Egypt. J. Bot., **Vol. 55**, No, **2**. pp. 207-230 (2015)

Pollen Morphology and Protein Pattern of *Nitraria retusa* and Some Selected Taxa of Zygophyllaceae in Egypt

H. EL-Atroush[*], A.E. EL-Shabasy[**], M.A. Tantawy[*] and H.M.S. Barakat[*]

[*]*Botany Department., Faculty of Science, Ain Shams University and* [**]*Botany Department, Faculty of Education, October University, A.R.E.*

POLLEN morphology and pattern of *Nitraria retusa* (Forssk.) Asch. were studied and compared against eight selected taxa of Zygophyllaceae *viz*. *Fagonia arabica*, *F. cretica*, *Peganum harmala*, *Tribulus terrestis*, *Zygophyllum album*, *Z. coccenium*, *Z. decumbens* and *Z. simplex* using Scanning Electron Microscope (SEM) and sodium dodecyle sulphate-polyacrylamide gel electrophoresis technique (SDS-PAGE). A wide range of measurements were obtained from digitized SEM images of whole pollen grains and exine pattern. Twenty nine protein bands were obtained with 100% polymorphism among the species examined. *F. arabica* and *T. terrestis* were characterized by one positive unique band with a molecular weight 56.8 and 24 kDa respectively. *P. harmula* was characterized by two positive unique bands with a molecular weight 62 and 42 kDa. *N. retusa* was characterized by three positive unique bands with amolecular weigh 64.6, 60.7 and 36 kDa. A dendrogram was constructed based on the similarity data matrix by unweighted pair group method using arithmetic averages cluster analysis. SEM and protein pattern analysis recommended the separation of *Nitraria retusa* (Forssk.) Asch. besides *Fagonia* sp. into a distinct family.

Zygophyllaceae is a small family with 27 genera and 250 species, distributed in the arid and semi-arid regions of the tropics. Plants included are small trees, shrubs or sub-shrubs (Sheahan & Cutler, 1993 and Ghazanfar, 1994). *N. retusa* (Forssk) belongs to the family Nitrariaceae where it is the only genus of this family (Täckholm, 1974 and Boulos, 2000) or to the family Zygophyllaceae (Zohary, 1972). Zygophyllaceae belongs to Geraniales and is divided into seven subfamilies Engler, (1931). Ronse Decraene and Smets (1991), Ronse Decraene *et al*., (1996) separated *Nitraria retusa* from Zygophyllaceae based on the study of floral development and vascular anatomy (Hussein *et al*., 2009). More investigation of the morphology, palynology, cytology and biochemistry have shown the family to be very heterogeneous (Sheahan and Cutler, 1993). Zygophyllaceae in the classification of Sheahan and Chase (1996) is subdivided into five subfamilies and about 27 genera and they recommended recognizing *Nitraria* and *Peganum* as two distinct families, belonging to the order Sapindales and not being closely related to Zygophyllaceae based on morphological and anatomical data. According to Hutchinson (1967), Goldberg (1986) and Zohary

(1987) Zygophyllaceae was treated as a homogenous family including the Nitrariaceae, while the latter was treated by several authors as a distinct family (Täckholm, 1974; Sheahan and Cutler, 1993; Bolous, 2000 and Dahlgren, 1980; 1983 and 1989).

Taxonomists and paleobotanists have recognized the importance of pollen development and morphology in the identification and classification of plants (Martens and Fetz, 1980; Raj, 1983; Blackmore, 1981 and Doyle & Walker, 1975). The study of pollen grains and spores morphology has provided a great wealth of phylogenetically useful information which played an important role in problems of botanical identification especially angiosperm systematic and phylogeny (Van Campo, 1966; 1967 and Doyle and Walker, 1975).

Proteins are the primary products in the realization of hereditary information and reflect the genetic structure of the organism the most precisely (Konarev, 1983). One of the methods for detecting the molecular heterogeneity is SDS-PAGE, which is the most frequently employed techniques for separating and identifying the proteins according to their molecular weights (Haidar *et al*., 2013; Moradpour *et al*., 2014 and Savithiry, 2014). 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 and Badr *et al*., 1995 & 2000). Variation in seed protein electrophoretic patterns proved useful in re-assessing the species relationships in a number of genera; *Zygophyllum* (EL-Ghamery *et al*., & Khafagi, 2003) and *Tribulus* (Amal, 2006).

