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BIOCHEMICAL GENETIC FINGERPRINTING OF EIGHT *ACACIA* SPECIES FROM EGYPT

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Eight *Acacia* species with different levels of drought resistance were collected from three different phytogeographical regions; El-Naqab (Sinai Peninsula), Gabal-Elba (G-Elba) in the south and the Nile Valley of upper (southern) Egypt. Seeds of these species were used for biochemical analysis where proteins, polymorphic enzymes and glycinebetaine were analyzed to evaluate the biochemical genetic variants among these *Acacia spp.*

Results obtained from total protein analysis indicated that *Acacia* species exhibited a maximum number of 21 protein bands that had molecular weights ranged between 137.75 and 16.66 KDa. Results also revealed that specific protein bands with molecular weights of about 34.81 and 22.99 KDa were detected in different species which may be considered as positive markers associated with *Acacia spp.* Results also indicated that protein bands with molecular weights of 52.87 and 6.66 kDa were recorded in all species under study, irrespective of the riverine species *A. arabica* which may be also considered as a positive markers of drought resistance.

The polymorphic enzymes, two isozyme systems (esterase and acid phosphatase) were used to study variations in gene expression of the studied eight *Acacia* species. In this concern, the electrophoretic bands showed that all true xerophytes *Acacia* species gave the maximum genes expression of concerned isozymes, but differ in their intensities, pointing to their growth under different ecological conditions. Whereas, *A. arabica* (the riverine species) gave a minimum genes expression. Meanwhile, all *Acacia* species, which represent an intermediate stage of drought stress, exhibited genes expression falling between true xerophytes and riverine *Acacia* species.

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Concerning glycinebetaine content, both *A. gerarrdii and A. tortilis* attained the highest value of it compared with other species (36 and 35 μg/g, respectivity). These two species proved to be the most drought resistant among the concerned *Acacia spp.*

Keywords: drought resistance, Gebel Elba, El-Naqab, riverine, biochemical nanalysis, total protein, polymorphic enzyme and lycinebetaine.

Species of the genus *Acacia* are of distinguished economic and ecological importance among the desert vegetation. They are important timber from which Bedouins make different tools. Ancient Egyptians used wood of *A. ehrenbergiana* to break stones. They are of considerable importance as fodder plants, particularly during the summer months (Scholte, 1992). They are also the best fuel for cooking, charcoal production and their cortex is used for tanning. Furthermore, they provide shade, hedges and soil stabilization (Baumer, 1990 and Ghabbour and Ayyad, 1990).

In Egypt there are 13 species of *Acacia* (Tackholm, 1974). Although *Acacia* scrub is presumably the natural climax vegetation of the wadis dissecting Egyptian deserts (Kassas and Imam, 1954). Species of this genus show different degrees of drought resistant. For example, *A. raddiana* grows mostly on the softer deposits, compared to *A. tortilis*, which may suggest that the latter species is more drought resistance. Furthermore, Zahran (1964) reported that although the two species grow together in Abu Ghussan district of the Eastern desert most of the very abundant *A. raddiana* shrubs were dry, dead or almost dead whereas shrubs of *A. tortilis* were thriving almost normally. This observation was repeatedly noticed in a number of wadis where the habitat was subjected to a spell of rainless years. Meanwhile, *A. nubica* is known as a drought-deciduous species occurs in a few types of habitats within Gebel Elba (Zahran and Willis, 1992). *Acacia albida, A. arabica* and *A. laeta* grow in the deep silt habitat (+ 5 m above water level) of the river Nile islands. The main adaptive response of such group of *Acacia* species under such habitat conditions is the presence of a well-developed root system, capable of penetrating silt deposits down to fresh water supplies.

