Genetic Description of Acacia Species Based on Different Markers

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ABSTRACT: Five *Acacia* species: *Acacia Tortilis ssp. radiana, Acacia farnesiana, Acacia stenophylla, Acacia sclerosperma*and *Acacia saligna* were used in the current research. Morphological, biochemical and molecular markers used to describe the genetic variations among *Acacia* species. Data showed highly significant differences among the five species concerning the morphological parameters. *Acacia Tortilis ssp. Radiana* collected from (Siwa Oasis and Borg Al-Arab city) showed the highest values comparing with other species. The highest values for spine length (mm) were 28.75 and 19.25 mm, in respect. The lowest mean value 6.50 mm was recorded in *Acacia farnesiana*for Leaf length (cm) data showed that *Acacia sclerosperma* and *Acacia saligna* have the highest mean values (19.25 and 26.08 cm). While the lowest leaf length (2.85 cm) was recorded in *Acacia Tortilis ssp. Radiana* collected from Siwa.

One cathode common band (Pex.C1) was found for all the species. While, five anodal (Pex.A1; Pex.A2; Pex.A3, Pex.A4 and Pex.A5) bands were recorded for all species in different molecular weights. (Pex.A2, Pex.A3 and Pex.A5) was recorded in *Acacia tortilis*ssp*. radiana* (Siwa)*, (*Pex.A2,andA4) were recorded in *Acacia tortilis* ssp*. radiana* (Borg Al-Arab).

In molecular markers measured, out of 156 fragments, 5 fragments were produced for the primer OPA-18 in the six samples of *Acacia* species with molecular weights ranging from 251 to 832 bp. and polymorphism (40%). While, 18 fragments were observed with OPB-03 primer (11 unique) and 3 polymorphic with polymorphism (93.3%). Ten fragments with molecular weights from 326 to 1503 bp were recorded for OPC-02 primer and (70 %) polymorphism. Eleven fragments with wide molecular weight range extended from 299 to 3178 bp recorded to OPD-03. This primer revealed (72.7%) polymorphism. Finally, primer OPE-12 gave nine fragment bands with 77.8 % polymorphism.

Keywords: *Acacia*, morphology, biochemical, genetic markers

INTRODUCTION

Genetic markers represent genetic differences between individuals or species. There are three major types of genetic markers: (1) morphological (classical or visible) markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA or molecular markers, which reveal sites of variation in DNA (Jones, *et al*., 1997).

Morphological markers are usually visually characterized phenotypic characters such as flower colour, seed shape, growth habits or pigmentation.

Isozyme markers are differences in enzymes that are detected by electrophoresis using specific stains. The major disadvantages of morphological and biochemical markers are that they may be limited in number and are influenced by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995). DNA markers are widely accepted as potentially valuable tools for crop improvement in rice (Mackill, *et al.,* 1999), Wheat (Koebner and Summers, 2003), maize (Tuberosa, *et al*., 2003) and oilseeds (Snowdon and Friedt, 2004.

Genetic markers are of great value in genetic research and practical breeding programs, since they reflect the genetic variation among individuals. Morphological markers, or mutations in morphological markers, or mutations in genes with visible consequences, have been used in genetic studies since early in the twentieth century (Hussium, *et al*., 2000).Isozymes loci are excellent biochemical markers since they are usually co-dominantly inherited do show pleiotropic effects, rarely exhibit epistasis and are not affected by the environment. Isozymes have been used very successlly in certain aspects of plant breeding and genetics as nearly neutral genetic markers (Tanksley, *et al.,* 1989). Unfortunately, the number of genetic markers provided by isozyme assays is insufficient for most applications in plant breeding. As a result, even with the use of isozymes as biochemical markers, the fill potential of genetic mapping in plant breeding have not been achieved (Tanksley, 1983).

Proline is the only organic cytosolute which able to make the major contribution or osmotic adjustment at sever salinity in roots, while in shoots and spikes the contribution of Proline in osmoregulation might be reduced. We concluded that there is no stable situation in usage of organic or inorganic soluble components in osmotic adjustment in the cultivars and lines on different salinity levels. This is happened not only in different cultivars but also in different organs which conferring the contrasting opinions about the physiological significance of Proline which has remained controversial among physiologists. Many reports have pointed out that Proline is mostly accumulated when plants growth ceased (Joly, *et al.,* 2000).

RAPD is one of the widely used molecular markers, where it was applied in determination of paternity, gene mapping, identification of markers linked to traits of interest without the necessity for mapping the entire genome, plant and animal breeding, to understand the complexity of the transmission cycles of insects vectors and population and evolutionary genetics (Marcili *et al*., 2009 and Sharma *et al*., 2009). This wide range of applications due to that tiny amounts of DNA are sufficient for the amplification, no prior knowledge of a DNA sequence is required, commercially primers kits are available, simple, no expertise is required, low cost of the unit of assay, relatively quick, produce high number of fragments and able to distinguish between closely related individuals (Hadrys, *et al*., 1992 and Bardakci, 2000).

