A3].

**Table (3):** Similarity index of genetic distance values between different groups; Northern Egypt (A1, A2, A3), Southern Egypt (B1, B2, B3), Northern Iraq (C1, C2, C3) and Southern Iraq (D1, D2, D3), using RAPD-PCR method to find out phylogeny (Nei and Li coefficient).

| | A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 | C3 | D1 | D2 | D3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A1** | 1.000 | | | | | | | | | | | |
| **A2** | 0.174 | 1.000 | | | | | | | | | | |
| **A3** | 0.244 | 0.235 | 1.000 | | | | | | | | | |
| **B1** | 0.087 | 0.179 | 0.235 | 1.000 | | | | | | | | |
| **B2** | 0.146 | 0.157 | 0.217 | 0.314 | 1.000 | | | | | | | |
| **B3** | 0.171 | 0.133 | 0.100 | 0.133 | 0.150 | 1.000 | | | | | | |
| **C1** | 0.178 | 0.182 | 0.120 | 0.109 | 0.120 | 0.273 | 1.000 | | | | | |
| **C2** | 0.143 | 0.192 | 0.128 | 0.192 | 0.170 | 0.146 | 0.157 | 1.000 | | | | |
| **C3** | 0.125 | 0.143 | 0.108 | 0.095 | 0.108 | 0.129 | 0.146 | 0.105 | 1.000 | | | |
| **D1** | 0.089 | 0.109 | 0.160 | 0.109 | 0.120 | 0.091 | 0.111 | 0.157 | 0.098 | 1.000 | | |
| **D2** | 0.095 | 0.115 | 0.128 | 0.154 | 0.128 | 0.098 | 0.118 | 0.125 | 0.158 | 0.353 | 1.000 | |
| **D3** | 0.100 | 0.120 | 0.133 | 0.160 | 0.133 | 0.103 | 0.163 | 0.130 | 0.167 | 0.327 | 0.348 | 1.000 |

**RAPD:** Random Amplified Polymorphic DNA; **PCR:** Polymerase Chain Reaction




**RAPD:** Random Amplified Polymorphic DNA **; PCR:** Polymerase Chain Reaction

**Figure (3):** A phylogenetic tree [Dendrogram] of the genetic relationships of different groups; Northern Egypt (A1, A2, A3), Southern Egypt (B1, B2, B3), Northern Iraq (C1, C2, C3) and Southern Iraq (D1, D2, D3), using RAPD-PCR method to find out phylogeny (Nei and Li coefficient).

**B- ISSR-PCR method:**

Seven primers; 14A, 44B, HB-9, HB-10, HB-11, HB-12, and HB-13 were used. The phylomorphic amplification band sizes ranged from 100bp–3000bp with a total number of bands ranged from 6-50 bands (average 25 band/primer).

Maximum bands (50) appeared with HB-12 primer with 14 polymorphic bands and 36 unique bands followed by 41 bands of HB-13 primer with 14 polymorphic and 26 unique bands. Minimum bands (6 monomorphic bands) appeared with 14A primer, the primers 44B, HB-9, HB-10 and HB-11 represented by 11,27,25 and 19 total bands respectively (Table 4 and Fig. 4).

**Table (4):** Total amplified fragments by arbitrary nucleotide sequence ISSR primers (%); polymorphism and frequency.

| ISSR-Primers / Polymorphism | 14A | 44B | HB-9 | HB-10 | HB-11 | HB-12 | HB-13 |
|---|---|---|---|---|---|---|---|
| Monomorphic | 6 | 3 | 0 | 0 | 0 | 0 | 1 |
| Polymorphic | 0 | 4 | 6 | 5 | 7 | 14 | 14 |
| Unique | 0 | 11 | 21 | 20 | 12 | 36 | 26 |
| Total number of bands | 6 | 11 | 27 | 25 | 19 | 50 | 41 |
| (%) Polymorphism | 0.000 | 72.727 | 100.0 | 100.0 | 100.0 | 100.0 | 97.561 |
| Frequency | 1.000 | 0.371 | 0.105 | 0.106 | 0.149 | 0.113 | 0.138 |

