Phylogenetic taxonomy among Iraqi cacti taxa by using RAPD markers

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Abstract:

Analyzing the genetic diversity by using the Randomly Amplified Polymorphic DNA (RAPD PCR) technique to identify seven genotypes selected of cacti were *Ariocarpus kotschoubeyanus* (Lem.) K. Schum, *Gymnocalycium pflanzii* (Vaupel) Werderm., *Mammillaria elongata* DC., *Mammillaria erythra*, *Mammillaria grahamii* Engelm, *Mammillaria winterae*, *Stetsonia coryne* (Salm- Dyck) Britton & Rose., the outcome has been detected ten primers rise from 25 primers with yield 390. The primer OPC-05 yielded a high number of DNA fragments, 53. The primer OPA-11 was characterized by a heist polymorphism percentage of 100 % and the lowest percentage of polymorphism by primer OPAD-17 was 25%, depending on the similarity matrix the UPGMA analysis was done. the phylogenetic dendrogram showed the studied taxa divided into two main groups at a similarity value of 0.025, the first group included six taxa divided into two clusters (A, and B) with a similarity value was 0.013, the first cluster (A) separated into two clades (AI, and AII), the AI isolated *G. pflanzii* from other taxa, while AII divided to two-branched (1and 2), the (1) contain *M. elongata*, *M. erythra*, and *M. grahamii* with achieving closest genetic distance was 0.960, while (2) include *M. winterae*, *S. coryne* that have similarity average 0.831. The B group has only one genotype *A. kotschoubeyanus* which showed the highest molecular differences from other taxa, The results obtained from this study may be useful as a phylogenetic tool that can be combined with morphological, and anatomical features to make a better understanding of the systematic or taxonomy of cactus taxa.

Keywords: *Cactus; Molecular Taxonomy; phylogenetic tool; RAPD PCR*

1. Introduction

The variation in cactus is recognized by its remarkable phenotypic levels of diversity [1]. The Cactaceae family is considered one of the most attractive families globally, comprising about 127 genera with some 1750 species succulent and non-succulent species distributed throughout the world, cacti show many adaptations to dry environments, these dramatic modifications made it considering the highly succulent members observed in the plant kingdom with spectacular morphological adaptations (Edwards and Ogburn,2012). They are popular due to their spectacular nocturnal flowers and have some importance as crops grown for their edible fruits [2].

Despite the changes and mutations in cacti that have been studied over many years [3], but still, the changes and modifications are still not evolutionally clear, even more, studying cacti is considered a promising path to consider as biofuel food or fodder through studying the metabolic and genetic pathways and its ability to live in dry environments [4], also to find genetic engineering tools to modify crops to suit future threats of drought, desertification, and heat [5]. Many expected range threats facing the cacti [6] and [7], Hybridization,

species cohesion, and species delimitation challenges are initial signs of the evolutionary succulent syndrome nature [8,9], and [10], molecular data may help to reveal cryptic diversity or identifying smuggled living cactus. Even more, the family has a spectacular diversity of taxa and growing forms [11], in addition to a substantial interpopulation variation in morphology, which may cause taxonomic uncertainty [12].

Dependence on genetic indicators based on DNA is the criterion factor in identifying and diagnosing species because they are not provoked by external factors and are present in all stages of growth and all cells, so it was of interest to scientists, thus many evolutionary studies using genetic-based markers have been published in recent decades [13,14] to clear gene flow, evolutionary relationships, and phylogenetic taxonomy [12].

RAPD technique is one of the molecular markers that has proven its ability to separate taxa as well as simplicity of use, a low-cost, and the most common method, Specifically, we can increase the accuracy of the results to determine the genetic dimension and reveal the largest area of the genome by increasing primers numbers [15,16], indeed the RAPD was the primary marker used in evolutionary studies in cacti[12], thus our aim here to review of current knowledge achieved using RAPD molecular markers among some cacti taxa.

2. MATERIALS AND METHODS

A. Plant specimens

Cacti samples were seven taxa *Ariocarpus kotschoubeyanus* (Lem.) K. Schum, *Gymnocalycium pflanzii* (Vaupel) Werderm., *Mammillaria elongata* DC., *Mammillaria erythra*, *Mammillaria grahamii* Engelm, *Mammillaria winterae*, *Stetsonia coryne* (Salm- Dyck) Britton & Rose. were selected from nurseries for the period 2020-2021 seasons. Varies taxa in shape and size.

B. DNA extraction

DNA extracted from each sample 200 mg from fresh vegetative parts by Plant Genomic DNA Extraction Mini Kit from FAVORGEN from Korea.