Acacia is the common name for plants of the genus *Acacia* in the bean family, Leguminosae. This genus consists of approximately 1,100-1,200 species primarily of trees, but also including some shrubs and climbers. Globally distributed, *acacia* species are located in Asia, Madagascar, the Caribbean and

Pacic islands, the Americas, and most prominently in Australia and Africa in arid and semiarid tropical zones. Those tropical regions that have long, dry winters and short wet summers often support shrubby vegetation known as thorn scrub or savanna. *Acacia* trees constitute much of the woody vegetation in such plant communities (Ross, 1981). Because of the wide distribution of *Acacias* in the arid lands, and their multiple uses that include fodder, fuel, medicine besides the environmental values of soil fixation and fertility (Shaw, *et al.*, 2002). *Acacia* is the second largest genus in the family Leguminosae with about 1350 species (Maslin, 2003 and Maslin *et al.,* 2003).The current classification of *Acacia* differentiates three subgenera (Ross,1979 and Maslin *et al.,* 2003): *Acacia*, Heterophylum and Aculeiferum, *Acacia raddiana* belongs to the acacia subgenus. The base chromosome number in the genus *Acacia* is x=13 with polyploidy occurring in several species, (Blakesley *et al.,* 2002; Khatoon and Ali, 2006). There are 129 *Acacia* species in Africa. They are intermediate in plant succession and colonize degraded land. They restore soil fertility and can be maintained indefinitely in agricultural systems. Despite their benefits, they are disliked for their thorns and invasiveness (Barnes, 2001). *Acacia* are allelopathic, and their toxic aqueous leachates are used to detect differences in the patterns of expression of cytoplasmic root proteins in crop plants, indicative of biochemical alterations at the cellular level (Bukhari, 2002).The main objective of the current research is genetic description and phylogenetic relationships among *Acacia* species in Egypt based on different markers by calculate the morphological variation among *Acacia* species, assay peroxidase activity and proline content, estimate the level of polymorphism via RABD-PCR markers.

MATERIALS AND METHODS

A- Morphological Marker:

Leaves of five *Acacia* species: *Acacia Tortilis* ssp*. radiana, Acacia farnesiana, Acacia stenophylla, Acacia sclerosperma* and *Acacia saligna* were collected completely random from 20 individual *Acacia* trees natural habitats along different localities in Egypt i.e. Abis Station Farm, Faculty of Agriculture Saba Basha., Borg Al-Arab, Marsa Matroh City and EL-Gara (Siwa Oasis). Leaf lengths (cm), pinna length (cm), leaflet length (mm), spine length (mm) were measured. In addition to, some qualitative characters recorded such as leaves type, growth form, crown shape, stem number and spine shape.

B- Biochemical Marker:

Leaves from each species were grounded separately, using a cooled mortar with a pestle, and adding 0.23 M Tris-acetate, pH 5.0. Homogenate was extracted by the solution containing Tris (27.7 g) and citric acid (11.0 g) in 1L volume adjusted with distilled water. Electrophoresis was carried out by the prescriptions recommending 1% agar-starch-polyvinyl-pyrrolidone gel and Tris-orate or Tris-acetate separation buffers. Electrophoresis was conducted at 270 v, 4ºC for 100 min. 100 ml of0.01 M acetate buffer pH 5.0, containing 0.1% benzidine and 0.5% hydrogen peroxide (H_2O_2) were layered over the gel immediately before staining.

Proline was determined according to the method of Bates *et al*. (1973) by 3% Aqueous Sulfosalicylic Acid, Acid Ninhydrin:1.25 g Ninhydrin ,30 ml glacial acetic acid,20 ml 6M phosphoric acid.

C- Molecular Marker:

RAPD has been developed, in which DNA is amplified by the polymerase chain reaction (PCR) using arbitrary short (10 nucleotides) primers (Williams *et al.* 1990). DNA extracted from 50 mg samples of leaves using either the DNeasy Plant System. RAPD analyze was carried out using 5 oligonucleotide primers (Table 1) that were selected from the Operon Kit (Operon Technologies Inc., Alabameda, CA). The polymerase chain reaction mixture (25µl) consisted of 0.8U of *Taq* DNA polymerase; 25pmol dNTPs; 25pmol of primer and 50ng of genomic DNA. PCR amplification was performed in a Biometra *T1* gradient thermal cycler for 40 cycles after initial denaturation for 3 min at 94°C. Each cycle consisted of denaturation at 94°C for 1min; annealing at 36°C for 1min; extension at 72°C for 2min and final extension at 72°C for 10min (Williames, *et al.* 1990). Amplification products were separated on 1% Agarose gels at 100 volts for 1.30 hrs with 1 x TBE buffer. To detect ethidium bromide/DNA complex, Agarose gels were examined on ultraviolet transilluminator (302nm wavelength) and photographed.