The aim of present study was to reveal the characterization and relationships among nine species of Zygophyllaceae *viz*. *N. retusa*, *F. arabica*, *F. cretica*, *P. harmala*, *T. terrestis*, *Z. album*, *Z. coccenium*, *Z. decumbens* and *Z. simplex* by using SEM and SDS-PAGE.

Material and Methods

A- Pollen morphology

Pollen grains of the selected plant species were collected from different regions according to Table 1 and identified according Täckholm (1974) and Boulos (1999).

Ideally, to obtain the maximum amount of systematic data from SEM studies of pollen, non acetolyzed materials were studied. Valuable systematic characters may be lost in acetolysis and true pollen shape may be greatly changed. The non-acetolysis technique was recommended owing to some of the pollen characters may be affected during the acetolysis technique such as pollenkitt (Hess, 1981 a; b) exineless pollen (Kress and Stone, 1982; 1983) and aperture morphology (Thanikaimoni, 1986). Study of fresh (non-acetolyzed pollen) can give greater

insight into the functional significance of pollen characters which, in turn, can contribute to better systematic treatment (Tantawy *et al*., 2005).

Preparation of non-acetolyzed materials

mature pollen grains at anthesis were investigated using SEM (Skvarla, 1966). For SEM investigation conventionally air or critical point dried material was sputter coated (Hess, 1986). The terminology used in the description of pollen grains is generally based on that of Erdtman (1952), Faegri (1956), Kremp (1968) and Punt *et al*. (1994).

TABLE 1. Collection data.

No.	Species	Locality
1	*Fagonia arabica*	Cairo-Suez road
2	*F. cretica*	Burg El-Arab City-Alexandria
3	*Nitraria retusa*	Pharaoh's Basins-Sinai
4	*Peganum harmala*	Burg El-Arab City-Alexandria
5	*Tribulus terrestis*	October 6 City-Giza
6	*Zygophyllum album*	Rasheed City-Bahera
7	*Z. coccenium*	Cairo-Suez Road
8	*Z. decumbens*	Hagole Valley- Cairo-Suez Road
9	*Z. simplex*	Cairo-Suez Road

B- SDS-PAGE of soluble seed storage protein

Characterization and molecular mass determination of soluble seed storage proteins was carried out using one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Polyacrylamide slab gel (12%) was prepared according to Laemmli (1970).

Extraction of protein

Samples were prepared for electrophoresis by grinding seeds to fine powder with pestle and mortar. Ten mg of the resulting powder was transferred to centrifuge tube, supplemented with 400 µl extraction buffer and placed into a refrigerator for one hr. The extract was then centrifuged at 13000 rpm for 10 min. at room temperature. The supernatant was transferred to new tube and used directly for electrophoresis or kept in a deep freezer until use within few days.

Polyacrylamide gel preparation

SDS polyacryamide gel slabs were prepared in the volumes given in Table 2.

TABLE 2. Volume of solutions and buffers used for preparing 12% SDS polyacrylamide gel.

Stock solution	Separating gel	Stacking gel
Acrylamide stock solution	10 ml	1.33 ml
Separating gel buffer (1.5 mM Tris/HCl pH 8.8)	7.5 ml	—
Stacking gel buffer (0.5 mM Tris/HCl pH 6.8)	—	2.5 ml
10% SDS	0.3 ml	0.1 ml
Distilled water	12 ml	6.1 ml
10% ammonium persulphate	150 µl	5 µl
TEMED (N, N, N`, N`-Tetramethyl Ethylene diamine)	10 µl	5 µl
Final volume	30 ml	10 ml

Electrophoresis of protein

Protein samples were prepared by mixing 10 µl of clear protein extract with the same amount of 2X extract buffer (2.5 ml stacking gel buffer, 2 ml glycerol or sucrose, 4 ml 10% SDS and 1 ml β-mercaptoethanol made up to 10 ml distilled water) and denatured by heating at 100°C for 2-3 min. The samples were left to cool and 3 µl of Bromophenol blue dye were added. Electrophoresis was carried out in gel running buffer (15.15 g Tris base, 0.5 g SDS, 7.2 g glycine in 500 ml distilled water) using a vertical gel electrophoresis unit (Model SE-400) trapped gas bubbles were purged away. Slab gel was prepared by pouring the separating gel between clamped glass plates, leave it for at least half hour to polymerize. After polymerization of the main gel, stacking gel solution was poured as an overlay to the separating gel to improve the tightness of protein banding. The gel was attached to the vertical electrophoresis tank system and equal amount of the samples (12 µl) was loaded through electrode buffer into wells in the stacking gel layer. The upper tray was filled with the same buffer. Molecular weight marker (5 µl) composed of 18- 66 KDa proteins (Fermentas protein ladder) were also loaded. Electrophoresis was carried out at 140 V for the first 15 min. followed by 150 V until the indicator dye reached the bottom of the gel. Gels were stained overnight in 20 ml Coomassie blue R 250 and de-stained by shaking overnight in de-staining solution (500 ml methanol and 100 ml acetic acid made up to 1 Liter with dist. water). Gels were photographed, scanned and analyzed using Gel doc 2000 Bio–Rad system.