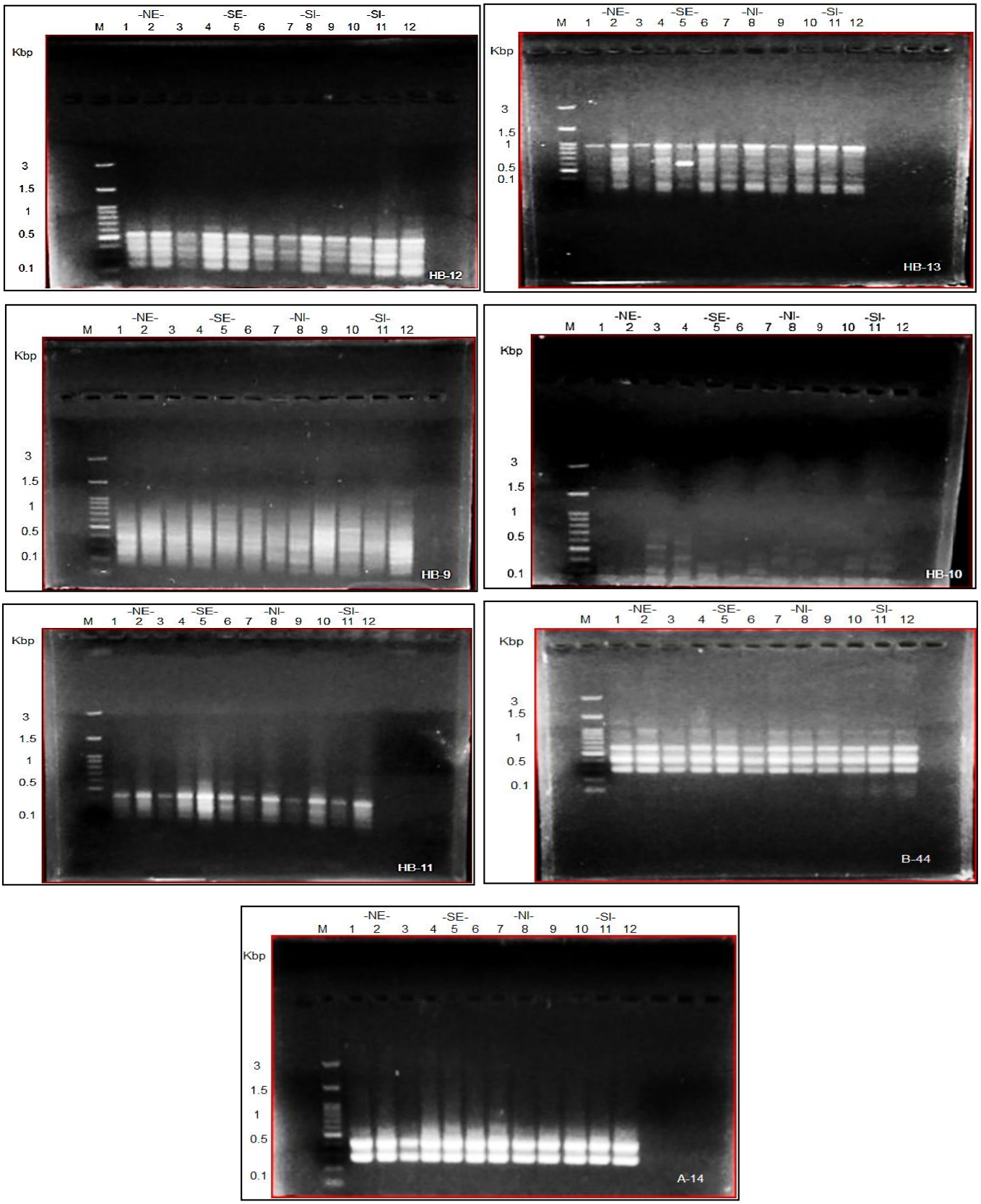
**ISSR:** Inter-Simple Sequence Repeat

**Similarity coefficient [index]:**

The genetic distance and relationship were obtained by similarity coefficient of the studied groups by ISSR-PCR method, ranged from 0.323 to 0.528, the similarity coefficient of samples C1 and C3 was highest at 0.528, whereas similarity coefficient of samples C1 and both D1 and B1 was lowest at 0.323, which showed the biggest genetic distance (Table 5).