Table 1. Primers name and their oligonucleotide sequences used in the study

primer number	primer Code	Sequence $3^{/}$---$5^{/}$
1	OPA-18	AGGTGACCGT
2	OPB-03	CATCCCCCTG
3	OPC-02	GTGAGGCGTC
4	OPD-03	GTCGCCGTCA
5	OPE-12	TTATCGCCCC

RESULTS AND DISCUSSION

A- Morphological Markers:

The morphological variations among the five *Acacia* species were calculated using; spine, pinna, leaf and leaflet length. The results were recorded in Table (2). Analysis of the variance showed high significant differences among the different species concerning the marker measured. As for spine length (mm) data , it indicates clearly highly significant variations among different species. The highest values were recorded in *Acacia Tortilis ssp. Radiana* collected from(Siwa Oasis), followed by *Acacia Tortilis* ssp*. Radiana* (collected from Borg Al-Arab city) in means 28.75 and 19.25 mm, respectively. The lowest mean value was recorded in *Acacia farnesiana,* the mean 6.50 mm with LSD=1.36. No spines were observed in the other three species. Data showed inverse relationship between the spine and pinna length, epically in the desert localities, these species grew in very hard conditions such as drought, salt etc. the plants try to make modification in increasing the spine length and vice versa decrease the pinna length to subject the

biotic and Abiotic stress. The results showed that *Acacia Tortilis ssp. Radiana* collected from Siwa and Borg Al-Arab, have the lowest values of pinna length (0.68 and 0.98cm), respectively, compared with the highest value of (4.28 cm) in *Acacia farnesiana.*

For Leaf length (cm), data in Table 2 showed that *Acacia sclerosperma* and *Acacia saligna* have the highest mean values (19.25and 26.08 cm), in respect. While the lowest leaf length was recorded in *Acacia Tortilis* ssp*. Radiana* collected from Siwa(2.85 cm), flowered by *Acacia Tortilis* ssp*. Radiana* collected from Borg Al-Arab by mean (3.15 cm) and finally *Acacia farnesiana* with mean (3.80 cm), while, no significant differences were observed among other species, the means ranged from (2.0 to 2.50 mm) in the *Acacia Tortilis ssp. Radiana* collected from(Siwa=2mm), followed by *Acacia Tortilis* ssp*. Radiana* collected from Borg Al-Arab=2.50) and *Acacia farnesiana*=2.25mm*.*

High similarities were found between *Acacia farnesiana and Acacia Tortilis* ssp*. Radiana* in leaves type it were pinnately compound. On the other hand, *Acacia saligna, Acacia sclerosperma* and *Acacia stenophylla* had simple leaves (Table 3).The same trend for growth form Shrub/small tree compared with other spices were shrub or tree. For stem number, the highest number recorded for *Acacia farnesiana* (2-5 stems) forward by both *Acacia Tortilis* ssp by (1-4 stems) and finally *Acacia stenophylla* usually one stem. Concerning to spine shape, data in Table 3 showed that, spine shape was small in *Acacia farnesiana* compared with long white straight in both *Acacia Tortilis* ssp which collected from Siwa and Borg EL-Arab.

Table 2. Morphological variations of *Acacia* species: spine length, pinna length, leaf length and leaflet length

Species	Spine length(mm)	Pinna length (cm)	Leaf length (cm)	Leaflet length (mm)
Acacia farnesiana	*6.50^{c}	4.28^{a}	3.80^{d}	2.25^{a}
Acacia Tortilis ssp.*Radiana* (Borg)	19.25^{b}	0.98^{b}	3.15^{e}	2.50^{a}
Acacia Tortilis ssp*. Radiana* (Siwa)	28.75^{a}	0.68^{c}	2.85^{e}	2.00^{a}
Acacia saligna	-	-	23.28^{b}	-
Acacia sclerosperma	-	-	26.08^{a}	-
Acacia stenophylla	-	-	19.25^{c}	-
L.S.D 0.05	1.36	0.252	0.508	0.705

*Mean followed by the same letter is not significantly different at 0.05 levels (-) not found