Application of biochemical genetic techniques have an important potential to provide a new tool for the study of evolution and migration of plant species from their gene pool centers. Electrophoretic techniques have been effectively used to determine total plant protein and isozyme polymorphism for cultivars identification in several crops (Goodman and Stuber, 1980; Abdelsalam *et al.*, 1998; Vladova *et al.*, 2000 and El-Rabey *et al.*, 2002). These techniques are considered rapid and accurate test to identify and characterize plant species. Since *Acacias* are well known as true

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xerophytic plants, it is possible to determine the biochemical fingerprint (total proteins and polymorphic enzymes) for each *Acacia* species to distinguish its identity and their properties.

The present work was designed to elucidate some biochemical characteristics, which influence the occurrence and distribution of a considerable number of *Acacia spp.* in the diverse habitats of the Egyptian deserts and the Nile valley.

MATERIALS AND METHODS

Eight *Acacia* species were concerned in this study. They were arranged in a descending order according to their drought resistance, as indicated from reported ecological observations, as follow:

Three true xerophytic species; *A. tortilis, A. raddiana* and *A. gerarrdii*. Two moderately drought resistant species; *A. albida* and *A. nubica* Two species behave like annuals and inter in dormancy during dry periods; *A. mellifera* and *A. laeta*. One species associated with riverine affinity; *A. arabica*. Riverine species are those confined in their distribution in Egypt to the banks of the River Nile and its islands.

Seeds of the studied species were collected from naturally growing stands at El-Naqab, Southern Sinai (*A. gerarrdii and A. raddiana*), Gebel Elba in the south eastern corner of Egypt (A. *tortilis, A. albida, A. nubica, A. mellifera and A. laeta)* and the Nile Valley in upper Egypt (*A. arabica*). Fine powder of seeds was used for biochemical analyses.

Biochemical Analyses

Protein Electrophoresis

Extraction of grain protein, gel preparation and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were used according to the method outlined by Laemmli (1970) and modified by Studier (1973).

Isozymes Electrophoresis

Seed powdered samples were extracted either in cold distilled water or in Tris-borate buffer. Electrophoretic runs were performed according to Stegemann *et al.* (1985).

The staining solution of esterase isozyme

-100 mM Na-phosphate, pH 6.0	50 ml
-α-naphthyl acetate	25 mg
- Fast blue RR salt	50 mg

(Wendel and Weeden, 1990)

The staining soluation of Acid-phosphatase isozyme

- 50 mM Na-acetate buffer, pH 5.0	50 ml
- Na- α -naphthyl acid phosphate	50 mg
- MgCL2	50 mg

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-Fast Garnet GBG salt 50 mg
(Jonathan and Wendel, 1989)

Gel analysis

All gels resulted from protein and isozyme electrophoresis were scanned using Gel Doc-2001 Bio-Rad system. The densitometric scanning of the bands were performed on three directions characters. Each band is recognized by its length, width and intensity. Accordingly, relative amount of each band quantity could be measured and scored.

Glycinebetaine content

Rapid assay for determination of glycinebetaine compound was applied. This was carried out according to the method described by Grieve and Grattan (1983). The periodide method for quaternary ammonium salts was modified to permit rapid screening of plant samples.

RESULTS AND DISCUSSION

Protein electrophoretic pattern

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is widely used to fractionate the proteins according to their molecular weights. Seed protein SDS succeeded in partial discrimination of different species according to the occurrence, intensity and /or density of bands.

Electrophoretic profiles for seed total protein of the different eight *Acacia species* growing under different habitat conditions are shown in figure (1) and tabulated in table (1).