**Phylogenic (Kinship) tree:**

Phylogenetic tree obtained from ISSR-PCR results showed the genetic relationships (similarities and differences) between groups, in which a wide overlap between different groups was detected; samples interfered with each other for example; the groups A, A3 interfered with the group B; the groups B, B1, where they overlapped with the group C, the group C, C2 clearly overlapped with the group D, either group D, D2 overlapped with group A (Fig. 5).



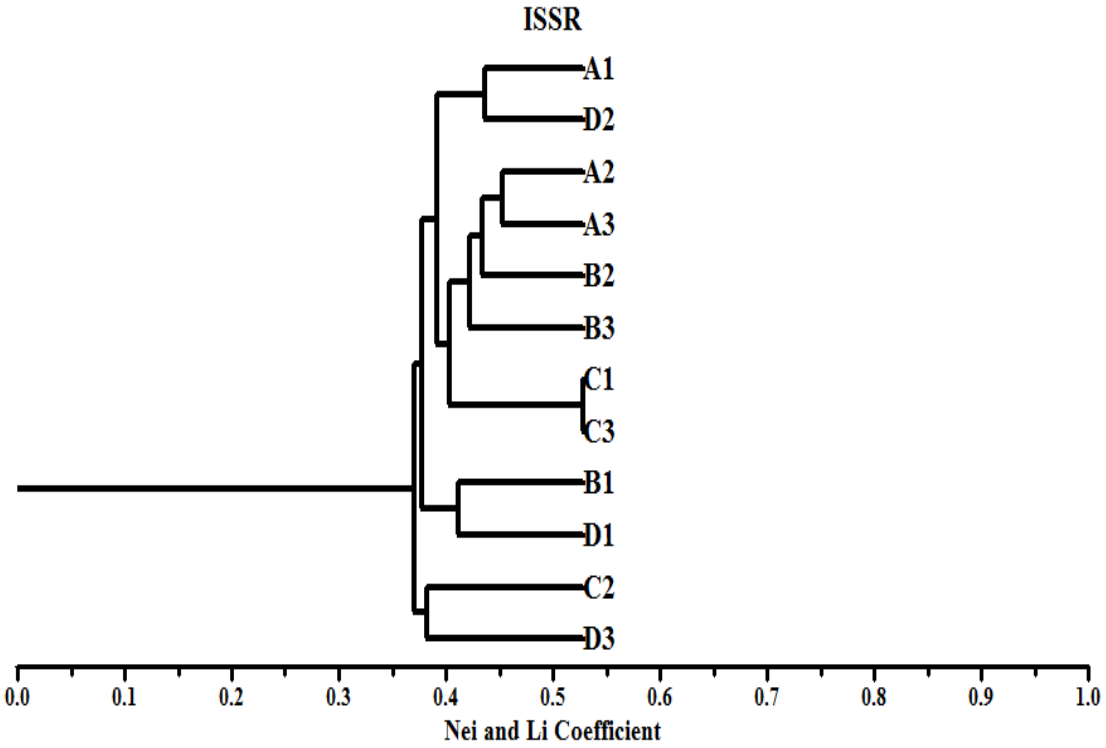

**NE:** Northern Egypt; **SE:** Southern Egypt; **NI:** Northern Iraq; **SI:** Southern Iraq; **ISSR:** Inter-Simple Sequence Repeat; **PCR:** Polymerase Chain Reaction

**Figure (4):** Agarose gel electrophoresis of representative results (banding patterns) of ISSR-PCR finger-printing method using the seven specific primers [HB-12; HB-13; HB-9; HB-10; HB-11; A-14 and B-44].

