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# PHYLOGENETIC RELATIONSHIPS AMONG EUPHORBIA SPECIES ACCORDING TO RANDOM AMPLIFICATION OF POLYMORPHIC DNA (RAPD-PCR) TECHNIQUE

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**ABSTRACT**
Phylogentic relationships among six genotypes of *Euphorbia* were analyzed by RAPD-PCR markers, DNA of fresh young leaves was extracted from each sample for RAPD method, twenty primers were tested, but just four decamer primers clarify the diversity among genus. A total produced bands were fifty two, which fifty bands were polymorphic, The matrix reveals that Euphorbia taxa separated to main groups (I and II) with similarity range 1.18, the first group includes two species were *Euphorbia milii* and *Euphorbia tirucalli* they have been closest genetic distance matrix among species under study with similarity range 0.29, while group II contains 4 species it had divided to 2 clusters with similarity value 1.09, The results gained from this study can be used a phylogenetic tool in combination with other morphological , anatomical, and palynological characters to better understanding to systematic study of this genus.

**KEYWORDS:** Iraqi Euphorbia**,** molecular, RAPD, genetic distance.

## INTRODUCTION

*Euphorbia* rising up as a second-largest genus of flowering plants and considering a typical genus appears several evolution lines in Euphorbiaceae family and is comprised with over than 2150 species worldwide[1] and [2]. The genus has a complex biogeographic history leading to its nearly worldwide distribution[3-6]. These evolutionary and biogeographic forms make *Euphorbia* an ideal system for the study of evolution and adaptation of plants to different environments. Distributed worldwide and varying in habit from with highest diversity in the arid, semi-arid, Mediterranean, and tropical regions, associated with diversity morphological patterns in this genus includes geophytes[7]. In Iraq there are about 40 taxa belongs to Euphorbia genus including wild and cultivates [8] and [9]. Molecular evidence have become useful tools to identify the evolution processes, reflect the true phylogeny, and the knowledge of genetic relationship among genotypes is an important consideration for systematic[10] as well as provide a basic background knowledge to implement conservation genetics programmers mutation rate or in dominance characteristics [11-13]. Despite Euphorbia is one of the richest genera in list of taxa, there are only a few studies have deal with phylogenetic diversity within this genus, but over the past decades, phylogenetic studies have been given us understanding the broad scale relationships within *Euphorbia* such as[6] using internal transcribed spacer (ITS) sequences and plastid phylogenies; [3],[14], but[10] using random amplifying polymorphic DNA marker (RAPD), but the phylogenetic relationships among its constituents stay poorly understood. The random amplified polymorphic DNA (RAPD) have been universally used in DNA fingerprinting gene mapping as a fast, reliable and simplest technique and to isolate phylogenetic relationships of many organisms taxonomy [15, 16] and [17] with tiny plants material and its ability to reveal high degree of polymorphism it has successfully been applied as a identifiably molecular technique within many plant families [18-23]. However it just [10] study prevent data about the genetic diversity within Euphorbia species in small area. The objectives of this study were attempt to investigated and identify the level of genetic distinct among Euphorbia species based on DNA profiling using RAPD technique and provide an taxonomical key based on genomic features.

## MATERIALS & METHODS

### Plants materials

Fresh young healthy Euphorbia leaves of six species were *Euphorbia aleppica, Euphorbia cyathophora, Euphorbia milii, Euphorbia peplus, Euphorbia pulcherrima,* and *Euphorbia tirucalli* were collected during May 2016 from Baghdad province.

### DNA extraction

Approximately 50 to 100 mg tissue of fresh leaf put in 1.5 ml tube, homogenized the tissue with liquid nitrogen using a conical hand tissue grinder. Genomic DNA was isolated from leaf specimens using the procedure described by DNeasy Plant Mini Kit Protocol (Bioneer, Korea).

### Screening of PCR

twenty various10mers RAPD primers were tested in this study (table 1) supplied by Bioneer company were screened, four primers which had previously been shown indicated results of band patterns, multi master mix were used, the thermos profile for the PCR reaction was: 95°C for 5 minutes, then 35 cycles of 95°C for 30 sec, 37°C for 1 minute, and 72°C for 5 minute. Genotypes were

visualized on 1% agarose gel, 1x TBE, 0.5 mg/ ml ethidium bromide stained, with 1500 bp ladder (SibEnzyme Ltd. Russia) and 100 v for 45 min visualized and photographed under a UV transilluminator and scored as 1 or 0 based on presence or absence of a band, within the size range of 100-1300 base pairs (bp).