Results

A- Palynological characters

The main palynological characters of the studied species are listed in Table 3 and 4. Pollen grains were monad, isopolar and radially symmetrical. They showed diversity in shape, size, aperture type and exine characters.

Pollen shap: the shape of pollen grains varied from perprolate, prolate and sub-prolate (Plate 1-4).
Pollen size: the studied pollen grains were either minute or small grains.
The amb shape: it was elliptic or circular to elliptic.
Apertures: tricircumcolporate except *T. terrestis* which appeared as monocryptoporous.
Aperture length and width: ranged from 4.56 to 12.7µm and from 0.05 to 5.8 µm respectively.
Aperture surface level: either opening furrow, deeply slit or sunken.
Aperture memberane: granulated or not.
Exine sculpture: reticulate in all species except in *N. retusa* his striate.
Metareticulum: present or not.
Brochus type: homobrochate or heterobrochate.
Pollen kitt: either conspicuous, weak conspicuous or non-conspicuous.
The polar axis (P): the mean length of the polar axis (P) was ranged from 5.74µm in *Z. simplex* to 19.73µm in *T. terrestis*.
The equatorial axis (E): the mean equatorial diameter was ranged from 3.31µm in *Z. simplex* to 16.9µm in *T. terrestis*.
P/E axis type: ranged from longiaxe (P/E >1.8) and semi-equiaxe (P/E<1.8).
Apocolpium index: ranged from 0.33 µm in *F. cretica* and 2.02 µm in *Z. simplex* and absent in *T. terrestis.*
Mesocolpium: ranged from 0.65 in *Z. decambens* and 6.2 µm in *N. retusa* respectively and absent in *T. terrestis*.
The muri: granulated or not, appeared as angustimurate, latimurate or lira.
The muri size (thickness): ranged from 0.08 µm in *Z. simplex* to 2.11µm in *T. terrestis* but absent in *N. retusa*.
The sculptural density per unit area: present in *N. retusa*, ranged from 1 in *T. terrestis* to 29 in *Z. simplex*.
Lumina: generally irregular or circular in outline.
Lumina width (area): ranged from 0.13 µm in *Z. decambens* to 0.73 µm in *T. terrestis* but was absent in *N. retusa*.

B- Seed protein panding profile

Seed protein banding profile is illustrated in Fig. 1. The total number of bands was 29 bands. The molecular weight of these bands ranged between 20 to 90.4 kDa. The bands detected in the nine studied species expressed as present (1) or absent (0) are given in Table 4. The highest number of bands was 14, detected in *P. harmala*, while the lowest number of bands was five detected in *F. cretica*. All the bands were polymorphic giving a 100% polymorphism among the species examined. The protein assay permitted the identification of only four species by unique positive markers. It shows that *F. arabica* and *T. terrestis* were characterized by one positive unique protein band with molecular weight of 56.8 and 24 kDa respectively, while three positive unique bands with molecular weight 64.6, 60.7 and 36 kDa associated *N. retusa*. On the other hand, species *P. harmala* was characterized by two positive unique bands with a molecular weight of 62 and 42 kDa.

