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Analysis of molecular diversity among *Zygophyllum*, *Tribulus* and *Fagonia* species by PCR-RAPD in Saudi Arabia

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Abstract — *Zygophyllum* species are succulent plants that are drought resistant and/or salt tolerant, growing under severe, dry climatic conditions. There is little information concerning molecular variations among species of this genus. Genetic diversity was assessed, using RAPD primers, of 6 populations collected from various locations in Saudi Arabia. Yong leaves were used for DNA extraction. Molecular taxonomy for these studied plants were performed by Polymerase Chain Reaction- Random Amplification of Polymorphic DNA (PCR-RAPD). RAPD reactions are PCR reactions, but they amplify segments of DNA which are essentially unknown to the scientist (random). PCR was used to amplify a known sequence of DNA. Thus, we choose the sequence we wants to amplify, then designed and makes primers which will anneal to sequences flanking the sequence of interest. Thus, PCR leads to the amplification of a particular segment of DNA. The obtained dendrogram from our results was based on Nie's UPGMA cluster analysis of the genetic differences. These results revealed that the obtained dendrogram based, *Z. simplex* represents the closest relationship with *Z. migahidii*. Phylogenetic distances show that *F. glutinosa* and *F. indica are* far from *T. terrestris* and *T. macropterus* and also, has completely different pattern of RAPD profiles compare to other samples.

Key words—*Zygophyllum* species, PCR-RAPD, Molecular studies, Nie's

Introduction

Zygophyllaceae (Caltrop family) is a family of approximately 25 genera and 240 species adapted to semi desert and Mediterranean climates [Hammoda et al 2013]. *Zygophyllum* belongs to the Zygophyllaceae family Species belonging to this genus represent a group of succulent plants that are drought resistant and/or salt tolerant, living under severe, dry climatic conditions. The abundance of species related to this genus could be attributed to their high tolerance to environmental stresses in addition to their unpalatability. The growth and distribution of *Zygophyllum* species are attributed to their dependence on the chemical nature of the soil of their habitats [Hammad et al 2010]. Saudi Arabia possesses a unique genetic diversity in the form of ecotypes of tree species. The western and southwestern regions of the country are rich in native plant flora of cultivated crops and medicinal plants (Anonymous, 1995). Around 300 species of medicinal plants are used in traditional medicine in Saudi Arabia (Abbas, 2002).

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Our approach involved the collection of *Zygophyllum simplex, Zygophyllum migahidii, Tribulus terrestris, Tribulus macropterus, Fagonia glutinosa* and *Fagonia indica* from *Zygophyllaceae* from Saudi Arabia and at the time of collection, a pressed specimen will be prepared and retained in the Herbarium of the Department of Botany and Microbiology, King Saud University. The classical taxonomic parameters of these species would be investigated to clarify the morphometric variations between the studied species. Molecular techniques based on the polymerase chain reaction (PCR) can be achieved to differentiate between the selected species. Also extraction and identification of phytochemical compounds derived from native plants belonging to family Zygophyllacea in Saudi Arabia. Plants are collected primarily from variant extreme climatic conditions habitats. The aim of the present study was to investigate the molecular taxonomy of selected plants in Saudi Arabia by Polymerase Chain Reaction-Random Amplification of Polymorphic DNA (PCR-RAPD).

Materials and methods

DNA-RAPD analysis

DNA was extracted from the plant species using the kit method and used for the PCR-RAPD analysis. Herbarium specimens from the plants were used for DNA extraction, frozen in liquid nitrogen, and stored at –80°C until extraction. Total genomic DNA was isolated using a CTAB method (Saghai-Maroof, 1984) with minor modifications. DNA quantity and quality were measured with a UV spectrophotometer (ultrospec 2100 pro-Amersham Biosciences, England). RAPD assay were performed using 25 random 10-mer oligonucleotide primers from Integrated DNA technologies (Belgium). After screening 25 primers, RAPD analysis was conducted using 8 most polymorphic primers tabulated

in Table 1. PCR (Amersham- Pharmacia Biotech, France) reaction were carried out in a 25 µL reaction mix containing approximately 15 ng template DNA, 2.5 pmol of each dNTP (Amersham-Gene), 5 pmol of a single 10-mer primer, 1 unit of Taq DNA polymerase (Amersham-Gene). The buffer [10 mm Tris-HCl, PH 8.8 at 25°C, 1.5 mm $MgCl_2$, 50 mm KCl, 0.1% Triton X-100] used as per instructions of the manufacturer. The PCR reaction were run in a Eppendorf thermal cycler (Eppendorf, Germany) programmed for an initial denaturation step of 3 min at 94°C followed by 35 cycle of 30 s at 94°C, 30 s at 34°C and 1.5 min at 72°C. A final elongation step of 5 min at 72°C was included. The PCR products were separated on 1.5% agarose gel run in 1 X TBE and stained with ethidium bromide. The gels were visualized with a UV trans-illuminator. All PCR reactions were run in duplicate and only reproducible and clear bands were scored.