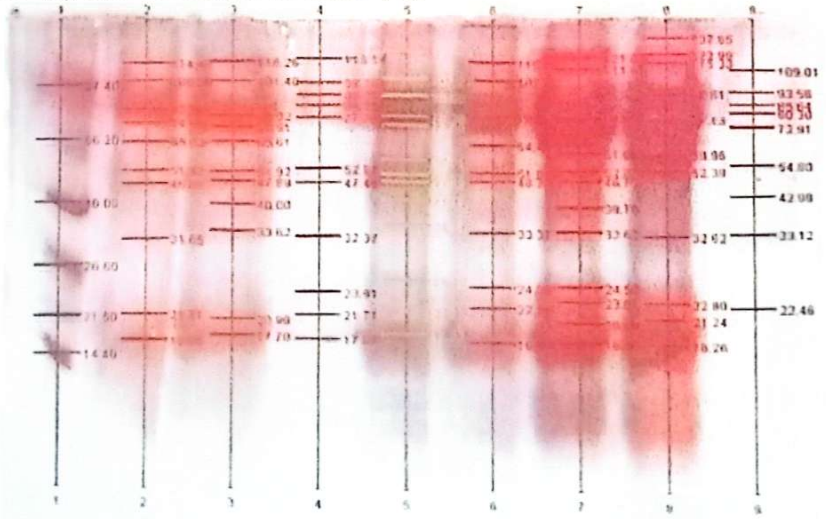

Fig. (1). SDS-PAGE profiles of the studied eight *Acacia* species.

1 Molecular marker
**2,3 and 4 A. tortilis, A. raddiana and A. gerarrdii*
**5 and 6 A. albida and A. nubica*
**7 and 8 A. mellifera and A. laeta*
** 9 A. arabica*

TABLE (1). SDS –PAGE of the studied eight *Acacia* species.

Band No.	Mid Mol. Marker	RF.	1	2	3	4	5	6	7	8	Mr. KDa
1		0.041							+		137.65
2		0.073				+		+	+		123.90
3		0.093	+	+	+						116.00
4		0.112								+	109.01
5	97.40	0.132	+	+	+						102.22
6		0.161			+	+			+	+	92.81
7		0.185	+		+					+	85.64
8		0.205					+			+	80.30
9		0.215		+	+	+	+	+	+		77.66
10	66.20	0.227	+	+							74.69
11		0.268	+	+							65.02
12		0.285						+	+		61.05
13		0.305				+				+	56.81
14		0.324	+	+	+	+	+	+	+		52.87
15		0.349	+	+	+	+	+	+			48.32
16	40.00	0.390		+		+		+		+	41.47
17		0.444	+	+	+	+	+	+	+	+	34.81
18	26.60	0.561				+					25.10
19		0.573			+		+	+			24.50
20	21.50	0.607	+	+	+	+	+	+	+	+	22.99
21	14.40	0.695	+	+	+	+	+	+	+		16.66

*1, 2, and 3 *A. tortilis, A. raddiana and A. gerarrdii*
*4 and 5 *A. albida and A. nubica*
*6 and 7 *A. mellifera and A. laeta*
* 8 *A. arabica*

Results obtained revealed that the protein (Mr) molecular weights of the studied eight *Acacia spp.* Ranged between 137.65 and 16.66 KDa and exhibited a maximum number of 21 bands which were unnecessarily present in all *Acacia* species. The results also showed that all the studied species shared the specific protein bands No. 17 and 20 with molecular weights of about 34.81 and 22.99 KDa, respectively. Therefore, these protein bands may be considered positive markers associated with *Acacia spp.* In addition, *A. tortilis, A. raddiana* and *A. gerarrdii* had two specific bands No. 3 and 5 with molecular weights of about 116.0 and 102.22 KDa, respectively. Also, *A. tortilis* and *A. raddiana* had two specific bands No. 10 and 11 with molecular weights of about 74.69 and 56.02 KDa, respectively. However,

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the presence of these specific bands were different with respect to their intensities, where they got the pattern of descending order of *A. tortilis*< *A. raddiana* < *A. gerarrdii*. From these former results, it may be concluded that the production of these stress adaptive proteins may represent a common mechanism, which enables xerophytes to withstand the harmful effects of drought. On the other hand, protein bands of molecular weights 52.87 and 16.66 KDa were observed in all studied species except for *A. arabica*. The absence of such bands in the riverine species may be taken into consideration in the evaluation of its drought resistance. Furthermore, band No. 4 with molecular weight of 109.01 KDa was observed only in *A. arabica*, this may be regarded a negative marker of drought resistance. Moreover, *A. albida, A. nubica, A. mellifera* and *A. latea* species may be considered as intermediate candidates between true xerophytic species and *A. arabica* the riverine species. In this concern, Zhang *et al.* (1998) reported that a large number of genes are induced and accumulated in response to stress conditions, many of which have known to have a role in the accommodation to stress habitats. It is apparent therefore, that drought stress induced *de novo* synthesis of stress protein suggesting that plant cells were able to monitor different levels of stress intensity and modulated gene expression. These results agreed, in part, with those obtained by Cherry (1994).

