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ORIGINAL ARTICLE

Genetic relationships to ten species of *Euphorbia* (Euphorbiaceae) in Iraq using PCR-Rapd Technique

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ABSTRACT
The article studied the genetic relationships for ten species of the genus Euphorbia L. using the Random Amplified Polymorphic DNA (RAPD) technique ,the study depended on extraction the genomic DNA using the dray leaves.20 random primers using the produced for many polymorphic bands among the 10 species. It was noted the appearance of a number of bands of Polymorphic to each species: Euphorbia anachoreta, Euphorbia analalavensis , Euphorbia analamerae, Euphorbia andrachnoides, Euphorbia angrae, Euphorbia angularis, Euphorbia angulata, Euphorbia angusta, Euphorbia ankaranae , Euphorbia annamarieae which tested in RAPD analysis. The band size ranged from 150 - 1550 bp. The total band obtained was 48 in the primer OPC-07, So the highest number of polymorphic bands (33) was found by the primer OPAO-05but the lowest number of polymorphic bands was (9) by the primer OPC-07.
Key words: Euphorbia, PCR-Rapd, euphorbiaceae, morphology, Genetic

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INTRODUCTION

One of the largest genera of flowering plants is *Euphorbia* continent about 2,000 species (Geltman,2015).*Euphorbia* is a very large and diverse genus in flowering plants, commonly called spurge, in the spurge family (Euphorbiaceae) [1, 2]."Euphorbia" always used for ordinary English referring all members of Euphorbiaceae [3]. Some euphorbias are commercially widely available. Some are commonly cultivated to ornamentals, or collected and highly valued for the aesthetic appearance to their unique floral structures, like crown for thorns plant (*Euphorbia milii*) [4]. The plants are annual, biennial or perennial herb, woody shrubs, or trees. The roots were fine or thick and fleshy or tuberous. Many species are more or less succulent, thorny, or unarmed. The main stem and mostly also the side arms for the succulent species are thick and fleshy, 15–91 cm (6–36 in) tall. The leaves either opposite, alternate, or in whorls [5]. In succulent species, the leaves are mostly small and short-lived. The stipules are mostly small, partly transformed for spines, glands or missing.In *Euphorbia*, flowers occur in a head, called the cyathium (plural cyathia). Each male or female flower in the cyathium head has only its essential sexual part, in males the stamen, and in females the pistil. The flowers do not have sepals, petals, or nectar to attract pollinators, although other nonflower parts for the plant have an appearance and nectar glands with similar roles [6] called the cyathium (plural cyathia). Each male or female flowers in the cyathium head have only its essential sexual part, in males the stamen, and in females the pistil. Euphorbias are the only plants characterized by this kind for flower head [7].

MATERIAL AND METHODS

Ten species of *Euphorbia* which are they: *Euphorbia anachoreta*, *Euphorbia analalavensis* , *Euphorbia analamerae*, *Euphorbia andrachnoides*, *Euphorbia angrae*, *Euphorbia angularis*, *Euphorbia angulata*, *Euphorbia angusta*, *Euphorbia ankaranae* , *Euphorbia annamarieae* tested in RAPD analysis. The leaves of the plants used to isolated the DNAs in RAPD-PCR experiment , and using modified

