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Taxonomic Significance of Seed PProteins and Iso- enzymes in *Tribulus* (Zygophyllaceae)

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ABSTRACT

Relationships among nine *Tribulus* species (Zygophyllaceae) were re-assessed by numerical analysis of comparative data on seed morphology, electrophoretic patterns of seed storage protein and iso-enzymes of esterase (EC. 3.1.1.1), perioxdase (EC. 1.11.1.7), acid phosphatase (EC. 3.1.3.2) and superoxide dismutase (EC. 1.15.1.1). The species were divided into two main groups: (i) *T. macropterus*, *T. megistopterus* and *T. mollis,* and (ii) *T. terrestris*, *T. spurius*, *T. bimucronatus* var. *inermis*, *T. pentandrus, T. parvispinus* var. *parvispinus* and *T. kaiseri.* Group ii was further subdivided into two subgroups (ii-a) consisting of *T. terrestris* and *T. spurius* and (ii-b) encompassing the rest. This arrangement was compared with previous classification based entirely on morphological characters.

Key Words: *Tribulus*; Seed morphology; Seed protein; Iso-enzymes

INTRODUCTION

Tribulus L. (Zygophyllaceae) comprises about 25 species (Boulos, 2000) in tropical and warm regions. The Egyptian flora included nine species. Oliver (1868) divided the genus *Tribulus* into two groups based on characters of ovary and stigma. Engler and Prantl (1931) put *Tribulus* in tribe Tribuleae within the subfamily Zygophylloideae. Tãckholm (1956, 1974) divided *Tribulus* species into three groups on the basis of carpel characters. Porter (1974) recommended that *Tribulus* should be moved into a separate subfamily Tribuloideae. On the other hand, El-Hadidi (1975, 1977) proposed a new family Tribulaceae based on Engler's tribe Tribulae. EL-Hadidi (1978) divided the genus *Tribulus* into three sections on the basis of persistence of calyx and mature carpel characters: Terrestris (*T. terrestris*, *T. parvispinus* & *T. spurius*), Alata (*T. macropterus, T. megistopterus* & *T. pentandrus*) and Inermis (*T. mollis*, *T. kaiseri* & *T. bimucronatus*).

Sheahan and Cutler (1993) suggested that the tribuloid genera (including *Tribulus*) should be separated from the zygophylloid genera at the subfamily level. Later, Mohammed (1997) and Ahmed (2005) supported the keep of the genus in tribe Tribuleae within the Zygophylloideae.

Electrophoretic separation of seed proteins is a powerful and efficient tool in addressing taxonomic and evolutionary relationships at both species and subspecies levels (Ladizinsky & Hymowitz, 1979). Variation in seed protein electrophoretic patterns proved useful in re-assessing the species relationships in a number of genera; *Vicia* L. (Sammour, 1989*), Sesbania* L. (Badr *et al*., 1998); *Zygophyllum* L. (EL-Ghamery *et al*., 2003) and Khafagi (2003) and *Anagallis arvensis* L. (Aboel-Atta, 2004).

Previous classifications of *Tribulus* species were based only on morphological and floral characters (Engler & Prantl, 1931; Oliver, 1868; El-Hadidi, 1975, 1978). Intensive search of several databases concerning protein patterns of plants (e.g. http: www.ebi.uniprot.org/uniprot-serv) showed that seed protein of *Tribulus* species have not as yet been analyzed. It, therefore, seemed worthwhile to benefit from this novel source of characters in re-assessing the taxonomic relationships within this genus by subjecting comparative data on seed morphology, seed storage proteins and iso-enzymes of esterase (EC. 3.1.1.1), perioxdase (EC. 1.11.1.7), acid phosphatase (EC. 3.1.3.2) and superoxide dismutase (EC. 1.15.1.1) to cluster analysis.

MATERIALS AND METHODS

Nine *Tribulus* species were collected either fresh or as herbarium material and accurately identified according to Boulos (2000). Collection data are given in Table I.

Homogenous polyacrylamid gel electrophoresis (PAGE) was conducted as the method outlined by Stegemann *et al.* (1988) for iso- enzyme analysis. The gels were stained using the formulae described by Jonathan and Wendel (1990) for esterase and acid phosphatase, Graham *et al.* (1964) for peroxidase and Siciliano and Shaw (1976) for superoxide dismutase.

Sodium dodecyl sulfate polyacrylamid gel electrophoresis (SDS-PAGE) was performed for banding of seed proteins, according to the method of Laemmli (1970) as modified by Studier (1973). Gels were photographed, scanned and analyzed using Gel doc 2000 Bio–Rad system.

All the examined specimens were used as operational taxonomic units OUT′s and a total of 33 characters morphological and electrophoretic were analyzed by means of Hierarchical Cluster analysis using similarity matrix (Romesburg, 1984). The relationships between the studied specimens of *Tribulus* were demonstrated as dendrogram (Fig. 1) by using the statistical program PRIMER (Ver. 5.0).