**Table (5):** Similarity index of genetic distance values between different groups; Northern Egypt (A1, A2, A3), Southern Egypt (B1, B2, B3), Northern Iraq (C1, C2, C3) and Southern Iraq (D1, D2, D3), using ISSR-PCR method to find out phylogeny (Nei and Li coefficient).

| | A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 | C3 | D1 | D2 | D3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 | 1.000 | | | | | | | | | | | |
| A2 | 0.421 | 1.000 | | | | | | | | | | |
| A3 | 0.435 | 0.453 | 1.000 | | | | | | | | | |
| B1 | 0.407 | 0.333 | 0.436 | 1.000 | | | | | | | | |
| B2 | 0.393 | 0.444 | 0.423 | 0.400 | 1.000 | | | | | | | |
| B3 | 0.407 | 0.393 | 0.440 | 0.413 | 0.433 | 1.000 | | | | | | |
| C1 | 0.377 | 0.367 | 0.449 | 0.323 | 0.373 | 0.386 | 1.000 | | | | | |
| C2 | 0.345 | 0.369 | 0.407 | 0.358 | 0.344 | 0.419 | 0.426 | 1.000 | | | | |
| C3 | 0.440 | 0.386 | 0.435 | 0.339 | 0.429 | 0.407 | 0.528 | 0.345 | 1.000 | | | |
| D1 | 0.373 | 0.364 | 0.400 | 0.412 | 0.369 | 0.349 | 0.323 | 0.358 | 0.441 | 1.000 | | |
| D2 | 0.436 | 0.355 | 0.431 | 0.406 | 0.393 | 0.339 | 0.345 | 0.349 | 0.364 | 0.375 | 1.000 | |
| D3 | 0.333 | 0.418 | 0.393 | 0.377 | 0.364 | 0.375 | 0.349 | 0.382 | 0.333 | 0.377 | 0.369 | 1.000 |

**ISSR:** Inter-Simple Sequence Repeat; **PCR:** Polymerase Chain Reaction




**ISSR:** Inter-Simple Sequence Repeat; **PCR:** Polymerase Chain Reaction

**Figure (5):** A phylogenetic tree [Dendrogram] of the genetic relationships of different groups; Northern Egypt (A1, A2, A3), Southern Egypt (B1, B2, B3), Northern Iraq (C1, C2, C3) and Southern Iraq (D1, D2, D3), using ISSR-PCR method to find out phylogeny (Nei and Li coefficient).

**C- Combined analysis using RAPD and ISSR-PCR methods:**

The genetic distance and relationship were obtained by similarity coefficient using combined RAPD & ISSR-PCR methods; the highest value of similarity index among different groups was 0.365, which fell within the group D1and D2, followed by 0.362 between B1 and B2, and the lowest value was

0.222, which fell within the group B1 and C1 (Table 6).

**Phylogenic (Kinship) tree:**

Phylogenetic tree obtained from the results of combined RAPD and ISSR-PCR methods showed excellent shape for each group from the origin of the tree without overlaps, apart from a simple overlapping between the groups B3, with group C (Fig. 6).

**Table (6):** Similarity index of genetic distance values between different groups; Northern Egypt (A1, A2, A3), Southern Egypt (B1, B2, B3), Northern Iraq (C1, C2, C3) and Southern Iraq (D1, D2, D3), using both ISSR and RAPD-PCR methods to find out phylogeny (Nei and Li coefficient).

| | A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 | C3 | D1 | D2 | D3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 | 1.000 | | | | | | | | | | | |
| A2 | 0.311 | 1.000 | | | | | | | | | | |
| A3 | 0.345 | 0.346 | 1.000 | | | | | | | | | |
| B1 | 0.267 | 0.262 | 0.340 | 1.000 | | | | | | | | |
| B2 | 0.289 | 0.316 | 0.327 | 0.362 | 1.000 | | | | | | | |
| B3 | 0.315 | 0.283 | 0.289 | 0.296 | 0.320 | 1.000 | | | | | | |
| C1 | 0.286 | 0.278 | 0.283 | 0.222 | 0.257 | 0.337 | 1.000 | | | | | |
| C2 | 0.260 | 0.291 | 0.277 | 0.286 | 0.270 | 0.311 | 0.304 | 1.000 | | | | |
| C3 | 0.317 | 0.283 | 0.289 | 0.238 | 0.301 | 0.306 | 0.362 | 0.250 | 1.000 | | | |
| D1 | 0.250 | 0.248 | 0.286 | 0.276 | 0.261 | 0.243 | 0.224 | 0.271 | 0.300 | 1.000 | | |
| D2 | 0.289 | 0.246 | 0.286 | 0.293 | 0.278 | 0.240 | 0.239 | 0.252 | 0.280 | 0.365 | 1.000 | |
| D3 | 0.240 | 0.291 | 0.277 | 0.286 | 0.270 | 0.272 | 0.268 | 0.281 | 0.271 | 0.356 | 0.360 | 1.000 |