CTAB method Twenty random primers used in the present study which showed reproducible results: OPC-01(CCCAGTTGG), OPC-02(AATGGGTTC), OPC-03 (TCCCGAAGC), OPC-04(ATCGCAGTC), OPC-05(GAAGTTCGC), OPC-06(TTGTACGCG), OPC-7(ACAGTAGAG), OPC-08(TTGGCCTAG), OPA-9 (TATGACGCC), OPA-10 (GTAGTTGCC), OPAO-01 (TACAGTTCG), OPAO-02(TTTGCGTTC), OPAO-03(TCCGGATGC), OPAO-04(TTCGCAGTC), OPAO-05(GTTGTTCGC), OPAO-06(AAGTACGCG), OPAO-07(ACAGTAGAG), OPAO-08(AAGGCCTAG), OPAO-09(TATCACGCC), OPAO-10 (GAAGTTGCC). Each 20 µl volumes of PCR premix contained 2 µl of 10x buffer, 300 µMdNTPs, 1 µl of a 10 pM solution of each primer and 1 unit of HF DNA polymerase. One round of amplification consisted of denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 49°C for 1 min and extension at 72°C for 1 min, and a final extension for 5 min at 72 °C. PCR products were purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. The sequencing reaction was performed in a 10 µl final volume with the BigDye Terminator cycle sequencing kit (Perkin-Elmer, Applied Biosystems). Cycling conditions included an initial denaturation at 94°C for 5 min, followed by 30 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. The sequenced products were precipitated with 17µl of deionized sterile water, 3µl of 3 M NaOAc, and 70 µl of 95% EtOH. The capillary gel electrophoresis was conducted with Long Ranger Single Packs (FMC BioProducts) by an ABI 3100 automated DNA sequencer (Perkin-Elmer, Applied Biosystems). The sequences were analyzed by ABI Sequence Navigator (Perkin-Elmer/Applied Biosystems). Nucleotide sequences of both DNA strands were analyzed to ensure accuracy. The sequences were subjected to BLAST-searched.[8].

RESULTS AND DISCUSSION

RAPD-PCR technique was used to reveal DNA polymorphism in DNA of the studied Euphorbia spp. in order to search for the sources of differences that could be used as a DNA marker represent the Euphorbia, In this study, the DNA was obtained from 50 samples, The clear band and purity for DNA was extracted, So The DNA obtained from dried leaves samples, So the method selected accordingly to this for extraction the DNA. using commercial kit produced good quality and high purity of intact DNA to use in the RAPD-PCR analysis. In our studies the genetical features of the five species are summarized in table [1], this agreement with Doyle and Doyle [9].

Tables 1: Ten primers in ten species of *Euphorbia* variation

No.	primers	Sequence	Total band obtained	bp size range	Polymorphic bands
1	OPC-01	CCCAGTTGG	40	200-1200	17
2	OPC-02	AATGGGTTC	35	200-1000	10
3	OPC-03	TCCCGAAGC	26	250-950	20
4	OPC-04	ATCGCAGTC	28	300-1100	13
5	OPC-05	GAAGTTCGC	32	250-1500	21
6	OPC-06	TTGTACGCG	31	400-1000	22
7	OPC-07	ACAGTAGAG	48	200-1200	9
8	OPC-08	TTGGCCTAG	30	250-750	14
9	OPC-09	TATGACGCC	33	300-800	10
10	OPC-10	GTAGTTGCC	38	150-950	20
11	OPAO-01	TACAGTTCG	33	250-1550	30
12	OPAO-02	TTTGCGTTC	35	200-1050	22
13	OPAO-03	TCCGGATGC	29	350-1050	27
14	OPAO-04	TTCGCAGTC	43	100-1150	31
15	OPAO-05	GTTGTTCGC	44	150-1000	33
16	OPAO-06	AAGTACGCG	38	2000-900	32
17	OPAO-07	ACAGTAGAG	41	150-1100	26
18	OPAO-08	AAGGCCTAG	39	250-1150	22
19	OPAO-09	TATCACGCC	28	200-1300	24
20	OPAO-10	GAAGTTGCC	36	150-1400	31

The analysis of PCR amplified DNA fragments relies on several bases including the absence or presence of bands, differences in molecular weight also, there were distinct divergence in intensity of the bands. The 20 random primers showed distinguishable polymorphic bands, The bands can successfully using as genetic markers in identification the varieties. One of the advantages of the PCR techniques is the rapid DNA analysis of many plant samples using small quantities of DNA. The Choosing suitable primers are very important process to PCR-RAPD to get clear and good bands, this agreement with Zhao [10].

According to our results we found that the band size ranged from 150 - 1550 bp.(Tables 1),this was agree with studies of Gengler-Nowak [11].The highest total band obtained was 48 in the primer OPC-07but the lowest number of total band obtained was 28 in the primer OPC-03,his was agree with studies of Tokuoka and Tobe [12]. So the highest number of polymorphic bands was 33 found by the primer OPAO-05 but the lowest number of polymorphic bands was 9 by the primer OPC-07(Fig.1).According to our results , this agreement with the study of Yadav *et al.* [13].