RESULTS AND DISCUSSION

The data obtained from the electrophoretic pattern analysis of the seed proteins, iso-enzymes and seed morphology of the nine species are presented in Tables II and III.

The morphology of mature seeds showed variations in size, shape and color of the nine species of *Tribulus*. The total number of protein bands obtained by scanning of seed protein gel was ten. The number of bands varied from one species to another and ranged between 3 to 5 bands for each species. The highest number of total protein bands (5) was recorded in *T. macropterus, T. mollis, T. spurius, T. parvispinus* var. *parvispinus* and *T. kaiseri* and the lowest number (3) was recorded in *T. terrestris* and *T. bimucronatus* var. *inermis.*

The result also showed that all the studied species shared the specific band number 8 with 28.0 kDa mol. wt. Some of the examined species had specific band such as *T. terrestris* (band no. 4 with mol. wt. 44.69 kDa), *T. spurius* (band no. 9 with mol. wt. 24.0 kDa) and *T. macropterus* (band no.10 with mol. wt. 22.85 kDa).

The total number of iso-enzyme groups obtained by scanning the gel of studied species was seven bands of

Table I. Nine species of *Tribulus* and their collection data

Species	Locality	Date
1.*T. macropterus* Boiss.	Sinai	25-6-2004
2.*T. megistopterus* Kralik	Gebl Elba	26-7-2003
3.*T. mollis* Ehrenb.	Wadi Adendan*	22-11-1928
	Sinai	25-1-1987
4.*T. spurius* Kralik	South Banha ,Ballana*	1-12-1964
	Gabl Elba	7-12-1986
5.*T. parvispinus* Presl var.*parvispinus*	Gebl Hamata, Red Sea coast	7-5-1988
6.*T. terrestris* L.	Nasr city	20-6-1992
	Nasr city	18-2-2003
7.*T. kaiseri* Hosni	Wadi El-Siq*	5-6-1927
	Sinai	14-5-1986
8.*T. pentandrus* Forssk.	Kurkur Oasis*	18-2-1964
	Nasr city*	15-2-1976
9.*T. bimucronatus* Viv. var.*inermis* (Kralik) Hosni	Gabl Elba*	1932
	Haimur*	10-3-1963

*=Herbarium specimen (CAI)

Table II. List of 33 characters recorded comparatively for *Tribulus* species

Protein	*1-90.0kDa: present (1) /absent (0)*
	2-60.76kDa: present (1) /absent (0)
	3-54.04 kDa: present (1) /absent (0)
	4-44.69 kDa: present (1) /absent (0)
	5-36.95 kDa: present (1) /absent (0)
	6-34.00 kDa: present (1) /absent (0)
	7-31.00 kDa: present (1) /absent (0)
	8-28.00 kDa: present (1) /absent (0)
	9-24.00 kDa: present (1) /absent (0)
	10- 22.85 kDa: present (1) /absent (0)
Esterase	11- Group1: present (1) /absent (0)
	12- Group2: present (1) /absent (0)
	13-Group3: present (1) /absent (0)
	14- Group4: present (1) /absent (0)
	15- Group5: present (1) /absent (0)
	16- Group6: present (1) /absent (0)
	17- Group7: present (1) /absent (0)
Peroxidase	18- Group1: present (1) /absent (0)
	19- Group2: present (1) /absent (0)
	20- Group3: present (1) /absent (0)
	21- Group1: present (1) /absent (0)
Superoxide dismutase	22- Group1: present (1) /absent (0)
	23- Group2: present (1) /absent (0)
	24- Group3: present (1) /absent (0)
	25- Group5: present (1) /absent (0)
Acid phosphatase	26- Group1: present (1) /absent (0)
	27- Group2: present (1) /absent (0)
	28- Group3: present (1) /absent (0)
Seed morphology	29- Length: 2.0-3.0 mm. (1) / 3.5-4.0 mm. (2) / 4.0-5.0 mm. (3)
	30- Shape: Elliptic(1) / long obovate (2) / obovate (3)
	31- Color: Yellowish (1) / light brown (2) / dark brown (3)
	32-Texture: Smooth (1) / rough (2)
	33-Seed/locule:1-2 (1) / 2 (2) / 3 (3)

Fig. 1. Hierarchical representation of the relationships between 9 *Tribulus* species based on the characters recorded in Table III; names of species 1-9 are given in Table I

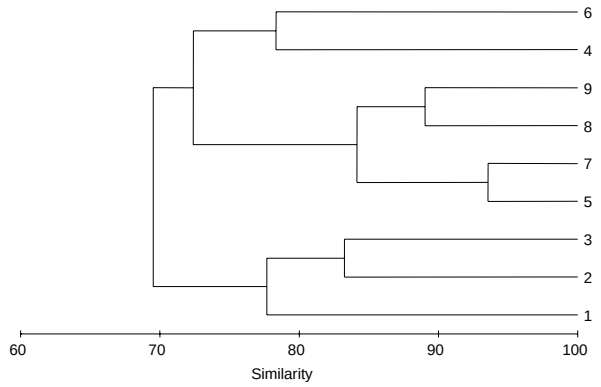

Table III. Comparative recording of the 33 characters listed in Table II for species 1-9 listed in Table I