Results

Molecular characterization

The preceding methods used for distinguishing our studied species were mainly morphological and biochemical. However, these traditional methods of characterization frequently inadequate in the identification of species. So, it is necessary to obtain a better understanding of species diversity, molecular methods should be adopted for species identification. In this study, analysis of six species by RAPD using oligonucleotide primers was made. PCR amplifications with some primers indicated that each primer-template yielded district, easily detectable bands of variable intensities (Figure 1). The bands used for fingerprinting were those reproducible over repeated runs with sufficient intensity to detect presence or absence with confidence.

Pair-wise comparison of all RAPD profiles revealed a similarity matrix. Simple matching coefficient (Ssm) and genetic distance (d), derived from RAPD banding patterns, are shown in the dendrogram. The genetic distance between the two samples of *Z. simplex* and Z. *migahiddi* was considered to be short (0.839) and their RAPD banding pattern were quite similar to each other. Also there is a close relationship between these two samples of *Zygophyllum* with *Tribulus* and *Fagonia* (0.854 and 0.849, respectively). The dendrogram constructed base on genetic distances, derived from RAPD analysis, shown in Figure 2. Clustering analysis was based on UPGMA.

Discussion

Saudi Arabia is lightly forested with less than 1% of forest cover with good resources of medicinal and aromatic plants. The use of herbal medicine has been increasing in the country since the 1990s. The government stresses the safety, efficacy and registration of traditional medicine. Traditional medicine in Saudi Arabia is based on herbal remedies and spiritual healing. It has been mainly inherited from old tribes and still continues to flourish despite widespread availability of allopathic medicine.

There are many studies in medicinal plants of Saudi Arabia, since the introduction of allopathic medicine in 1940, the health authorities have worked for its development and today the country enjoys a sound modern health care system. Many higher plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavor and fragrance ingredients, food additives, and pesticides (Balandrin & Klocke, 1988).

The search for new plant derived chemicals should thus be a priority in current and future efforts toward sustainable conservation and rational utilization of biodiversity (Phillipson, 1990). In the search for alternatives to production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites (Ramachandra Rao & Ravi Shankar, 2002). Cell suspension culture systems could be used for large scale culturing of plant cells from which secondary metabolites could be extracted.

Advances in tissue culture, combined with improvement in genetic engineering, specifically transformation technology, has opened new avenues for high volume production of pharmaceuticals, nutraceuticals, and other beneficial substances (Hansen & Wright, 1999). The active principles of many drugs are found in plants or are produced as secondary metabolites. The remarkable contribution of plants to the drug industry was possible, because of the large number of phytochemical and biological studies all over the world. Herbal remedies used in folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the

active principle by isolation and characterization of their constituents. The fruits of *Tribulus terrestris*, is a famous traditional Chinese medicine (Xie & Huang, 1988). *Tribulus terrestris* is also reported to have antimicrobial activity (Jit & Nag, 1986; Bose, 1964; Tomova, 1988; Sharma, 1977).

In this investigation, RAPD markers showed a high level of polymorphism and a high number of clearly amplified bands in Figure 3. Extensive DNA polymorphism has been reported using RAPD markers in several other crops plants (Hilu & Stalker, 1995; Iruela, 2002; Hou, 2005). The RAPD-based dendrogram of different genotypes displayed the genetic relationships between these accessions, which accorded with previous studies on different plant (Ahmad, 1992; Tayyar & Waines, 1996; Iruela, 2002). It was evident from the results that the dendrogram based on RAPD-markers was accord with the dendrogram based on morphological traits, as reported for other crops (Loarce, 1996; Fernandez, 2002).

Conclusion

Molecular taxonomy was carried out for studied plants PCR-RAPD finger print. The obtained dendrogram from our results was based on Nie's UPGMA cluster analysis of the genetic differences. These results revealed that the obtained dendrogram based, *Z. simplex* represents the closest relationship with *Z. migahidii*. Phylogenetic distances show that *F. glutinosa* and *F. indica are* far from *T. terrestris* and *T. macropterus* and also, has completely different pattern of RAPD profiles compare to other samples.

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Table 1: Oligonucleotide decamer primers along with their sequences used in the study. The most of the primers gave unique bands in all species. The primers, OPA04, OPA05, OPC15, OPC16, OPD11and OPD13discriminated species by the presence or absence of unique bands, which act as markers for species confirmation.