C. Polymerase Chain Reaction PCR

Materials were prepared to analyze molecular characters according to [17] RAPD method was performed in a thermal cycler (BIO-RAD USA) with 10-mer primers synthesized by (Integrated DNA Technologies, Jordan. Table (1). The PCR reaction was set at a final volume of 10µl of 2X PCR premix (HSPrim Taq Premix, Ge Net Bio, Korea), 3µl primer, 4µl grade eater, and 3µl (50µg of extracted DNA template).

Cycler thermo T100 (BIO-RAD, USA). The reaction of PCR was performed with a program containing an initial denaturation step at 95°C for 10 mins, followed by 35 cycles with 95°C for 45 seconds, 34°C for 30 seconds for primer annealing, and 72°C for 1 minute for primer extension. The DNA was cooled at 4°C after the reactions. The electrophoresis (Promega, USA) with 1.5 % agarose gel was used to separate the amplified products, each 5µl of PCR product was loaded into the wells of agarose gel with 2µl of loading dye. The electrophoresis was carried out using 1X TBE buffer (GeNetBio, Korea) at 100 v for 1 hour. The DNA fragments were compared with 4 µl with 100 bp DNA marker equipped by the Promega, USA, used as standard molecular weight.

TABLE **1. Sequences and names of primers that used** [18]

N.	Primer	Sequence (5' – 3')	GC Content %	Tm ºC
1.	OPA-01	CAG GCC CTT C	70	44.6
2.	OPA-05	AGG GGT CTT G	60	41.7
3.	OPA-07	GAA ACG GGT G	60	34.8
4.	OPA-10	GTG ATC GCA G	60	36.2
5.	OPA-11	CAA TCG CCG T	60	37.0
6.	OPAD- 17	GGC AAA CCC T	60	42.6
7.	OPAE- 07	GTG TCA GTG G	60	38.5
8.	OPAF- 13	CCG AGG TGA C	70	33.8
9.	OPAG- 14	CTC TCG GCG A	70	33.9
10.	OPAI- 06	TGC CGC ACT T	60	40.6
11.	OPAK- 14	CTG TCA TGC C	60	43.4
12.	OPB- 07	GGT GAC GCA G	70	45.1
13.	OPB- 09	TGG GGG ACT C	70	42.9
14.	OPB- 15	GGA GGG TGT T	60	37.0
15.	OPC- 05	GAT GAC CGC C	70	33.2
16.	OPC- 11	AAA GCT GCG G	60	39.6
17.	OPC- 16	CAC ACT CCA G	60	45.4
18.	OPD- 11	AGC GCC ATT G	60	35.7
19.	OPH- 13	GAC GCC ACA C	70	42.2
20.	OPK- 02	GTC TCC GCA A	60	44.4
21.	OPL- 12	GGG CGG TAC T	70	33.4
22.	OPS-02	CCT CTG ACT G	60	39.3
23.	OPT- 01	GGG CCA CTC A	70	33.2
24.	OPY- 03	ACA GCC TGC T	60	37.2
25.	OPZ- 06	GTG CCG TTC A	60	34.7

RESULT AND DISCUSSION

10-mer primers are used to detect goal genotype and distance genetics to define the genetic relationships and similarities of molecular. 25 primers were examined, and just 10 primers showed reproducible types with 390 DNA fragments generated, these bands cleared 80 positions containing polymorphic and monomorphic shapes. 295 bands were monomorphic, represented by 32 sites while 48 sites were exploited by 145 polymorphic bands, and 8 were absent bands, the absence of appropriate link sites for the initiator on the DNA strand can be used to construct differences among species [19], while 12 of the band registered as a unique band. Primer OPC-05 has the highest number of bands 53, but the primer OPAI-06 has been minimum number of bands 16. The size of the gene and primer sequence influence the number of bands [20]. primer OPAE-07 diagnoses the taxon A. kotschoubeyanus. With the complementary site in line (1550 bp), and the taxa *M. elongate* by line (520 bp), these data consider good molecular taxonomic indications and fingerprints as unique bands for taxa, absent bands did not appear. Data in table 2 and figure 1. Among the studied primers the primer (OPAI-06) characterized the S.coryne genus with two unique bands that have molecular size (500, 320 bp), indeed this can be used as fingerprints and molecular taxonomic markers for this genus. According to Table 2 and Figure 1 outcome, we can observe that the primer (OPC-05) reveals the highest efficiency was (13.5) while OPAI- 06 stabled a lower efficiency was (4.1).