Table 3 . Qualitative description of some *Acacia* species in Egypt

Species	Leaves type	Growth form	Crown shape	Number of stems	Spine shape
Acacia farnesiana	Pinnately compound	Shrub/small tree	often spread	2-5	small
Acacia Tortilis ssp. Radiana (Borg Al-Arab)	Pinnately compound	Shrub/small tree	irregular/ round	1-4	Long white straight
Acacia Tortilis ssp. Radiana (Siwa)	Pinnately compound	Shrub/small tree	irregular/ round	2-4	Long white straight
Acacia saligna	Simple	shrub or tree	spread	1	-
Acacia sclerosperma	Simple	shrub or tree	spread	1	-
Acacia stenophylla	Simple	shrub or tree	rounded	usually 1	-

These results are in the line with Fatima, *et al*., (2011) who studied the morphological variations on Acacia species in Morocco. The authors assessed the variability in eight pod traits of 300 genotypes (mother-tree) of *A. tortilis ssp. raddiana* (Savi) Brenan collected from southern regions of Morocco. The results showed that, in the analysis of variance, that *Acacia raddiana* have significant differences in all traits due to genotype within provenances, but only in pod length, seed weight per pod, seed number per pod, infected seed number per pod and 100-seed weight due to provenances. Results showed that analyses of the three traits showed significant species differences. Our study in agree with those Boxshall and Jenkyn (2001) and Wasowski and Wasowski, (2003) which studied the morphological variation in *Acacia stenophylla*, they proved whole description for this species via morphological parameters such as spreading shrub or small tree, tree tall, leaves, branches, leaf arrangement, leaf venation: pinnate, leaf margin: entire leaf apex: acute, leaf base: oblique, size notes. These results is alien with Boulos, (1999) and Orwa, *et al*.(2009) described that *Acacia tortilis* is a small to medium-sized evergreen tree or shrub that grows up to 21 m tall. Leaves glabrous to densely pubescent, glandular, short at 1.25-3.75 cm long; petiole 0.2-0.9 cm long, with a gland; rachis 0.3-2 cm long.

B. Biochemical Markers:

- Peroxidase assay:

Peroxidase iso-enzyme assay was applied as the most appropriate technique for the evaluation of wild *acacia* and domesticated *acacia* species .In contrast, as shown in (Table4), Peroxidase isozymes exhibited a wide range of variability among the different species at different localities. One cathode (Pex.C1) were found as common band for all the species. While, five anodal (Pex.A1; Pex.A2; Pex.A3, Pex.A4 and Pex.A5) bands were recorded for all species in different molecular weight. (Pex.A2, A3 and A5) was recorded in *Acacia tortilis*ssp*. radiana* (Siwa)*, (*Pex.A2,and A4) was recorded *Acacia tortilis* ssp*. radiana*(Borg).While, *Acacia stenophylla,* and *Acacia sclerosperma* showed (Pex.A3).and finally *Acacia saligna* showed Pex. A4. From the data it can be conducted that the peroxidase patterns in the *Acacia radiana* (Siwa) wild and the five domesticated *Acacia* plants leaves showed two kinds of banding profiles. First,

it was evident that all plants expressed the (Pex.C1) and the five domesticated plants exhibited the same banding profile containing the sesame loci. Indicated that, these one common locus was consistently monomorphic expressed. Second, the *Acacia radiana* (Siwa) wild types displayed one common locus (Pex.C1). The banding pattern activity of *Acacia* displayed a unique marker band at(Pex.A5) locus indicating that (Pex.A2, Pex.A3and Pex.A4) loci are polymorphic specifically.

Isozyme have been used as markers in a number of genetic studies, such as genetic diversity in *Brassica juncea* Persson, *et al.,* (2001). Peroxidase are enzymes related to polymer synthesis in cell wall (Bowles, 1990), as well as in the prevention of oxidative damage caused by environmental stress to the membrane lipids (Kalir, *et al.,* 1984). It was found that salt stress increased peroxidase bands intensity. *Acacia Tortilis* ssp*. radiana* (Siwa) showed higher band intensity compared with the other species. Plant peroxidase have been used as biochemical markers for various types of biotic and Abiotic stresses due to their role in very important physiological processes, like control of growth by lignifications, cross linking of pectins and structural proteins in cell wall, catabolism of auxins (Gaspar, *et al.,* 1982).