**ISSR:** Inter-Simple Sequence Repeat; **RAPD:** Random Amplified Polymorphic DNA; **PCR:** Polymerase Chain Reaction

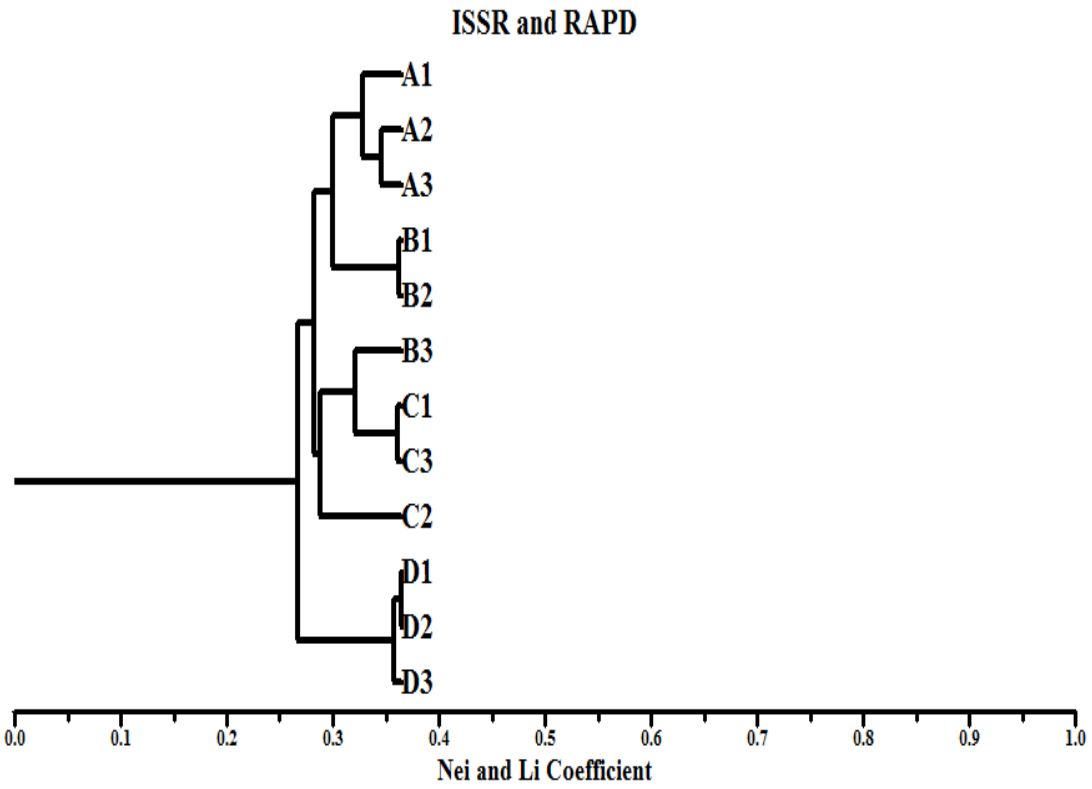



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**Figure (6):** A phylogenetic tree [Dendrogram] of the genetic relationships of different groups; Northern Egypt (A1, A2, A3), Southern Egypt (B1, B2, B3), Northern Iraq (C1, C2, C3) and Southern Iraq (D1, D2, D3), using both ISSR and RAPD-PCR methods to find out phylogeny (Nei and Li coefficient).

## IV. DISCUSSION

Forensic investigators such as anthropologists, pathologists, and geneticist, have many tools for human identification, one of the most useful methods is DNA fingerprinting (Goodwin, 2017). DNA technology has enriched forensic identification procedures from just detecting the base pairs repeat sequence to predicting the physical characteristics, geographical origin, and sex determination (Manjunath et al., 2011).