**Data analysis**

RAPD matrix analyzed by NTSYS-PC statistical package version 2.1. The data matrix was used to calculate the genetic similarity within and among species using Jaccard's similarity coefficients and a dendrogram shown relationship among the 6 genotypes was constructed by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

**TABLE 1.** The sequences of twenty RAPD primers used in this study including those produced amplified.

| Primer name | Sequence (5´- 3´) |
|---|---|
| OPA-01 | CAGGCCCTTC |
| OPA-02 | TGCCGAGCTG |
| OPA-03 | AGTCAGCCAC |
| OPA-13 | CAGCACCCAC |
| OPA-18 | AGGTGACCGT |
| OPB-01 | GTTTCGCTCC |
| OPB-02 | TGATCCCTGG |
| OPB-03 | CATCCCCCTG |
| OPB-08 | GTCCACACGG |
| OPC-08 | GAACGGACTC |
| OPC-12 | TGTCATCCCC |
| OPC-13 | AAGCCTCGTC |
| OPF-08 | GGGATATCGG |
| OPF-14 | TGCTGCAGGT |
| OPG-01 | CTACGGAGGA |
| OPG-02 | GGCACTGAGG |
| OPM-04 | GGCGGTTGTC |
| OPM-15 | GACCTACCAC |
| OPZ-01 | GAGCCCTCCA |
| OPZ-03 | CAGCACCGCA |

**TABLE 2.** The Total number and size range of amplified bands obtained for each primer

| Primer name | Sequence (5´- 3´) | AN | Size range of bands (bp) | PM | % | MM | % |
|---|---|---|---|---|---|---|---|
| OPA-13 | CAGCACCCAC | 9 | 100-1200 | 8 | 88.89 | 1 | 11.11 |
| OPB-08 | GTCCACACGG | 15 | 350-1300 | 15 | 100 | 0 | 0 |
| OPC-08 | TGGACCGGTG | 12 | 150-1300 | 11 | 91.67 | 1 | 8.33 |
| OPM-15 | GACCTACCAC | 16 | 190-1250 | 16 | 100 | 0 | 0 |
| | Total | 52 | | 50 | | 2 | |

AN = amplification number; PM = Polymorphic bands; MM = Monomorphic bands. %PM= PM/ANx100, % MM= MM/AN x100

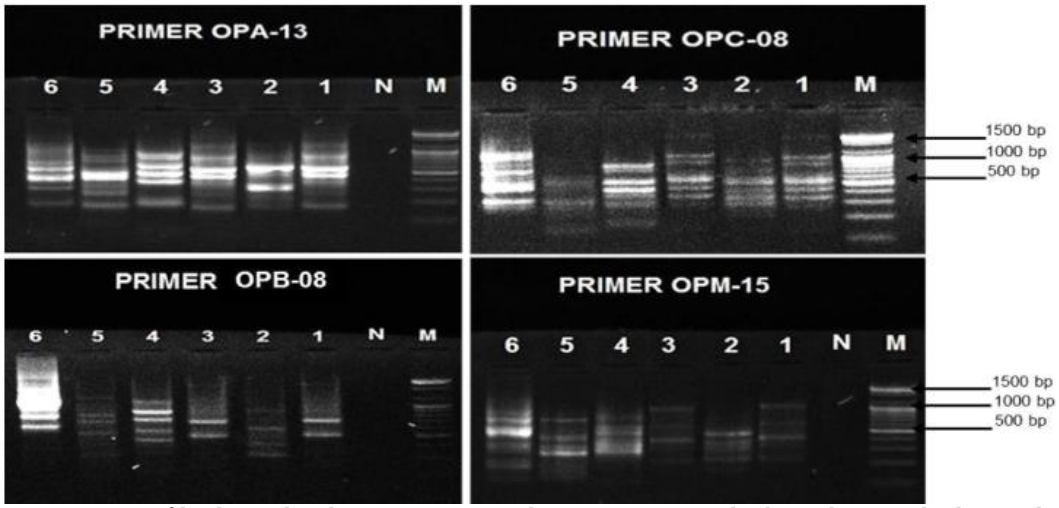


**FIGURE 1**. RAPD profile obtained with 6 primers. M-Marker1500-100; *1.Euphorbia milii, 2.Euphorbia cyathophora, 3.Euphorbia tirucalli, 4. Euphorbia aleppica, 5.Euphorbia pulcherrima,* and *6.Euphorbia peplus.*