Table 4 . Different loci of peroxidase activity among *Acacia* species

Genotypes	Pex.C1	Pex.A1	Pex.A2	Pex.A3	Pex.A4	Pex.A5
Tortilis ssp.*radiana*(Siwa)	1	0	1	1	0	1
Tortilis ssp.*radiana*(Borg)	1	0	1	0	1	0
Acacia farnesiana	1	1	0	0	0	0
Acacia stenophylla	1	0	0	1	0	0
Acacia sclerosperma	1	0	0	1	0	0
Acacia saligna	1	0	0	0	1	0

- Proline content (µmoles / g fresh weight)

Proline is an amino acid and compatible solute commonly accumulates in many plants exposed to various stress conditions such as salinity. Under stress condition, Proline is synthesized from glutamate due to loss of feedback regulation in the Proline biosynthetic pathway (Boggess and Stewart, 1980). Data in (Figure 1) indicated clearly that, *Acacia Tortilis* ssp*. Radiana* (Siwa) had the highest value of proline content was 43.4 µmoles / g fresh weight compared with the lowest one 7.6 µmoles / g fresh weight for *Acacia sclerosperma.* There were highly significant variations among all species in relation to proline content and this formula is gained by the environmental effects and conditions. Siwa oasis had special conditions in addition to increase the level of salt soil compared with other localities. *Acacia Tortilis ssp. Radiana* (Borg Al-Arab) had the second value in proline content by 23.1 (µmoles / g fresh weight). The aforementioned results supported the conclusion that proline was more accumulated in the salt, dry soil genotype, and may be useful as a possible salt injury sensor in plants. This variation of proline could be useful in selection for salt tolerance and used as a marker of salt tolerant plants. Similar results were obtained by Shen and Shen, (1992). They observed that under high NaCl concentrations, the percentage of free

proline in total amino acids markedly increased in barley seedlings. Genotypic variations in proline accumulation have been observed in many studies and attempts were made to correlate its accumulation with tolerance of plants to stress. This apparent correlation between proline accumulation and environmental stress suggests that proline could have a protective function (Ahmed and Hasan, 2011).

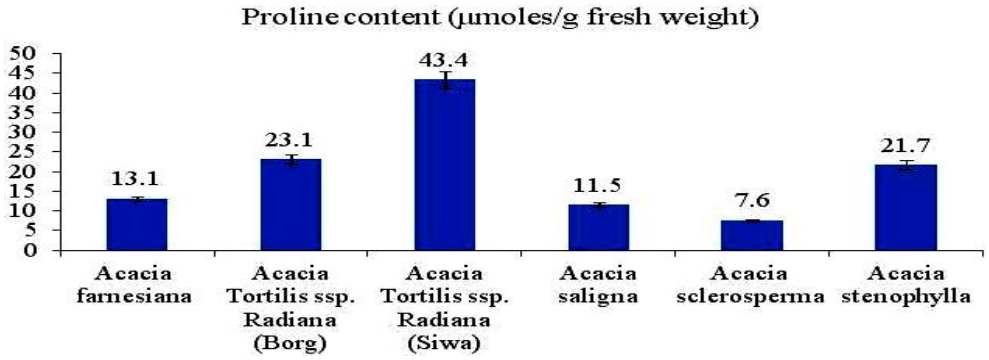

Figure 1. Proline content (µmoles/g fresh weight) in some *Acacia* species

C- Molecular Markers:

Plant molecular geneticists are currently used RAPD markers routinely to identify genetic variations (Irwin *et al*., 1998 and Sun *et al*., 1998). RAPD markers have been also used successfully in various taxonomic and phylogenetic studies (Wilkie, *et al*., 1993). In addition, it locates regions of the genome linked to agronomically important genes (Pillay and Kenny 1996). Furthermore, it facilitates introgression of desirable genes into commercial accessions (Lavi *et al*. ,1994).

In a total of 156 fragments, DNA banding pattern OPA-18 primer is presented in Table (5). Five fragments were produced in the six samples of *Acacia* species with molecular weight ranging from 251 to 832 bp. Three monomorphic and two polymorphic bands were recorded with polymorphism degree reach to 40%.While 18 fragments were observed with OPB-03 primer. Molecular weight ranged from 469 to 2264 bp. one monomorphic DNA bands, eleven unique bands and three polymorphic bands were recorded with polymorphism degree reach to 93.3%. Ten fragments with molecular weight 326 to 1503 bp were recorded for OPC-02 primer and polymorphism degree reach to 70 %. Three monomorphic DNA band, four unique DNA bands and three polymorphic bands were recorded.

The amplified DNA fragments of the studied of *Acacia* species with primer OPD-03 are tabulated in Table (5). Eleven fragment with wide molecular weight extended from 299 to 3178 bp. Three monomorphic bands, four unique bands and four polymorphic bands were observed. This primer revealed polymorphism degree reach to 72.7%. Finally, primer OPE-12 gave nine fragment bands with the six samples of *Acacia* species their molecular weightwere 295 to 1600 bp. two monomorphic bands, two unique bands and five polymorphic bands were recorded with polymorphism level 77.8 %.

