

# Genetic Polymorphism among Species belong to Amaranthaceae Collected from Baghdad Province-Iraq by using RAPD –PCR

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**Abstract:** *Plymorphism* was detected by using RAPD-PCR method in a systematic study aimed to reveal the genetic relationships among 6 collected species belonging to 4 genera of *Amaranthaceae* family collected from Baghdad province, which are *Amaranthus retroflexus* L.,*A. spinosus* L.,*A. albus* L. *Alternanthera sessilis* (L.) R.Br. ex DC. and *Gomphrena globosa* L. *Iresine herbstii* Hook.ex Lindl.

DNA of fresh leaves was extracted using modified protocol of CTAB,six chosen random primers were used for amplifying the DNA bands.

All primers were showed polymorphism in the RAPD gel profile. 195 bands were produced with many polymorphic bands, the results arise two main groups, the first one which separated at the similarity value of (0.54) included *Amaranthus retroflexus* and *A. spinosus*, while the second group included the other *Amaranthus* species and contained two subgroups, the first subgroup included *A. albus*, and the second subgroup that appeared related correlation at similarity range of (0.41) contained 2 species ; *Alternathera sessiles* and *Gmphrena globosa*, while the second subgroup of the second group included *Iresine herbstis* only, furthermore, this study considered as a first study at the molecular level revealed the differences and the similarities at the molecular level for this plant in Iraq.

**Keywords:** *Amaranthus*, RAPD-PCR, plant genetic variation, genetic polymorphism,DNA

## 1. Introduction

The Amaranthaceae family belongs to Caryophyllales order which is one of the largest plant orders with eleven developed families [1].A large number of wild plants and halophytes in the world belong to this order [2].

Most of the Amaranthaceae family plants are wild herbs, or cultivated shrubs [3].This family contains about 165 genera and 2,040 species and it is widely dispersed in temperate and tropical zone [4].Phenotypic variations of Amaranthaceae genera tend to have partly caused by many environmental factors. [5].The phylogeny studies based on morphological traits has limitation in data accuracy because of high subjectivity [6].Hence the importance of genetic studies, especially those related to genetic and evolutionary relations among plant varieties and species. The most important studies for determining the existence or absence of these relations among species and their source, provides workers in the field, plant classification and systematic with important information that denies or confirms their hypotheses and scientific observations in their taxonomic studies or diagnostic or anatomical of different types of plants. Efficient utilization of the most important plant genetic resources to use for nutrition and crop improvement requires clear systematic understanding of the most important traits [7].

Random amplified DNA polymorphic-PCR (RAPD-PCR) is one of the most important technics that are still widely used in the genetic relations studies among plants species and it can be used in the phylogeny studies and

polymorphism detection, especially in the case of abundance of species, and the absence of documented information of the genetic sequences for the source of these types of approved sources. It can be used to survey large number of loci and to discover unambiguous genetic and taxonomic relationships among different taxa [8, 9]. Therefore, RAPD primers can distinguish the traits accessions more effectively than study only the phenotypic markers study [7].

Number of genera belonging to Amaranthaceae family were found in Iraq were *Alternanthera sessilis* (L).R. Br.ex De C., *Amaranthus albus* L., *Amaranthus caudatus* L., *Amaranthus retroflexus* L., *Amaranthus spinosus* L., *Amaranthus viridis* L., *Celosia argentea* L., *Celosia cristata* L., *Gomphrena globosa* L. and *Iresine herbstii* Hook. Ex Lindl [10].

Six species belonging to 4 genera of Amaranthaceae family are *Amaranthus retroflexus* L., *A. spinosus* L., *A. albus* L. *Alternanthera sessilis* (L.) R.Br. ex DC. and *Gomphrena globosa* L. *Iresine herbstii* Hook.ex Lindl, Were collected for this study.

Some literatures assumes that Amaranth family is the future food with high nutritional value and produces betalain as colorant [11], and it is a phytochemical marker for Amaranthaceae [12, 13]. Thus members of this family considered as medicinal plants because this colorant and widely used as antifungal, anti-malarial, and antiviral. Beside that, this colorant can be used as healthy food colorant. Many Phylogenetic studies of Amaranthaceae used to find an alternative plant that produces high level of Betalain [14].

## 2. Materials & Methods

### 2.1. Plant Sampling

Samples were collected from orchards and nurseries as well as some agricultural areas in Baghdad - Iraq, during March – June, 2016 and were diagnosed and classified using the experts of plant taxonomy as well as the records of the classification of Arab and global sources on the Web. Six species belongs to four genera of Amaranthaceae family were collected from different regions (Table 1).

TABLE I: The list of Species under Study.