Researches on genetic variation using DNA polymorphism, has permitted preferable comprehension of human population history and diversity, in addition to supply investigative tools in forensics (Zhang et al., 2013). Several molecular marker systems were used for identification and analysis of genetic diversity, of which RAPD and ISSR are reliable PCR-based markers with a high power to detect genetic variations (Li-Wang et al., 2008).

Phylogenetic analysis is widely used to determine the evolutionary relationships, which is generally displayed by phylogenetic tree that is mainly based on sequence comparison (Qian and Luan, 2018). To establish a meaningful genetic relationship between the studied population groups; the present work assessed the used primers, determined the similarity index and developed the phylogenetic trees based on the different used techniques which were compared to the geographic locations of the studied population groups.

The results of the current work found that RAPD-PCR method was more valuable in detecting genetic polymorphism when compared to ISSR-PCR method as in RAPD-PCR method, RAPD primers had large numbers of total bands (20-50), with only zero to one monomorphic band. Also, wide genetic variations were observed (similarity indices) ranged from (0.087-0.353), that indicate more power of discrimination of genetic distance. Regarding the phylogenetic tree, it was observed that all groups showed clear results (relatively consistent), except for some little overlapping. Meanwhile in ISSR-PCR method, a clear appearance of bands one to six monomorphic bands was observed, with a highest similarity index 0.528 and low range of genetic variation (0.323-0.528), as well as a wide overlap (inconsistent phylogenetic tree) between all studied groups was detected.

Prabhakar et al. (2019) stated that RAPD is the preferred method for detection of genetic variations, as it is more reliable, reproducible and it only requires tiny amount of the DNA (about 10 ng per reaction) for PCR technique and it can scan and amplify the genome of these minute DNA repeats. Also, Kalpana et al. (2012) concluded that the polymorphism created by the RAPD primers was higher as compared to ISSR primers, as RAPD gives DNA markers that are scattered all over the genome and they are effortless in analysis.

Earlier, Kumar and Gurusubramanian (2011) concluded that RAPD markers exhibit feasible speed, cost and efficiency compared with other methods as well as RAPD can be carried out in a moderate laboratory, because it requires no DNA probes or sequence reference for the designing of specific primers, and it includes no blotting or hybridization procedures. One of the most important characteristic features of the RAPD-PCR technique is that it can be performed without previous knowing of the

genome sequence data of the tested sample, so, it gives a simple, fast and sensitive fingerprinting procedure (Lee and Chang, 1994).

In contrast, Nybom et al. (2014) stated that RAPD and ISSR-PCR techniques are usually arrived at quite similar results as regard genetic diversity and genetic distances, moreover RAPD-PCR has been criticized for problems with reproducibility and competitive priming, and these problems are less pronounced for ISSR-PCR. Also, in a former research done by Srivastava et al. (2004) an elevated ratio of genetic variation was detected by ISSR marker as compared to RAPD markers. This controversy can be explained on basis that ISSR-PCR technique is valuable for detection of the similarity within and between groups in addition to detect the genetic overlapping, which was obvious in the results of the current work.

The present study showed that combined analysis using both RAPD and ISSR-PCR methods gave a more clear indication and understanding of genetic variation (polymorphism) among the studied population groups, in comparison to either RAPD or ISSR-PCR techniques, as the obtained phylogenetic tree by the combined analysis showed excellent shape for each group from the origin of the tree without overlaps (highly consistent), apart from a simple overlapping between the group B3 and group C. Mei et al. (2017) concluded that RAPD and ISSR are dominant markers for DNA fingerprinting, and the improved RAPD and ISSR-PCR methods can provide a better understanding of genetic variations than either of them alone. Earlier, Mei et al. (2014) illustrated that combined RAPD and ISSR-PCR techniques can amplify more DNA products and show better banding patterns with high level of genetic variations between different populations. On the other hand, Poyraz (2016) illustrated that RAPD-PCR and ISSR-PCR techniques are more appropriate for studying high genetic diversity populations. They are more precise in revealing the genetic variations among nearer populations with closer homelands. i.e., the recognition power of these two methods relatively lowered when inhabitants become far in location.