RAPDs are generated by applying the polymerase chain reaction (PCR) to genomic DNA samples, using randomly constructed oligonucleotides as primers. Since the technique is relatively easy to apply to a wide array of plant

and animal taxa, and the number of loci that can be examined is essentially unlimited, RAPDs are viewed as having several advantages over RFLPs and DNA fingerprints. When the primers are of intermediate size (on the order of 10 base pairs), multiple amplifiable fragments (from different loci) are usually present for each set of primers in each genome. The fragments can be separated by size on a standard Agarose gel and visualized by ethidium bromide staining, eliminating the need for radio labeled probes. Since the primers consist of random sequences, and do not discriminate between coding and nonbonding regions, it is reasonable to expect the technique to sample the genome more randomly than conventional methods.

Morphological and genetic diversity among *Acacia aroma*, *A. macracantha*, *A. caven*, and *A. furcatispina* were studied with morphometric, isozymal, and RAPD approaches by Paola *et al*. (2002). The analysis of seven isozyme systems revealed 21 loci, and RAPD analysis showed 34 loci. Most of these loci allowed us to differentiate the species, with the exception of *A. aroma* and *A. macracantha*, the two most similar species. The levels of genetic variability estimated by isozymes were higher than those obtained from RAPD analyses. Morphometric

characters showed highly significant differences among the species, although *A. aroma* and *A. macracantha* are differentiated only by thorn length.

Table 5. Polymorphism of the *Acacia* species amplified with different primers.

	M.W.	*A.Tortilis* (wild)	*A. Tortilis* (domes.)	*A. Farnesiana*	*A. Stenophylla*	*A. Sclerosperma*	*A. Saligna*	Poly/Mon/Un.
OPA-18	832	1	0	0	0	1	0	polymorphic
	783	0	1	1	1	0	1	polymorphic
	524	1	1	1	1	1	1	Monomorphic
	438	1	1	1	1	1	1	Monomorphic
	251	1	1	1	1	1	1	Monomorphic
	2264	0	0	0	1	0	0	Unique
	2058	0	0	0	0	0	1	Unique
	1706	0	1	0	1	0	0	Polymorphic
	1616	1	0	0	0	0	0	Unique
	1601	0	0	0	0	1	0	Unique
	1479	0	0	0	0	0	1	Unique
	1374	0	0	0	1	0	0	Unique
	1218	0	0	0	0	0	1	Unique
	1103	0	0	1	0	0	0	Unique
	1029	1	1	0	1	1	1	Polymorphic
OPB-03	932	0	0	1	0	0	0	Unique
	793	0	1	0	0	0	0	Unique
	763	1	0	1	1	1	1	Polymorphic
	609	0	0	1	0	0	0	Unique
	469	1	1	1	1	1	1	Monomorphic
	1503	0	1	0	1	1	1	Polymorphic
	1240	0	1	0	1	1	1	Polymorphic
	951	1	1	1	1	1	1	Monomorphic
	759	1	1	1	1	1	1	Monomorphic
	625	0	0	1	0	0	0	Unique
	577	1	1	0	1	1	1	Polymorphic
OPC-02	542	0	0	1	0	0	0	Unique
	483	0	0	1	0	0	0	Unique
	428	1	1	1	1	1	1	Monomorphic
	326	0	0	1	0	0	0	Unique
	3178	1	0	0	0	0	0	Unique
	3035	0	0	0	0	1	0	Unique
	2150	1	1	1	0	1	0	Polymorphic
	1595	1	1	1	0	1	0	Polymorphic
	1123	1	1	1	0	1	0	Polymorphic
	933	1	1	1	1	1	1	Monomorphic
	833	0	1	0	0	0	0	Unique
	732	1	1	1	0	0	0	Polymorphic
	622	0	1	0	0	0	0	Unique
	461	1	1	1	1	1	1	Monomorphic
	299	1	1	1	1	1	1	Monomorphic
OPD-03	1600	1	0	0	0	0	0	Unique
	1264	1	1	1	0	0	1	Polymorphic
	763	1	1	1	0	1	1	Polymorphic
	623	1	1	1	1	1	1	Monomorphic
	488	0	0	0	0	1	1	Polymorphic
	453	1	1	1	1	0	0	Polymorphic
	392	1	1	1	1	1	1	Monomorphic
	344	0	0	0	0	1	1	Polymorphic
	295	0	0	0	0	1	0	Unique

The phonogram obtained from isozyme data is consistent with morphological data. The RAPD phenogram based on allelic frequencies showed agreement with morphological and isozymal approaches only at the intraspecific levels, while the RAPD phenogram based on Nei and Li's similarity measures agreed with the phenograms constructed from isozyme and morphological data.

High similarities and high indirect gene flow were found between *A. aroma* and *A. macracantha*, results that call the relationship between them into question.