Isozyme electrophoresis

Isozyme analysis by electrophoresis offers a well-defined and effective environment for free detection of genetic differences among individuals. Two isozyme systems (esterase and acid phosphatase) were used to study variations in gene expression under different environmental conditions of the concerned eight *Acacia* species.

Esterase (Est.)

Electrophoretic patterns for the eight studied species are presented (Table 2) and illustrated (Figure 2). These patterns indicated that a total of eight bands were identified in the studied *Acacia* species, which were present in some species and absent in the others (polymorphic). Exceptional being the bands No.1, 3,5,6 and 7 (monomorphic), which were present in all species that grow in Gebel Elba and El-Naqab regions under drought stress and may be considered positive markers. Meanwhile, these bands were absent in the riverine species (*A. arabica*), which grow on River Nile banks and its islands at Upper Egypt and detected as a negative marker.

In general, the electrophoresis bands showed that all true xerophytic species gave the maximum genes expression of esterase isozymes, but differ in their intensities, in spite of their growth under different ecological conditions. Meanwhile, *A. arabica* gave a minimum genes expression of esterase isozymes, whereas, all species, which represent the intermediate

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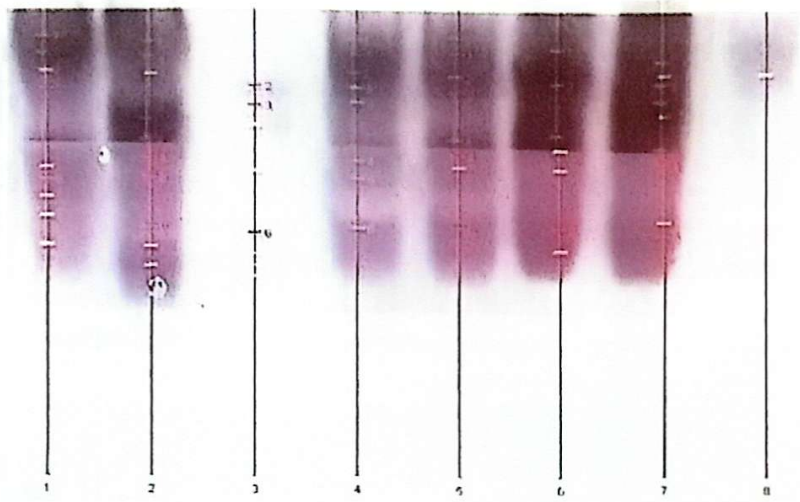
grade of drought stress exhibited gene expression falling between true xerophytes and the riverine species.

The same conclusion was reached by Al-Jibouri and Adham (1990 a and b) who found that no differences in banding patterns were detected between plants within a variety for esterase isozyme, but there were clear differences between varieties in such characteristics as number, relative mobility of isozymes and intensity of the bands.



*1, 2, and 3 A. tortilis, A. raddiana and A. gerarrdii
*4 and 5 A. albida and A. nubica
*6 and 7 A. mellifera and A. laeta
* 8 A. arabica

Fig. (2). Electrophoretic patterns of Esterase isozyme for the studied *Acacia* species.

TABLE (2). Electrophoretic patterns of Esterase isozyme for the studied *Acacia* species.

