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Genetic relationships to five species of *Acacia* (Fabaceae) in Iraq using PCR-Rapd Technique

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Abstract

The aim of this study is to assess the genetic relations among species and to know the extent of convergence and divergence between them. The phylogentic relationships for five genotypes of*Acacia* analyzed with RAPD-PCR (Random Amplified Polymorphic DNA) technique, The DNA for fresh and dry young leaves isolated from each sample for RAPD method, ten primers was studiedAnd used to identify the species of converged and similar in their characteristics or species diverged due to the difference of most of their qualities. The species were *Acacia nilotica*, *Acacia seyal*, *Acacia tortilis*, *Acacia farnesiana*, *Acacia reniformis*, And the primers which used in this study were:A01: CCCAATTGG, A02: AACGGCTTC, C03: TCCCGAAGC , C08: AACGCTGTC , D05: GAACTTCGC, OPA-1: TTGAACCCG , OpA-2: ACAGTTGAG,OpA-3: TAGGCGTAG ,OpA-4: TATGTCGGC, OpA-5: ATAGTAGCC, were tested in this study. In this study The band size ranged from 150 - 1500 bp, So the total band ranged from 18-38 and the polymorphic bands ranged from 9 in the primer OpA-2 to 22 in the primer OPA-1.We suggest that chemical, geographic and genetic studies support and support the morphological studies of plant species and help us to differentiate among plant species.

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Introduction

The Fabaceae or Leguminosae, which commonly known as legume, pea, or bean family, and it considered the large and economically family for the all flowering plants in the world(Haerinasab and Rahiminejad,2012) This family Includes trees, shrubs, and perennial or annual herbaceous plantsin world (Özbek *et al.* 2014). Fabaceae or Leguminosae, family found in all over the world, and considered the third-largest land plant family in species numbers, after the Orchidaceae and Asteraceae (Wang and Grusak, 2005).

The term molecular systematic using for express the macromolecular systematic- the use for DNA and RNA inferring the relationships among organisms (Ding *et al.* 2005). Molecular data that created our view for phylogenetic relationships, although not to the reasons initially suggested. (Souza, 2008).

Early proponents of molecular systemaics explained the molecular data which were more likely showing the true phylogeny than morphological data. (Rami and Eisa, 2008). The discovery of PCR with random primers using for amplify a set of randomly (Verbeken *et al.* 2003).

The molecular phylogenetic analyses didnt extensively use as a systematic tool for study species differentiation and evolution on the genus level (Niknejad *et al.* 2009). The purpose of the study was measureing the pure genetic relationships among these species using RAPD-PCR based markers (Masi*et al.* 2003), so for determining the degree of genetic similarity and variability between cultivars (Miller and Bayer, 2001).

Materials and methods

Plant material

The leaves of five species of *Acacia* were collected from the from different localities of Iraq with dry specimens from some Iraqi herbaria during the period march 2018 - August 2018.These specimens are identified according to Townsend and Guest (1974).

PCR mix

About 12.5 µl of the PCR ready mix was added when the final reaction volume was 25µl to obtain a final concentration 1X as recommended by provider and sterile distilled water was used to achieve a total volume of 25 µl after added each of primers and DNA template.

Amplification reaction

Amplification of random fragments of genomic DNA was preformed with the following master amplification reaction. RAPD-PCR master mix (final reaction volume = 25 µl) (Table 1) distributed loci for any genome (Gherardi *et al.*, 1998).

Results and discussion

Ten primers had been tested with same DNA samplesin the optimum conditions. The DNA was extracted from 50 tree samples, the clear and purity band for DNA was obtained, from dried and fresh samples.

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. In our studies the genetical features of the five species are summarized in table (2).

The 10 random primers showed polymorphic bands, the bands can successfully using as genetic markers in identification the varieties. Primers A01, A02, C03, C08, D05, OPA-1, OpA-2,OpA-3,OpA-4, OpA-5 were tested in this study, The choosing suitable primers are very important process to PCR-RAPD for get clear and good bands. This agreement with this agreement with Khadidiatou *et al.* (2008) and Rashmi *et al.* (2008).

The number of bands generated by each primer varied, according to our results we found that the primer OpA-5recorded the highest number of total band which was 38 (Fig 1), while the PrimerC08recorded the lowest number which was 18, so the band size ranged from 150 - 1500 bp.

Table 1. RAPD-PCR master mix.

Material's concentration and manufacturer	Final concentration	Volume for (1) tube
D.W	_____	9.5µl
Promega Green Mix (2X)	1X	12.5µl
Primer (10 pmol/µl)	10 pmol/µl	1µl
Total reaction volume		23µl

Table 2. Ten primers in five species of *Acacia* variation.

No.	primers	Sequence	Total band obtained	pb size range	Polymorphic bands
1	A01	CCCAATTGG	20	200-1200	17
2	A02	AACGGCTTC	25	200-1000	10
3	C03	TCCCGAAGC	26	250-950	20
4	C08	AACGCTGTC	18	300-1100	13
5	D05	GAACTTCGC	22	250-1500	21
6	OPA-1	TTGAACCCG	31	400-1000	22
7	OpA-2	ACAGTTGAG	28	200-1200	9
8	OpA-3	TAGGCGTAG	30	250-750	14
9	OpA-4	TATGTCGGC	33	300-800	10
10	OpA-5	ATAGTAGCC	38	150-950	20

The highest number of polymorphic bands (22) was found by the primer OPA-1 but the lowest number of polymorphic bands was (9) by the primer OpA-2. BARI According to our results, this was agreed with studies of Casiva*et al.* 2002. We found the species *Acacia nilotica* and *Acacia seyal* were very closely related to our data but the species *Acacia nilotica* was far away from *Acacia reniformis* (Fig 2). There are morphological or anatomical qualities that may be the cause of this convergence or divergence this agreement with Hamza *et al.* (2009) and the study for researcher Boer (2002).