To study the genetic similarities and phylogenetic relationships among the six tested samples of *Acacia* species were based RAPD-PCR. The obtained data were subjected to cluster analysis with dice equation by using SPSS (ver.15) computer program to calculate proximity matrix and design dendogram.Genetic similarity values generated from RAPD marker varied between 0.60 and 0.78 with an average of 0.69. Dendogram based on similarity values (Table 6) from RAPD was constructed to reveal similarities between the five different *Acacia species*. The dendogram (Figure 2) demonstrated that the sample of *Acacia species* fall into two main groups. The first one was divided into two clusters containing 4 and the second continue 5 and 6 in similarity from 73 to 77%. The second one divided into two sub clusters. According to similarity, the first one contained 3 and the second continue 1 and 2 in similarity from 71 to 74 %.

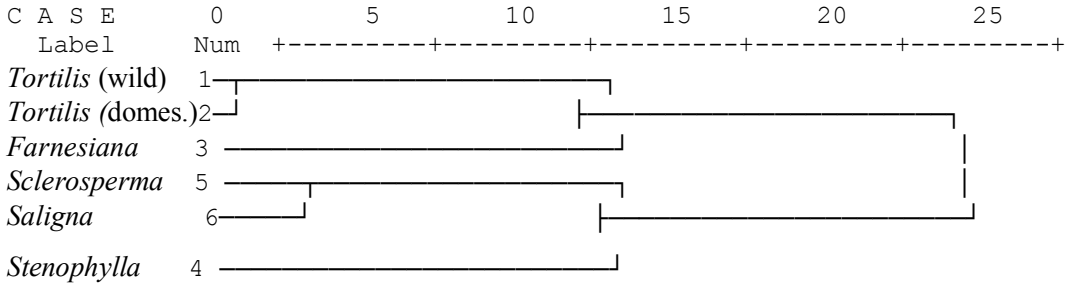

Figure 2. Dendrogram of similiratiy of different *Acacia* species based on 5 RAPD primers.

Table 6. DNA specific unique markers based on RAPD-PCR primers of different *Acacia* species.

Species	DNA specific unique marker Length (bp)	Total
1-*Tortilis ssp. radiana* (wild)	1600, 1616 and 3178	3
2-*Tortilis ssp. radiana* (domesticated)	793,833 and 622	3
3-*Farnesiana*	326, 483, 542, 609, 625, 932 and 1103	7
4-*Stenophylla*	1374 and 2264	2
5-*Sclerosperma*	295, 1601 and 3035	3
6-*Saligna*	1218, 1479 and 2085	3
Total		21

The characterization of a DNA sample by Random Amplified Polymorphic DNA (RAPD) analysis, which is often referred to as DNA "fingerprinting", has attracted considerable attention in the last ten years. RAPD is possibly the simplest test of all recently applied DNA-based tests for date palm identification (Trifi *et al.,* 2000).RAPD as a molecular marker system has also been successfully applied in cultivar identification. RAPD analysis is normally found to be easy to perform but

has the major disadvantage that reproducibility is difficult to achieve between different laboratories and often even between different people in the same laboratory (Jones *et al.,* 1997). Any diagnostic laboratory, which intends to use RAPD analysis as a quality control tool, has, therefore; firstly to ensure constant detection of identical DNA amplification products by several-fold repeated experiments preferably by different people. Elimination of possible variation in both DNA concentration and purity and assurance of consistent reaction conditions maybe a first step to overcome difficulties with assay reproducibility (Williames *et al.,* 1990).

Fagg and Allison (2004) reported variation in chemical composition, molecular as well as morphological characteristics between Ugandan and Sudanese populations of *A. senegal*. Our results in a lien with Shrestha *et al*. (2000) on *A. raddiana* populations and reported that there are a high degree of polymorphism, contrary to the conventional expectation of small, isolated populations. It is a maxim of conservation biology that the maintenance of genetic variation is important because future evolutionary adaptation depends on the existence of genetic variation.

Isozyme studies have also indicated that the West African provenances of *A. senegal*var. *Senegal* show little variation (Boer, 2002). Lower *H* values were also obtained in four Argentinean species of *Acacia* by Casiva *et al.* (2002) using isozymes and RAPD markers. Similar to the range of our *H* value, Playford *et al*. (1993) found high levels of genetic diversity (0.208) in *Acacia melanoxylon* population in association with a great genetic differentiation among geographic areas.

The percent polymorphic loci (*P)* values obtained in this study were by far higher than those observed in *Acacia caven* (29.4%) (Casiva *et al.,* 2002), *Acacia anomala* (43%) (Coates, 1988) and *Faidherbia albida* (42.7%) (Dangasuk and Gudu, 2000). However, similar results were obtained in *Haloxylon ammodendron* (74.9%) by Sheng *et al*. (2005) using ISSR markers, in *Changium smyrnioides* (69%) by Fu, *et al*. (2003) using RAPD markers and in *F. albida* (90%) reported by Joly, *et al*. (1992) using isozymes.