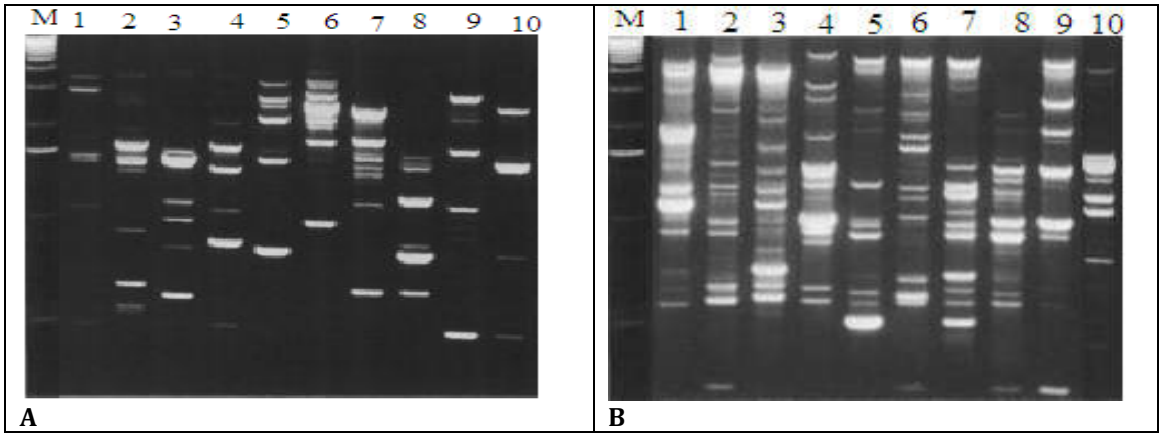

Fig.1 Rapid profile taken from ten species of *Euphorbia* variation :A: OPAO-04, B: OPAO-05

REFERENCES

1. GeltmanV. (2015). Phytogeographical analysis of Euphorbia subgenus Esula (Euphorbiaceae). Polish Botanical Journal 60: 147–161.
2. Genc I, Kültür, S. (2016). Euphorbia akmanii (Euphorbiaceae), a new species from Turkey. Phytotaxa 265: 112–120.
3. Fayed A, Al-Zahrani, A. (2007). Three new spiny Euphorbia (Euphorbiaceae) species from western Saudi Arabia. Edinburgh Journal of Botany 64: 117–129.
4. Horn J, Van W, Morawetz J, Riina R, Steinmann V, Berry E, WurdackJ. 2012. Phylogenetics and the evolution of major structural characters in the giant genus *Euphorbia* L. (Euphorbiaceae). Molecular and Phylogenetic Evolution 63: 305–326.
5. SheidaiM, Ghazei M, Pakravan M. (2010). Contribution to cytology of the genus Euphorbia in Iran. Cytologia 75:477–482.
6. Zokian S. (2011). Biosystematics of four species of Euphorbia L. grown in Baghdad university campus- jadiriyah. A Ph.D. thesis, College of Science- Baghdad University.
7. Rudall J, Bateman M. (2006). Morphological phylogenetic analysis of Pandanales: testing contrasting hypotheses of floral evolution. Systematic Botany.31:223–238.
8. Gherardi M, Mangin B, Goffinet B, Bonnet D, and Huguet T. (1998). A method to measure genetic distance between allogamous populations of alfalfa (*Medicago sativa*) using RAPD molecular marker. Theor. Appl. Genet. 98, 406-412.
9. Doyle J, Doyle L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 19:11–15.
10. Zhao F. 2014. Isolation and characterization of polymorphic microsatellites in the perennial herb Euphorbia kansui using paired endIllumina shotgun sequencing. Conservation Genet Resour. 6:841–843.
11. Gengler-Nowak M.(2003). Molecular phylogeny and taxonomy of Male sherbiaceae Systematic Botany. 28:333–344.
12. Tokuoka T, Tobe H. (2006). Phylogenetic analyses of Malpighiales using plastid and nuclear DNA sequences, with particular reference to the embryology of Euphorbiaceaesens. str. Journal of Plant Research. 119:599–616.
13. Yadav C, Pande M, Jagannadham V. (2006).Highly stable glycosylated serine protease from the medicinal plant Euphorbia milii. Phytochemistry. 67:1414-1426