In the present work, genetic variations and similarities were detected among different studied population groups; the highest genetic overlap "similarity" (shared from origin) was between Southern Egypt and both Northern Iraq and Northern Egypt, respectively. The biggest genetic distance (variation) was between Southern Iraq and both Northern Iraq and Northern Egypt. The genetic similarity of population within the same group was highest within Southern Iraq, followed by Southern Egypt, then Northern Iraq and finally Northern Egypt.

Genomic studies of the Greater Middle Eastern populations (Gulf region, North Africa and Central Asia) detected many old originator inhabitants and continental mishmash; these studies also concluded that the ancestral Arab inhabitants from Arabian Peninsula are detected in almost all of the Greater Middle Eastern geographic regions possibly due to the Arabian occupation in the seventh century (John et al., 2017).

## V. CONCLUSION:

1. RAPD-PCR method was more valuable in detecting genetic polymorphism as compared to ISSR-PCR method.
2. Combined analysis using both RAPD and ISSR-PCR methods gave a clear

indication of genetic variations than either RAPD or ISSR-PCR techniques.

3. Genetic variations and similarities were detected among different studied population groups, as follows:
   - The highest genetic overlap "similarity" (shared from origin) was between Southern Egypt and both Northern Iraq and Northern Egypt, respectively.
   - The biggest genetic distance (variation) was between Southern Iraq and both Northern Iraq and Northern Egypt.

## VI. LIMITATIONS & RECOMMENDATIONS:

due to financial reasons, this study was conducted on a small number of subjects, therefore a large-scale sample study is needed.

## VII. ACKNOWLEDGMENT

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# البصمة الوراثية لتحديد شجرة التطور الوراثي: دراسة جينومية على عينات من السكان المصريين والعراقيين

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يستخدم التحليل النشوئي الوراثي على نطاق واسع لتحديد العلاقات التطورية، ويتم التعبيرعنه بواسطة شجرة النشوء والتطور. ويهدف العمل الحالي إلى التعرف على شجرة النشوء والقرابة الوراثية لعينات من السكان المصريين والعراقيين، وكذلك المقارنة بين تقنيات بصمة الحمض النووي المستخدمة فى الدراسة، وهى ال (ISSR-PCR) ، وال ( RAPD-PCR).تم إجراء الدراسة الحالية على ٤٨ متطوعًا صحيحًا غير ذي علاقة أو قرابة ببعضهم البعض من مناطق جغرافية مختلفة (شمال مصر، وجنوب مصر وشمال العراق وجنوب العراق) ، إثنا عشر متطوعًا من كل منطقة. تم استخدام سبعة من البوادئ: OP-A3، OP-A9 ، OP-B3 ، OP-C3 ، OP-D1 ، OP-C15 و OP-K2 فى تقنية ال RAPD-PCR .وتم استخدام سبعة أخرى من البوادئ: 14A ، ٤٤ B، HB-9 ، HB-10 ، HB-11 ، HB-12، HB-13 فى تقنية ال ISSR-PCR. وقد كشف معامل التشابه وشجرة النشوء والقرابة الوراثية عن المسافة والعلاقة الوراثية بين المجموعات المدروسة على النحو التالي: كان أعلى نسبة من التشابك الوراثي "التشابه" بين مجموعة جنوب مصر و وكلٍ من مجموعتي "شمال العراق ، و شمال مصر" على الترتيب .وكانت أكبر مسافة وراثية (اختلاف) بين مجموعة جنوب العراق وكلٍ من مجموعتي " شمال العراق وشمال مصر". وقد خلصت الدراسة أيضًا إلى أن طريقة RAPD-PCR كانت أكثر قيمة في الكشف عن تعدد الأشكال الوراثية بالمقارنة مع طريقة ال ISSR-PCR ، كما أعطى التحليل المركب باستخدام كلٍ من طريقتي" ال RAPD و ISSR-PCR معًا" مؤشرًا واضحًا على التغيرات الجينية والعلاقات الوراثية من استخدام أيٍ من تقنيات RAPD-PCR أوISSR-PCR منفردة".