This is one of the goals of this genetic study which can be applied such methods in the diagnosis of the rest of *Acacia* species existing in Iraq.

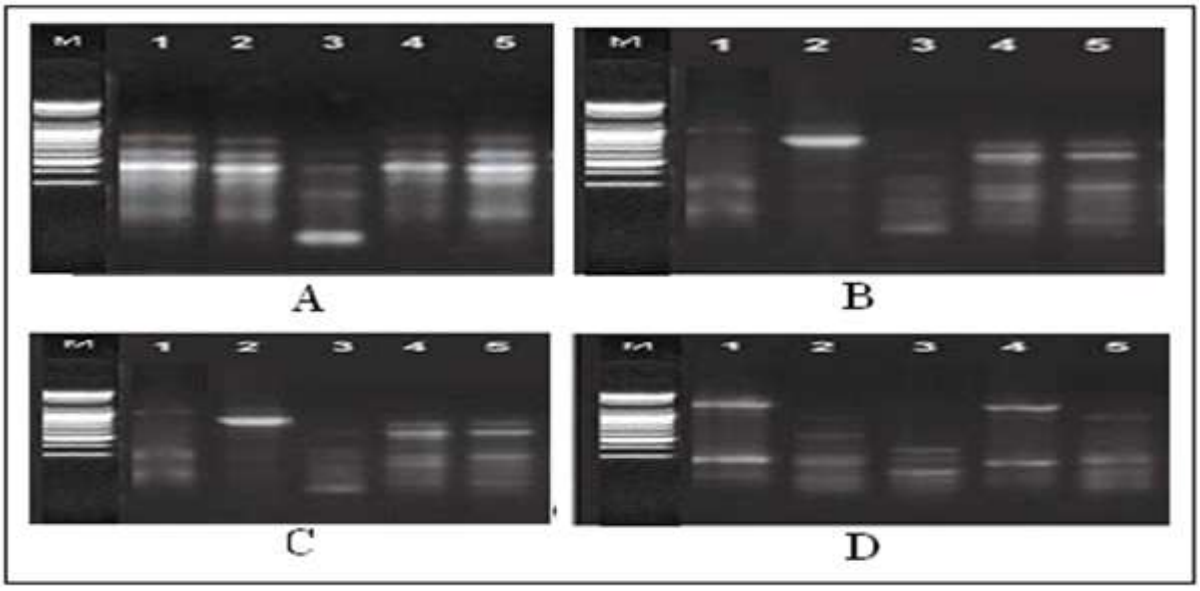

Fig. 1. Rapid profile taken from five *Acacia* variation: A: OpA-5, B: OpA-4, C: OpA-3, D: OpA-2.

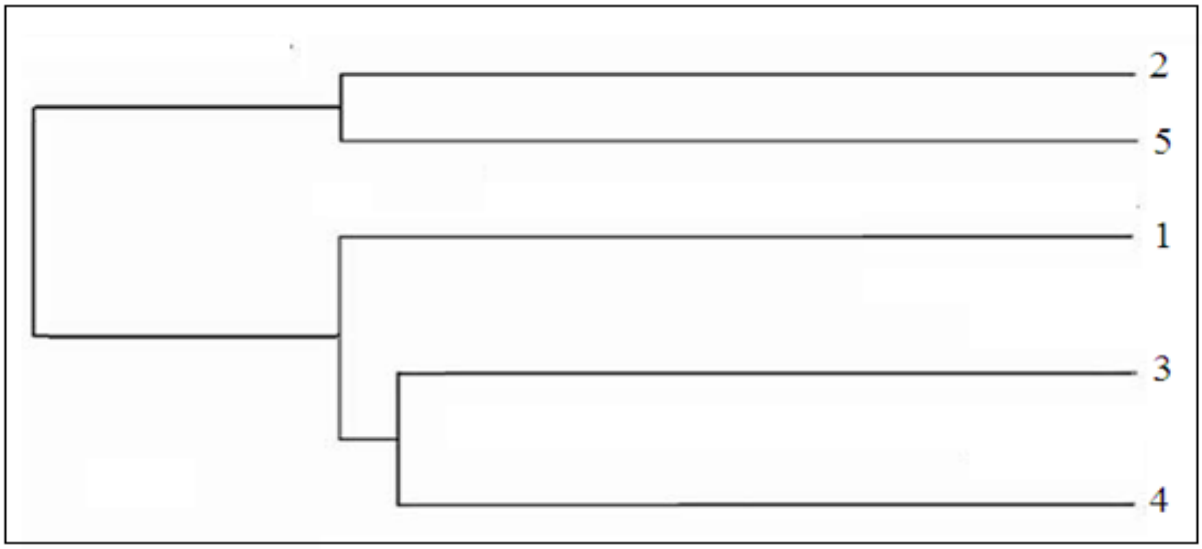

Fig. 2. A dendogram of genetic distance summarizing the data on differentiation among five species varieties, according to RAPD analysis, 2: *Acacia nilotica*,5: *Acacia seyal*, 1: *Acacia tortilis*, 3: *Acacia farnesiana*, 4: *Acacia reniformis*.

On the other hand, RAPD markers had been useful as the first step to produce a genetic map in plants with unknown or much or less known genetic serie.

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