Several authors have studied the taxonomy of *Acacia* using morphological characters (Vassal, 1972; Guinet and Vassal, 1978; Cialdella, 1984, 1997and Pedley, 1986), in the last ten years some have used biochemical and molecular markers instead (Playford, *et al*., 1992; Bukhari, 1997a and Clarke*, et al*., 2000). Biochemical and molecular studies have been conducted on African and Australian *Acacia* species to provide markers useful for plant breeding and conservation programs (Moran, *et al*., 1989a, b; Muona, *et al*., 1991; Joly, *et al*., 1992; Sedgley, *et al*., 1992; Playford, *et al*., 1993; Fagg, *et al*., 1997 and McGranahan, *et al*., 1997). However, no population genetic studies have been carried out so far on Argentinean species of *Acacia*.

Isozyme electrophoresis and random amplified polymorphic DNA (RAPD) analysis are broadly used in plant population genetic studies (Soltis and Soltis, 1990; Avise, 1994; Soltis, *et al*., 1998 and Hollingsworth, *et al*., 1999). Mainly, RAPD has allowed the resolution of complex taxonomic relationships (Voigt, *et al*.,

1995; Comincini, *et al*., 1997; Cottrell, *et al*., 1997and Wolff and Richards, 1999).

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الملخص العربي

التوصيف الوراثي لأنواع الأكاسيا باستخدام أنواع مختلفة من الماركر

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أجري هذا البحث على خمسة أنواع من الأكاسيا وهي:

Acacia Tortilis ssp*. radiana, Acacia farnesiana, Acacia stenophylla, Acacia sclerosperma and Acacia saligna.*

ولإظهار الاختلافات الجينية بين انواع الاكاسيا أستخدم الوصف المورفولوجى والتحليل البيوكيميائي و الجزيئى.

وأوضحت النتائج أن هناك اختلافات معنوية عالية بين الانواع المختلفة وذلك فيما يخص الوصف المورفولوجى . فقدأظهر نوع *Acacia Tortilis* ssp*. radiana*والذي جمع من واحة سيوة ومدينة بر ج العرب أعلي القيم بالمقارنة بباقي الأنواع من حيث طول الشوكة فتراوحت بين ٢٨.٧٥ و ١٩.٢٥مم علي التوالي بينما كانت أقل قيمة سجلت في *Acacia farnesiana* بمتوسط ٦.٥ مم . كما أظهرت نتائج طول الورقة أن *Acacia sclerosperma* و *Acacia saligna* كانتا لهما أعلى قيمة للمتوسط (١٩.٢٥ – ٢٦.٠٨ سم)، بينما أقل طول ورقة كان في *Acacia Tortilis* ssp*. Radiana* والتي جمعت من واحة سيوة بمتوسط (٢.٨٥سم).

أما فيما يخص التحليل البيوكيميائي فقد تم العثور علي حزمة واحدة (Pex.C1) مشترك لجميع العينات، بينما الخمسة حزم (Pex.A1:Pex.A2:Pex.A3:Pex.A4:PexA5)سجلت في كل الأنواع بأوزان جزيئية مختلفة، كما وجدت حزم (Pex.A2, Pex.A3 and Pex.A5) في النوع*Acacia Tortilis* ssp*. radiana*(سيوة)، بينما وجدت حزم (Pex.A2, Pex.A4) في النوع *Acacia Tortilis* ssp*. radiana* (برج العرب) .

بالنسبة للوراثة الجزيئية فقد تحصل علي ١٥٦حزمة، وكان عدد الحزم للستة عينات أكاسيا خمسة حزم لبريمر(OPA – 18) بوزن جزيئي تراوح من (٢٥١– ٨٣٢bp) ودرجة polymorphism (٤٠%). بينما أعطي بريمر (OPB - 03) ثمانية عشرحزمة منهم احد عشر حزم unique، وثلاث حزم بولي مورفيك، ودرجة polymorphism تصل إلى ٩٣.٣%.

في حين أن البريمر (OPC- 02) أظهر عشر حزم لها أحجام جزيئية تتراوح بين (٣٢٦–١٥٠٣ bp)، ودرجة polymorphism تصل إلى ٧٠%.

كما أنتجت أنواع الاكاسيا باستخدام البريمر (OPD-03) إحدى عشرة حزمة مع حجم جزيئي واسع يمتد من (٢٩٩ إلى ٣١٧٨ bp) بدرجة polymorphism تصل إلى (٧٢.٧%).

أخير ً ا، نتج عن البريمر (OPE-12) تسع حزم بدرجة polymorphism تصل إلى (٧٧.٨%).