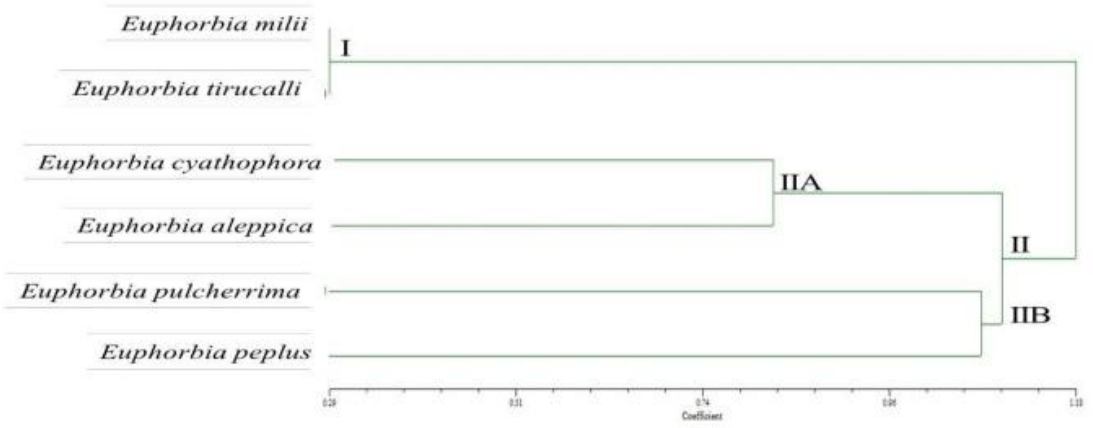


**FIGURE 2.** Dendrogram of *Euphorbia* species they were tested

## RESULTS

Only clear and repeatable amplification products were calculated, 52 amplified RAPD bands were obtained from our study ranging from 100 bp to 1.3 kb in size were showed from the 6 genotypes. The number of RAPD bands varied from 9 (primer OPA-13) to 16 (primers OPM-15) fifty polymorphic bands were outcome (table 2).

The dendrogram reveals the genetic linkages among the genotypes (Figure 2) shown that Euphorbia species were mainly separated into 2 basic groups with a similarity value at 1.18, the first (group I) consisted of 2species were *Euphorbia milii* and *Euphorbia tirucalli* they have been isolated from other genotype and have been closest genetic distance matrix among species under study with highest value similarity 0.29, while group II contains 4 species it had divided to 2 clusters(A and B) with similarity value 1.09, which were separated to 2 sub clusters, ( AII) were including 2 species were *Euphorbia aleppica*, and *Euphorbia cyathophora* by value similarity of0.83, but *(BII)* contained *Euphorbia peplus*, and *Euphorbia pulcherrima* that separated at the similarity value of 1.05.

## DISCUSSION

Experiments with Euphorbia species have been appeared by RAPD markers as a raped ad useful method for isolated different species and clustering genotypes in Euphorbia species. The present study identifies species according bands to each primer depends on sequence of primer and extent of variation in specific genotype[24-26]. There are primers non amplification producing were gave, similar results were detected in different studies [27-30].

In this study, the general similarity ratio were found between 0.29- 1.18 this associated with may be this ascribable to strong relationship among the selected species, high levels of polymorphisms were gene-rated in this study among species of *Euphorbia* generate unique bands that could be used as a DNA marker to distinguish among the local species of *Euphorbia* [10] different authors agree that genetic diversity has been attributed by two factors, the geographic origin of varieties, morphological characters and the somatic mutations [31, 21, 32], and [33]. Relationships within this group have been difficult to discern due mainly to homoplasious morphological characters and inadequate taxon sampling in previous phylogenetic studies [3, 4), 34, 6, 35]. *Euphorbia milii* and *Euphorbia tirucalli* shared by semi succulent stem, margin of leaf to both of species was entie, cyathium shape , nectar number in both species was 5, and isolated from rest species by have paracytic type of stomata, moreover the distributed founded in mid of Iraq. Despite the morphological study separated these species[32] but our phylogenetic analysis of these geophytic species suggests that geophytes have evolved independently multiple times in these species [4] and [36]. By comparing our results with another morphological key the *Euphorbia aleppica,* and *Euphorbia cyathophora* distinguished by root color (brown to light brown) , and grooves on their seeds were tuberculate regulose from rest species and shearing in another characters [37]. Our results clearly supported by [38] that indicates the several morphological traits and the taxonomic composition was based on a phylogenetic study. *Euphorbia peplus,* and *Euphorbia pulcherrima* separated from other species under study by length of branches root, and distribution of these species in mid and south Iraq. The RAPD analyses of data sets might partly be explained the cooperation of genes with biological processes such as hybridisation or lineage sorting this confirmed the results of [26].

The stronger phylogenetic relationship among species under study referred the relationships within the Euphorbia genus are still incompletely resolved and an evolutionary and taxonomic standpoint, is a need to develop a comprehensive proposed a sectional classification question involves an ecologically and diverse set of approximately 34 Euphorbia taxa to resolve the relationships of Iraq species. However, from this study we can provide a new data were combined with existing morphological, anatomical, chemical, and geographical data it will be significant for phylogenetic taxonomical key, and identification in Euphorbia species.

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