Groups of isozyme	1*	2*	3*	4*	5*	6*	7*	8*
Est. 1	+	+	+	+	+	+	+	-
Est. 2	+	+	+	-	-	-	+	+
Est. 3	+	+	+	+	+	+	+	-
Est. 4	+	+	+	+	-	-	-	-
Est. 5	+	+	+	+	+	+	+	-
Est. 6	+	+	+	+	+	+	+	-
Est. 7	+	+	+	+	+	+	-	-
Est. 8	+	+	+	-	-	+	-	-

*1, 2, and 3 *A. tortilis, A. raddiana and A. gerarrdii*
*4 and 5 *A. albida and A. nubica*
*6 and 7 *A. mellifera and A. laeta*
* 8 *A. arabica*
Est.= Esterase

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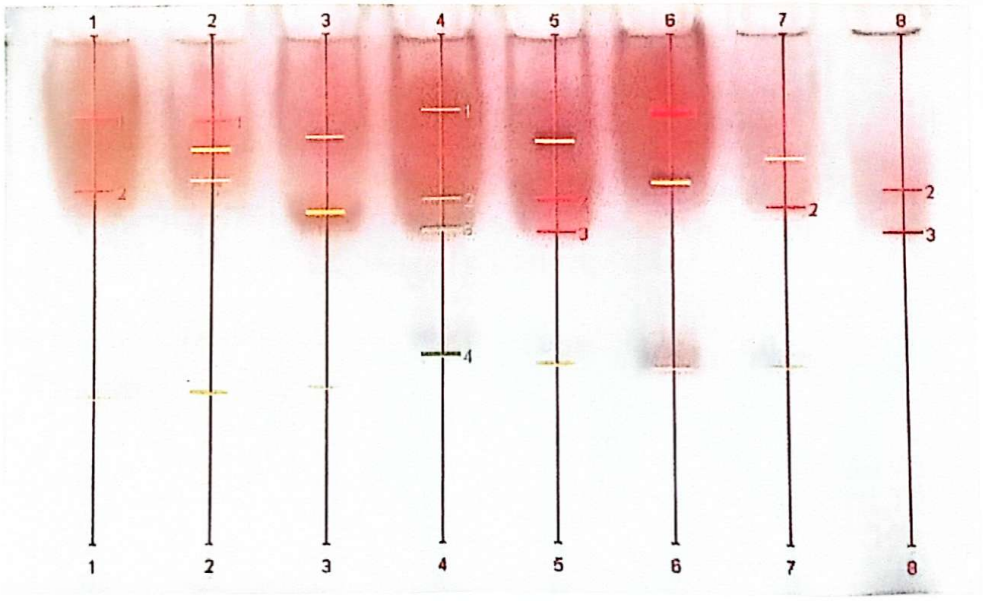
Acid phosphatase (Acp)

Electrophoretic patterns of acid phosphatase isozyme exhibited a maximum of four bands with different densities and intensities. The exhibited bands were not necessarily present in all species (Table 3 and Figure 3). These results showed that bands No.1 and 2 are present in all species (monomorphic), which grow under stress conditions, and hence they may be considered positive markers of such species, exceptional being *A. arabica* which may be regarded a negative marker of drought resistance. On the other hand, the remaining bands No. 3 and 4 are considered polymorphic ones. The four detected bands were present in the two moderately drought resistance species (*A. albida* and *A. nubica*), therefore, they might be used as marker bands for this isozyme system. In addition, band No. 3 was totally absent in the two species *A. mellifera* and *A. laeta*. This band was addressed as a negative marker for drought resistance. In other words, gene expression of acid phosphatase isozyme differ totally under different ecological conditions, where the maximum genes expression was associated with *A. albida* and *A. nubica*, while the minimum one was correlated with *A. arabica*.



**1, 2, and 3 A. tortilis, A. raddiana and A. gerarrdii*
**4 and 5 A. albida and A. nubica*
**6 and 7 A. mellifera and A. laeta*
** 8 A. arabica*

Fig. (3). Electrophoretic patterns of Acid phosphatase isozyme of the studied *Acacia* species .

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TABLE (3). Electrophoretic patterns of acid phosphatase isozyme of the studied *Acacia* species.