Characters	Tribulus species								
	1	2	3	4	5	6	7	8	9
1	1	1	1	0	1	0	1	0	0
2	0	0	0	0	0	0	1	1	1
3	1	1	1	1	1	0	0	0	0
4	0	0	0	0	0	1	0	0	0
5	1	1	1	0	1	0	1	1	0
6	0	0	0	1	0	1	0	0	1
7	0	0	1	1	1	0	1	1	0
8	1	1	1	1	1	1	1	1	1
9	0	0	0	1	0	0	0	0	0
10	1	0	0	0	0	0	0	0	0
11	0	0	0	0	0	1	0	0	0
12	0	0	1	0	0	0	0	0	0
13	1	1	1	1	1	1	1	1	1
14	0	1	0	0	0	0	0	0	1
15	0	1	0	0	0	0	0	1	1
16	1	1	1	1	0	0	0	0	0
17	1	1	1	0	0	1	0	1	0
18	0	0	1	0	0	0	0	0	0
19	1	1	0	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1
21	1	1	0	0	0	0	0	0	0
22	1	1	1	0	0	0	0	0	0
23	0	0	0	0	1	1	1	1	1
24	0	0	0	0	1	1	0	0	0
25	0	0	0	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1
27	0	0	0	1	1	1	1	1	1
28	0	0	0	1	1	1	0	0	0
29	3	2	2	1	1	1	1	1	2
30	1	3	2	2	2	3	2	2	2
31	2	3	1	1	1	1	3	3	3
32	1	1	2	1	1	1	1	1	2
33	1	1	2	2	2	3	1	1	1

Table IV. Summary for comparative study between the pervious and present studies

Species	Sections according to El Hadidi, 1978	Sections according to Mohammed, 1979	Sections according to Ahmed, 2005	Present study		
1.*T. macropterus* Boiss.	Alata	Alata	Alata	Group I		
2.*T. megistopterus* Kralik	Alata	Alata	Alata			
3.*T. mollis* Ehrenb.	Inermis	Terrestris	Alata			
4.*T. spurius* Kralik	Terrestris			Subgroup A		Group I I
5.*T. parvispinus* Presl var.*parvispinus*	Terrestris	Terrestris	Terrestris			
6.*T. terrestris* L.	Terrestris			1	Subgroup B	
7.*T. kaiseri* Hosni	Inermis	Pentandrus	Kaisandrus			
8.*T. pentandrus* Forssk.	Alata	Pentandrus	Kaisandrus	2		
9.*T. bimucronatus* Viv. var.*inermis*(Kralik) Hosni	Inermis					

esterase; four bands of each of peroxidase and superoxide dismutase and three bands of acid phosphatase. Esterase group (3) was recorded in all studied species. The highest number of esterase group (5) was found in *T. megistopterus*, which gave maximum gene /gene expression of esterase iso-enzyme. Meanwhile the lowest number (1) was found only in *T. parvispinus* var. *parvispinus*, which gave minimum gene/gene expression of esterase iso-enzyme. The highest number of acid phosphatase bands (3) was found in *T. spurius, T. parvispinus* var. *parvispinus* and *T. terrestris*, which gave maximum gene/gene expression of acid phosphatase iso-enzyme. The lowest number (1) was recorded in *T. macropterus*, *T. megistopterus* and *T. mollis*, which gave minimum gene/gene expression of acid phosphatase iso-enzyme.

The dendrogram resulting from cluster analysis (Fig. 1) segregated the species of *Tribulus* into two main groups. I and II at similarity level 69.53%. Group I included 3 species (*T. macropterus*, *T. megistopterus & T. mollis*) at 77.67% similarity level, while group II contained the rest at 72.4% similarity level. Group II was divided into two subgroups A and B at about 69.53% similarity level. Subgroup A comprised *T. parvispinus* var. *parvispinus*, *T.kaiseri*, *T. pentandrus* and *T. bimucronatus* var. *inermis* at 84.15% similarity level. Subgroup B comprised *T. terrestris* and *T. spurius* at 78.33% similarity level.

The groups presented in Table IV agree with those suggested by Mohamed (1997) and Ahmed (2005) but not with those given by El-Hadidi (1978).

The electrophoresis of four iso-enzymes in the studied species of *Tribulus* showed that esterase and superoxide dismutase could be considered as positive markers, while perixodase and acid phosphatase were detected as negative marker.

The morphology of mature seeds showed some variations in size, shape and color. It was found that the use of the electrophoretic pattern was able to distinguish between even the closely related species.

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