The dendrogram indicated the taxa under study split into two main groups with a 0.025 similarity value, the first group included six taxa divided into two clusters (A, and B) with a similarity value was 0.013, the first cluster (A) separated into two clades (AI, and AII), the AI isolated *G. pflanzii* from other taxa, our data agree with [21] that declared the conspicuous occurrence at the morphological level in cacti family occurs at the genomic level too, while AII divided to two-branched (1and 2), the (1) contain *M. elongata*, *M. erythra*, and *M. grahamii* with achieving closest genetic distance was 0.960, our result agrees with [22] that recorded low diversity among *Mammillaria* taxa due genetic drift among rinks, while (2) include *M. winterae*, *S.*

coryne that have convergence average 0.831. This diversity of taxa agrees with [23] that certain cacti taxa provide a model system for studying evolutionary processes. The B group has only one genotype *A. kotschoubeyanus* which showed unique in the number of sites and their molecular sizes with the highest molecular differences which made it stay away from everyone even though it was characterized by a distinctive pattern that differs clearly from other taxa, this result supported by phenotypic and anatomical expression as characteristics of the plant, this may indicate a mutation at a specific site that causes the initiator to perspire and the unique bands to appear [24] the result indicated that the physically isolated individuals and populations shoe wide genetic differentiation and divergence [25], and [26] high genetic similarity according to proximity degree, the differences in phenotype can be reverted to the presence of non–coding sites on the genes [27] cleared that some of the taxa in one group similar quantum genetic extent of these group**.**

Here, we obtain detailed molecular data and summarize it as a taxonomic reorganization for cacti taxa.

TABLE 2. Primers names with the Total number and size range of amplified bands obtained for each primer

N.	Primer name	Total sites	Monomorphic sites s	Polymorphic sitess	Number of Total band Polymorphic	Number of monomorphic band	Number of polymorphic band	Absent band	Unique band	Ability discriminatory %	Primer efficiency %	Percentage polymorphism %	Band size bp
1.	OPA-11	11	-	11	48	-	48	2	1	33.1	12.3	100	400-1620
2.	OPAD- 17	8	6	2	50	48	2	1	-	1.3	12.8	25	300-1000
3.	OPAE- 07	7	3	4	33	21	12	-	2	8.2	8.4	57	450-1550
4.	OPAF- 13	4	2	2	18	8	10	-	1	6.8	4.6	50	460-1090
5.	OPAG- 14	8	4	4	46	32	14	1	-	9.6	11.7	50	200-1240
6.	OPAI- 06	7	1	6	16	7	9	-	4	6.2	4.1	85	320-1500
7.	OPAK- 14	8	4	4	44	32	12	1	1	8.2	11.2	50	340-1480
8.	OPB- 15	6	3	3	31	18	13	1	1	8.9	7.9	50	300-1000
9.	OPC- 05	10	6	4	53	60	7	-	1	4.8	13.5	40	330-1250
10.	OPZ- 06	11	3	8	51	33	18	2	1	12.4	13	72	250-1440
	Total	80	32	48	390	259	145	8	12				