No.	Scientific name
1	<i>Amaranthus retroflexus</i> L.
2	<i>A. spinosus</i> L.
3	<i>A. albus</i> L.
4	<i>Alternanthera sessilis</i> (L.) R.Br. ex DC.
5	<i>Gomphrena globosa</i> L.
6	<i>Iresine herbstii</i> Hook.ex Lindl

### 2.2. DNA Extraction:

About 50 to 100 mg of fresh leaves tissue homogenized in 1.5 ml microfuge tubes using liquid nitrogen with hand tissue grinder.

Total genomic DNA was isolated using modified of cetyltrimethyl ammonium bromide (CTAB) method described by [15].the homogenized tissue powder was suspended in 2.5 ml of CTAB DNA extraction buffer (for 10 ml CTAB buffer ; 1ml Tris 1M,0.4ml EDTA 0.5 M,2.8ml NaCl 5M, 0.2g CTAB (cetyltrimethyl ammonium bromide),0.4g PVP(polyvinyl pyrrolidone) and 50 ml  $\beta$ -mercaptoethanol) just before use, then the suspension was mixed well, and incubated at 60°C for 20 min in order to homogenate, then followed by chloroform: isoamyl alcohol extraction (24:1), and precipitate with two volume of isopropanol at -20°C, then centrifuged for 5min to participate the isolated DNA. The sample then washed with 1ml of 70% ethanol and 10m M of ammonium acetate. The DNA resuspended by TE buffer ( 20m M EDTA, 0.1 M Tris- HCL p H= 8), then DNA detected by 1% agarose gel electrophoresis at 100V for 45 min. then stained with ethidium bromide [16].

### 2.3. PCR Technique

Six different RAPD primers were chosen and used in this study (Table 2), which supplied by Bioneer company/Korea, master mix from the same company was used.The program of thermal cycler reaction was:

denaturation at 95°C for 5 minutes, 35 cycles of 95°C for 30 sec, annealing at 37°C for 1 minute, and extension at 72°C for 5 minute and final extension at 72°C for 5 minutes.

Results were visualized on 1.5% agarose gel by using 1x TBE, 100 volts, for 2 hours, Molecular DNA ladder (100bp) was used (Bioneer).

TABLE II: The Sequences of RAPD Primers and Bands Amplification Results.

Primers	Primer sequence (5'-3')	NT	Size of bands (bp)	NU	%	NP	%	NM	%
OP-A01	CAGGCCCTTC	11	120-1200	3	27.27	6	54.54	2	18.18
OP-S238	TGGTGGCGTT	8	180 -800	2	25	5	62.5	1	12.5
OP-S147	AGCTGCAGCC	9	190-1000	2	22.22	4	44.44	3	33.33
OP-S253	GGCTGCTTCC	9	180-1000	2	22.22	5	55.55	2	22.22
R108	GTATTGCCCT	10	180-800	3	30	6	60	1	10
UBC90	GGGGGTTAGG	11	190-800	1	9.09	10	90.90	0	0
Total		58	120 → 1200	15	25.86	34	58.62	9	15.51

NT = Number of total bands.

NP = Number of Polymorphic bands.

NM = Number of Monomorphic bands.

NU = Number of Unique Bands.

Polymorphic = NP/NT×100

Monomorphic = NM/NT×100

#### 2.4. Data Analysis:

Obtained data from gel screening by RAPD—PCR technique results were analyzed using the Numerical Taxonomy and Multivariate Analysis System NTSYS-pc statistical package version 2.1[17].

This data matrix was used to calculate the similarity within and among Amaranth species based on Jaccard's similarity coefficients and genetic tree. The dendrogram displaying the genetic relationships among the genotypes was constructed by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) [18].

The detected polymorphic fragments data were analyzed. The amplification profile of all the studied species for the given primer were compared with each other, they scored as "1" for presence of band and "0" for the absence of the same band in the same size in other species. Only obvious clearly and reproducible amplified fragments were considered and calculated for genetic relationship analysis. The Genetic distances (G.D.) were estimated based on the data matrix obtained between pairs of the species according to [19] and according to the following formula:

$$G.D. = 1 - \{2Nab / (Na + Nb)\}$$

Where Na = total number of fragments of species 'a'; Nb = total number of fragments species 'b' and Nab = number of fragments shared by species 'a' and species 'b'.

### 3. Results & Discussion:

The total number of RAPD amplified fragments that produced using the 6 primers was 195 bands, (Figure 1) these bands ranged from 25 using primer OP-S238 to 37 with the primer OP-S253. The total number of bands that frequented with consider to molecular weights were 58 over 34 frequent polymorphic bands means that above 58 % of the bands were polymorphic (Table 2). It is worth mentioning that many unique bands appeared in the gel and for all the species studied, thus the total number of unique bands is 15(25.86%), while the total number of monomorphic bands is 9 (15.51%), which is less than the polymorphic bands percentage and that reveal the high genetic polymorphism of this Family[20].