Groups of isozyme	1*	2*	3*	4*	5*	6*	7*	8*
Acp. 1	+	+	+	+	+	+	+	-
Acp. 2	+	+	+	+	+	+	+	-
Acp. 3	-	-	+	+	+	-	-	+
Acp. 4	+	+	-	+	+	+	+	+

1, 2, and 3 A. tortilis, A. raddiana and A. gerarrdii
4 and 5 A. albida and A. nubica
**6 and 7 A. mellifera and A. laeta*
** 8 A. arabica*
Acp. =Acid phospatase

The obtained results are in agreement with those reported by (Abdelsalam *et al.*, 1998 and Rashed *et al.*, 1998). They showed that esterase and acid phosphatase isozyme profiles were good markers for the identification and discrimination between ten barley cultivars and nineteen wheat cultivars.

Glycinebetaine content

Glycinebetaine contents averaged 36 µg/g in *Acacia gerarrdii,* whereas low values of (32 µg/g) in *A. raddiana , A. nubica* and *A. arabica* . In *A. laeta , A. mellifera* and *A. tortilis* the determined average of glycinebetaine were 33,34 and 35 µg/g , respectively. It is evident, therefore that higher glycinbetaine contents were mostly associated with higher degrees of drought resistance. It is well established that increasing the concentrations of glycinebetaine may protect enzymes from heat stress (Nash *et al.*, 1982 and Gorham, 1992).

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البصمة البيوكيميائية الوراثية لثمانية أنواع من الأكاسيا فى مصر

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تمت الدراسة على ثمانية أنواع من الأكاسيا ذات مستويات مختلفة من حيث مقاومتها للجفاف جمعت من ثلاث مناطق بيئية مختلفة وهى النقب وجبل علبة ومصر العليا. استخدمت بذور هذه الأنواع لتحليلها بيوكيميائيا حيث تم التفريد الكهربى للبروتينات الكلية والإنزيمات والجليسين بيتين لدراسة الاختلافات الوراثية البيوكيميائية لهذه الأنواع من الاكاسيا.

أوضحت النتائج المتحصل عليها من التفريد الكهربى للبروتين أن أنواع الأكاسيا تحتوى على عدد ٢١ حزمة تتراوح أوزانها الجزيئية بين ١٣٧,٧٥ – ١٦,٦٦ كيلو دالتون. وأوضحت النتائج ايضا وجود حزم بروتين خاصة ذات أوزان جزيئية هى ٣٤,٨١ – ٢,٩٩ كيلو دالتون ويمكن ان تعتبر هذه الحزم دلائل ايجابية مصاحبة لبعض انواع من الأكاسيا. كما اوضحت النتائج ان حزم البروتين ذات الوزن الجزيئى (٥٢,٨٧ – ١٦,٦٦ كيلو دالتون) قد سجلت فى جميع انواع الأكاسيا المدروسة ما عدا الانواع الواردة من مصر العليا *A. Arabica* والتى تعتبر ايضا كدلائل ايجابية لتحمل الجفاف.

وبالنظر الى إنزيمات الاستيريز والاسيد فوسفاتيز الحامضى فقد استخدمت الاختلافات الوراثية كتعبير جينى لأنواع الأكاسيا المدروسة. وفى هذا الصدد أوضحت الحزم الناتجة من التفريد الكهربى أن كل أنواع الأكاسيا التى تتحمل الجفاف أعطت أعلى تعبير جينى فى الإنزيمات ولن تختلف فى كثافتها موضحة نموها تحت الظروف البيئية المختلفة وعلى العكس فإن أنواع الأكاسيا التى تم جمعها من مصر العليا *A. Arabica* أعطت أقل تعبير جينى فى حين أن الأنواع المتحملة الجفاف *A. gerarrdii, A. tortilis* بالمقارنة بالانواع الاخرى المدروسة لذا يعتبر هذين النوعين من الأكاسيا اكثر تحملاً للجفاف.

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