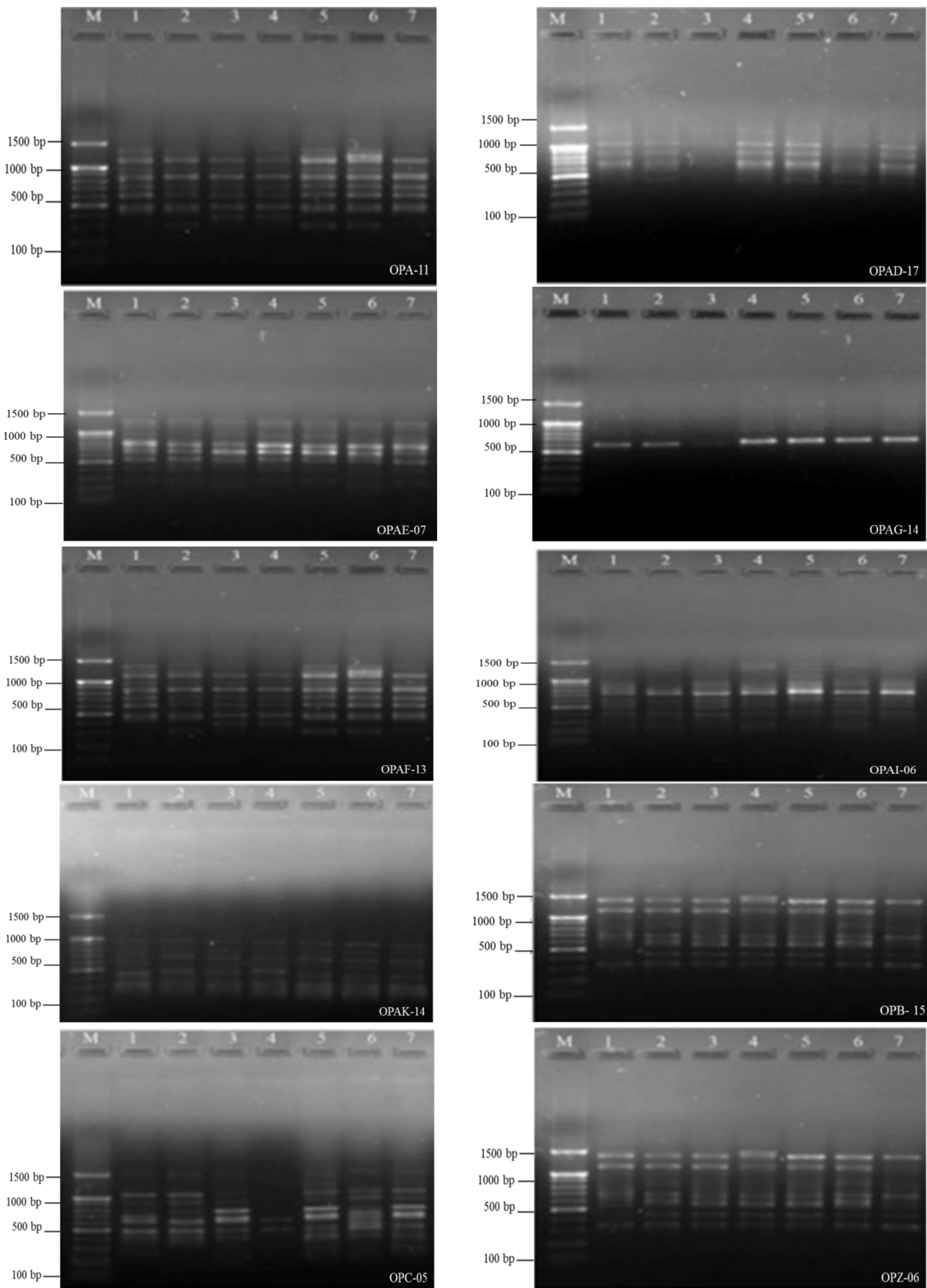

Fig 1. RAPD profile obtained with 10 primers. M-Marker1500-100; 1. *G. pflanzii*, 2. *A. kotschoubeyanus*, 3. *M. grahamii*, , 4. *M. erythra*, 5. *M. elongata*, 6. *M. winterae*, 7. *S. coryne*.

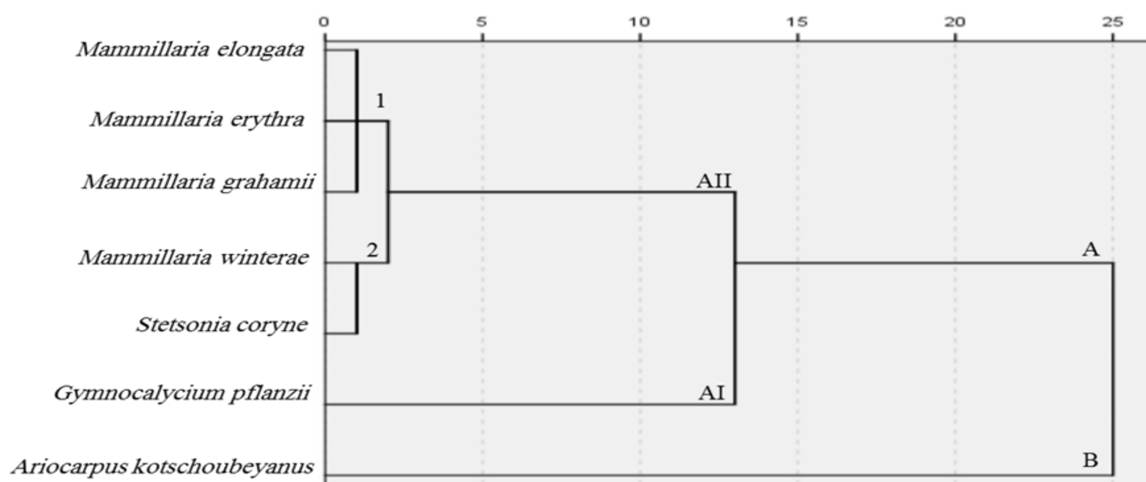

Fig. 2. The cacti taxa dendrogram using UPGMA analysis

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