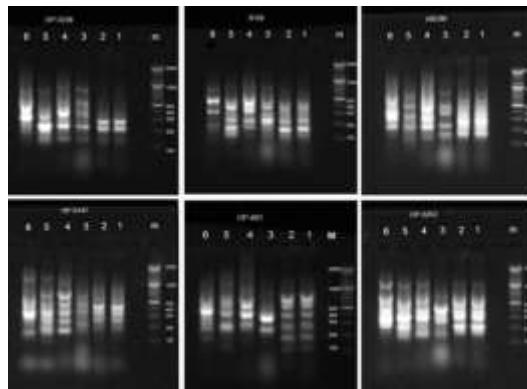


Fig. 1: Gel electrophoresis of DNA amplification bands for 6 studied species of Amaranthaceae family by using 6 different primers, m: molecular weight marker DNA Ladder (100-bp).

The studied species of the Amaranthaceae family showed high polymorphism due to the genetic differences among the four studied genera and from different tolerances as drought and salinity and Weather fluctuations in Iraq.

Representative polymorphisms that revealed by RAPD primers were presented in (Table 2), It is worth mentioning here that the dendrogram showed that the genetic relationships among the 6 Amaranth genotypes (Figure 2) and essentially divided into two main groups. The first one included *Amaranthus retroflexus* and *A. spinosus*, which separated at the similarity value of 0.54, while the second group included the other Amaranthus species and contained two subgroups, the first one included *A. albus*, and the second one that appeared related correlation at similarity range of about 0.41 contained 2 species, *Alternathera sessiles* and *Gmphrena globosa*, while the second subgroup of the second group included *Iresine herbstii* only.

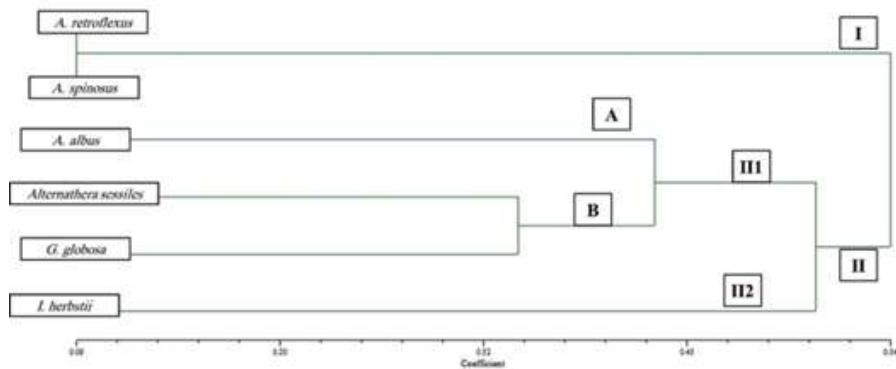


Fig. 2: Dendrogram Showing the Genetic Relationships Among Studied Species.

### 3.1. Genetic Distances:

The genetic distance value ranged from 0.334 (33%) between *Alternathera sessiles* and *Gmphrena globosa* to highest 0.934 (93%) between *Amaranthus retroflexus* and *A. spinosus* as showed in (Table 3).

TABLE III: Values of Genetic Distances Among Amaranthus Species Calculated According to Nei & Li's Formula (19).

<i>Amaranthus retroflexus</i>	1					
<i>A. spinosus</i>	0.934	1				
<i>A. albus</i>	0.587	0.645	1			
<i>Alternathera sessiles</i>	0.530	0.632	0.3578	1		
<i>Gomphrena globosa</i>	0.405	0.477	0.461	0.334	1	
<i>Iresine herbstii</i>	0.516	0.510	0.611	0.387	0.493	1

DNA amplification and species identification used to study the genetic diversity and the relationships among 6 Amaranthus species grown in Iraq which evaluated by RAPD-PCR method using 6 random primers obtained.

High percentage of polymorphic bands expressed by random primers in this study in comparison with the studies on the same plant, Popa et al. 2010 studied six Amaranth species from different geographic regions using RAPD molecular analysis showed relatively low intra- and inter-species polymorphism [21], however by using specific RAPD primers we can amplify some amplicons that, after further analysis, could be regarded as good molecular markers. [22].

In this study , high genetic polymorphism found agreed with the morphological and anatomical studies of some Iraqi Species of Amaranthaceae family [10].

Polymorphism between and within species may showed high level of polymorphism if plant sampling taken from far areas due to geographic effect to genetic traits [23].

## 4. Conclusion

The result indicate the high degree of correlation among 6 studied species according to RAPD-PCR technique and data analysis by the scientific methods used in the this study. These results gave an effective tool to understand the ralations among these species genetically.

## 5. References

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