Primer	Code	5`-3`	M.W.	Tm (°C)
61503250	OPA01	CAGGCCCTTC	2.964	36.4
61503265	OPA02	TGCCGAGCTG	3.044	40.7
61503266	OPA04	AATCGGGCTG	3.068	35.1
61503271	OPA05	AGGGGTCTTG	3.099	32.6
61503251	OPA11	CAATCGCCGT	2.988	36.7
61503252	OPA13	CAGCACCCAC	2.942	37.7
61503253	OPA18	AGGTGACCTG	3.068	32.8
61503254	OPB11	GTAGACCCGT	3.028	32.6
61503274	OPC15	GACGGATCAG	3.077	31.1
61503260	OPC16	CACACTCCAG	2.957	31.2
61503263	OPD11	AGCGCCATTG	3.028	37.1
61503264	OPD13	GGGGTGACGA	3.133	38.6

Figure 1: RAPD analysis carried out with different oligonucleotide primer on genomic DNA isolated from lane 1= *Zygophyllum simplex*; lane 2= *Zygophyllum migahidii*; lane 3= *Tribulus terrestris*; lane 4= *Tribulus macropterus*; lane 5= *Fagonia glutinosa* and lane 6= *Fagonia indica* collected from Saudi Arabia

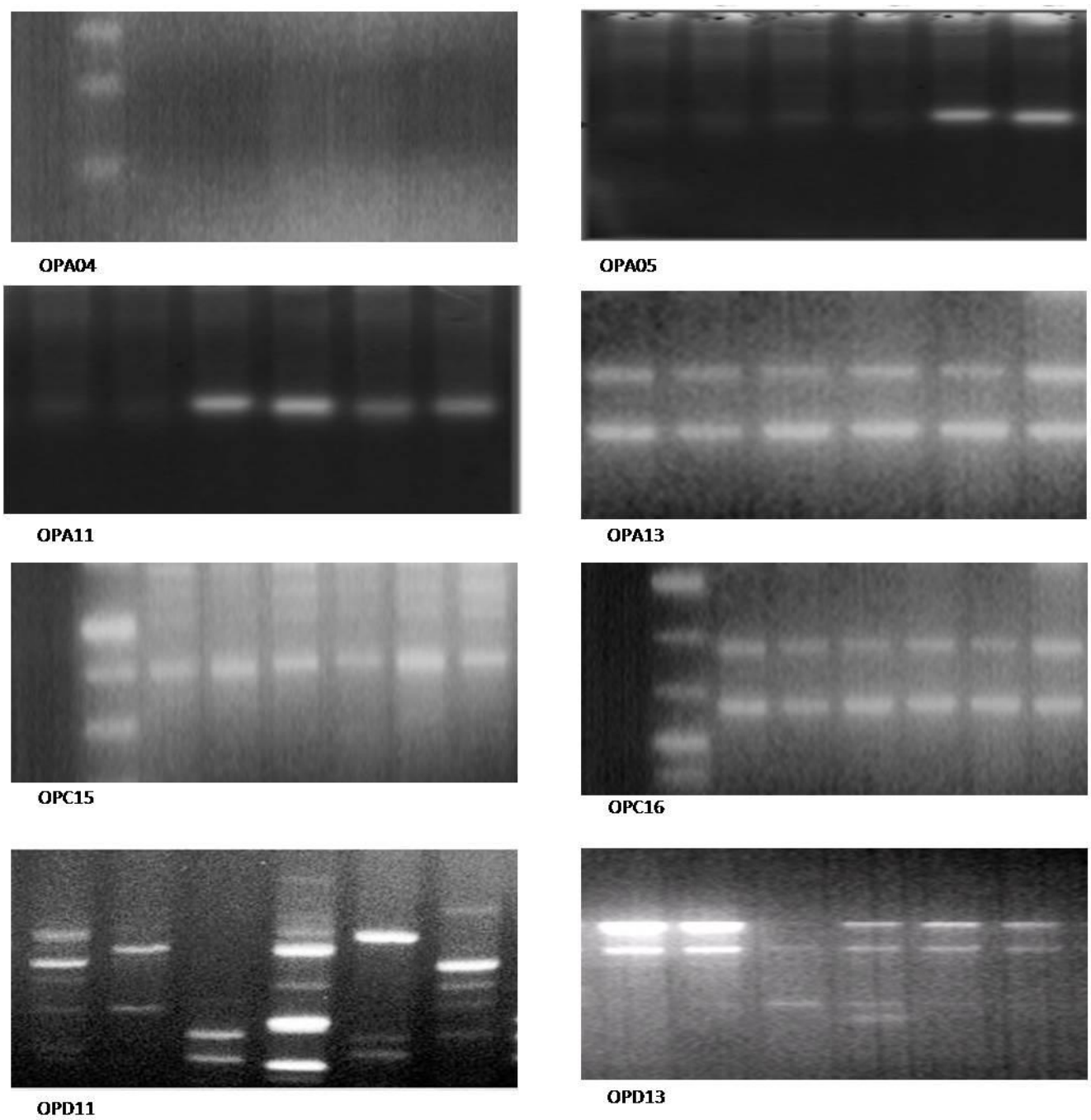

Figure 2: RAPD analysis carried out with mixed oligonucleotide primer (OPD11 and OPD 13) on genomic DNA isolated from; Lane M= 100-bP molecular weight marker Lane 1= *Zygophyllum simplex*; lane 2= *Zygophyllum migahidii*; lane 3= *Tribulus terrestris*; lane 4= *Tribulus macropterus*; lane 5= *Fagonia glutinosa* and lane 6= *Fagonia indica* collected from Saudi Arabia.