TABLE 3. Tabular summary showing the pollen grain measurements in micrometer (µm) for the studied species. (means value followed by range in parentheses)

Species / Characters	1	2	3	4	5	6	7	8	9
Polar axis (µm)	19.09 (18.2-20.7)	15.78 (14-17)	12.75 (10.8-14.4)	14.31 (13.21-15.71)	19.73 (17.62-20.48)	7 (6.71-7.57)	7.13 (6.5-7.8)	7.81 (7.29-8.71)	5.74 (5.2-6.5)
Equatorial axis (µm)	8.16 (7.14-9.6)	9.45 (8.8-10.6)	9.8 (9-10.8)	7.62 (6.79-8.57)	16.9 (16.19-17.67)	3.46 (3-3.86)	3.76 (3.2-4.3)	3.7 (3.43-4)	3.31 (2.7-3.6)
P/E	2.34	1.67	1.3	1.88	1.17	2.02	1.9	2.11	1.73
Aperture length (µm)	11.06 (9-12.86)	11.3 (9-15)	12.7 (12-13.7)	12.26 (10-13.57)	6.5	7.11 (6.86-7.71)	6.93 (6.5-7.24)	6.64 (6.14-7.29)	4.56 (3.81-5)
Aperture width (µm)	0.9 (0.36-1.6)	0.51 (0.4-0.67)	0.05	0.1	5.8	0.05	0.05	0.1	0.39 (0.3-0.48)
Apocolpium index	4.24(3.2-5.71)/8.16 =0.47	3.15(2.2-4.1)/3.15 =0.33	4.63(4.2-5)/9.8 =0.47	2.5(2.36-2.64)/7.62 =0.33	None	1.57(1.5-1.71)/3.46 =0.45	1.41(1.05-2.24)/3.76 =0.38	2.42(1.9-2.86)/3.7 =0.65	1.14(1-1.36)/3.31 =0.34
Mesocolpium (µm)	5.38 (5-5.8)	6.07 (4.8-6.8)	6.2 (6-6.4)	5.03 (4.4-5.71)	None	2.33 (2-2.57)	2.4 (2.2-2.7)	2.14 (1.86-2.57)	2.02 (1.8-2.2)
Muri size (µm)	0.29 (0.2-0.4)	0.27 (0.18-0.44)	None	0.14 (0.08-0.24)	2.11 (1.67-2.67)	0.1 (0.06-0.2)	0.13 (0.06-0.27)	0.11 (0.06-0.21)	0.08 (0.06-0.1)
Lumina width (µm)	0.25 (0.18-0.46)	0.27 (0.18-0.56)	None	0.26 (0.18-0.36)	0.73 (0.67-0.81)	0.16 (0.13-0.2)	0.19 (0.13-0.26)	0.13 (0.07-0.17)	0.14 (0.06-0.31)
Lumina/muri ratio	0.1	1	None	1.86	0.38	1.6	1.46	1.19	1.75
Sculptural density (meshes/ µm^2)	4	4	None	6	1	16	16	22	29

1: *Fagonia arabica*, 2: *Fagonia cretica*, 3: *Nitraria retusa*,
4: *Peganum harmala*, 5: *Tribulus terrestis*, 6: *Zygophyllum album*,
7: *Zygophyllum coccenium*, 8: *Zygophyllum decumbens* 9: *Zygophyllum simplex*.

TABLE 4. Tabular summary showing the pollen grain characters for the studied species.

		1	2	3	4	5	6	7	8	9
Polar axis	>12µm	+	+	+	+	+	-	-	-	-
	<12µm	-	-	-	-	-	+	+	+	+
Equatorial axis	>7µm	+	+	+	+	+	-	-	-	-
	<7µm	-	-	-	-	-	+	+	+	+
P/E axis type	longiaxe(>1.8µm)	+	-	-	+	-	+	+	+	-
	semi-equiaxe(<1.8µm)	-	+	+	-	+	-	-	-	+
Shape classes	perprolate	+	-	-	-	-	+	-	+	-
	prolate	-	+	+	+	-	-	+	-	+
	subprolate	-	-	-	-	+	-	-	-	-
Amb shape	elliptic	+	+	-	+	-	+	+	+	-
	circular to elliptic	-	-	+	-	+	-	-	-	+
Size classes	minute grains	-	-	-	-	-	+	+	+	+
	small grains	+	+	+	+	+	-	-	-	-
Pollenkitt	conspicuous	-	+	-	-	-	-	-	-	-
	non-conspicuous	+	-	+	+	+	+	+	-	+
	weak conspicuous	-	-	-	-	-	-	-	+	-
Aperture type	colpus	+	+	+	+	-	+	+	+	+
	porous	-	-	-	-	+	-	-	-	-
Aperture number	mono	-	-	-	-	+	-	-	-	-
	tri	+	+	+	+	-	+	+	+	+
Aperture site	circumaperture	+	+	+	+	-	+	+	+	+
	cryptoaperture	-	-	-	-	+	-	-	-	-
Aperture membrane		+	+	+	-	-	-	-	-	-
Aperture surface level	opening furrow	+	+	-	-	-	-	-	-	+
	deeply slit	-	-	+	+	-	+	+	+	-
	sunken pore	-	-	-	-	+	-	-	-	-
Colpus length	>7µm	+	+	+	+	-	+	-	-	-
	<7µm	-	-	-	-	-	-	+	+	+
Porous length		-	-	-	-	+	-	-	-	-
Colpus width	>0.1µm	+	+	-	-	-	-	-	-	+
	<0.1µm	-	-	+	+	-	+	+	+	-
Porous width		-	-	-	-	+	-	-	-	-
Apocolpium index	>0.4µm	+	-	+	-	-	+	-	+	-
	<0.4µm	-	+	-	+	-	-	+	-	+
Mesocolpium	>2.5µm	+	+	+	+	-	-	-	-	-
	<2.5µm	-	-	-	-	-	+	+	+	+
Exine sculpture	reticulum	+	+	-	+	+	+	+	+	+
	striate	-	-	+	-	-	-	-	-	-
Reticulum type	metareticulum	+	-	-	-	-	-	-	-	-
	non-metareticulum	-	+	-	+	+	+	+	+	+

TABLE 4. continued

		1	2	3	4	5	6	7	8	9
Muri size	faint <0.1µm	-	-	-	-	-	-	-	-	+
	Micro 0.1-0.2µm	-	-	-	+	-	+	+	+	-
	Normal 0.2-0.3µm	+	+	-	-	+	-	-	-	-
Muri type	granulated	+	+	-	+	-	+	-	+	-
	non-granulated	-	-	-	-	+	-	+	-	+
	angustimurate	+	+	-	+	-	+	+	+	+
	latimurate	-	-	-	-	+	-	-	-	-
	lira	-	-	+	-	-	-	-	-	-
Lumina width	0.1-0.2µm	-	-	-	-	-	+	+	+	+
	0.2-0.3µm	+	+	-	+	-	-	-	-	-
	>0.3µm	-	-	-	-	+	-	-	-	-
Lumina/muri ratio	<0.4	+	-	-	-	+	-	-	-	-
	0.4-1.5	-	+	-	-	-	-	+	+	-
	>1.5	-	-	-	+	-	+	-	-	+
Sculptural density	<4 meshes	-	-	-	-	+	-	-	-	-
	4-7 meshes	+	+	-	+	-	-	-	-	-
	8-16 meshes	-	-	-	-	-	+	+	-	-
	>17 meshes	-	-	-	-	-	-	-	+	+
Brochus type	hetero	+	+	-	+	+	+	+	+	-
	homo	-	-	-	-	-	-	-	-	+

1: *Fagonia arabica*, 2: *Fagonia cretica*, 3: *Nitraria retusa*,
4: *Peganum harmala*, 5: *Tribulus terrestis*, 6: *Zygophyllum album*,
7: *Zygophyllum coccenium*, 8: *Zygophyllum decumbens* 9: *Zygophyllum simplex*, + present, - absent.

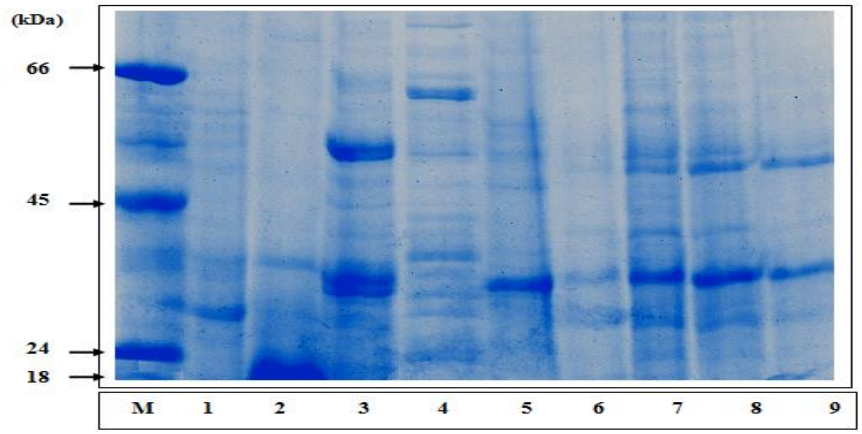


Fig. 1. Electrophoretic total soluble protein pattern of studies species M: marker,
1: *Fagonia arabica*, 2: *F. cretica*, 3: *Nitraria retusa*,
4: *Peganum harmala*, 5: *Tribulus terrestis*, 6: *Zygophyllum album*,
7: *Z. coccenium*, 8: *Z. decumbens* 9: *Z. simplex*.

Genetic relationships among the studied species as revealed by protein analysis

According to similarity matrix of protein analysis, the highest similarity value (0.93) was recorded between *Z. coccenium* and *Z. decumbens* indicating that these two species were closely related to each other. On the other hand, the lowest similarity value (0.38) was recorded between *P. harmala* and *Z. decumbens* indicating that these were distantly related species Table 5.

Cluster analysis

The out put of SAHN-clustering program was presented in the form of a phenogram by using the tree display graphic (TREE). The resulting dendrogram revealed by pollen morphology (Fig. 2) showed that the studied species have an average taxonomic distance of about 1.53. At this level *T. terrestis* is separated from other species. The remaining species are differentiated into two main lines at a distance of about 1.332. At group (1) *N. retusa* is split off at 1.178 level. At a distance close to 0.948, the remaining species; *F. arabica* and *F. cretica* are delimited as a sub-group which *P. harmala* is split off. On the other hand, at a distance close to 1.156, the remaining species; *Z. album*, *Z. cocenium and Z. decumbens* are delimited as a sub-group which *Z. simplex* is split off.

TABLE 5. Genetic similarity matrix of studied species for protein analysis.

	F. arabica	***F. cretica***	***N. retusa***	***P. harmala***	***T. terrestis***	***Z. album***	***Z. coccenium***	***Z. decumbens***	***Z. simplex***
F. arabica	1.00								
F. cretica	0.72	1.00							
N. retusa	0.45	0.45	1.00						
P. harmala	0.48	0.48	0.48	1.00					
T. terrestis	0.48	0.55	0.55	0.66	1.00				
Z. album	0.70	0.62	0.62	0.52	0.66	1.00			
Z. coccenium	0.66	0.52	0.45	0.41	0.55	0.76	1.00		
Z. decumbens	0.62	0.55	0.41	0.38	0.52	0.72	0.93	1.00	
Z. simplex	0.83	0.70	0.55	0.52	0.66	0.79	0.83	0.79	1.00

The resulting dendrogram, revealed by protein analysis (Fig. 3) shows that the studied species are presented as two main clusters at an average distance of 0.37. The first cluster includes *F. cretica* represented as a delimited species at 0.396. In the rest of cluster *F. arabica* at 0.6300 represented also as a separated species while the four *Zygophyllum* species are differentiated at 0.7515 into two sub cluster. The first sub-cluster includes *Z. album* and the second one includes the rest of *Zygophyllum* sp. at 0.9675. The second cluster is differentiated at 0.4350 into *N. retusa* and the rest of species; *P. harmala* and *T. terrestis* are represented as sub cluster at 0.6705.

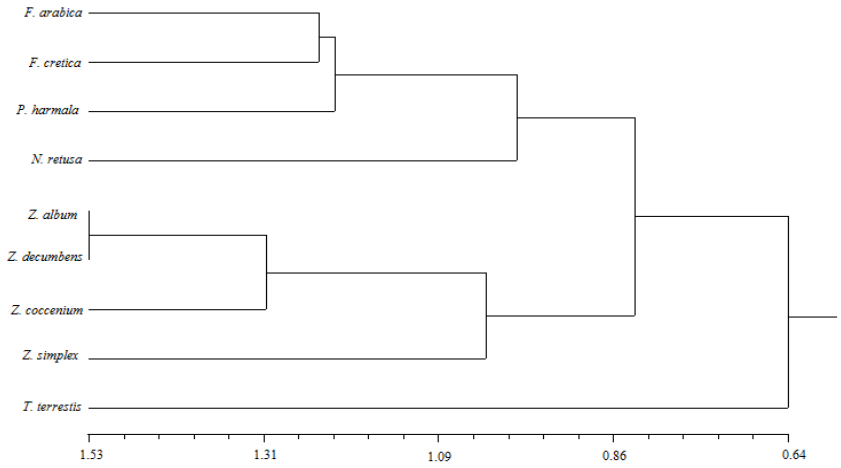


Fig. 2. Dendrogram of the studied species based on pollen grain characters.

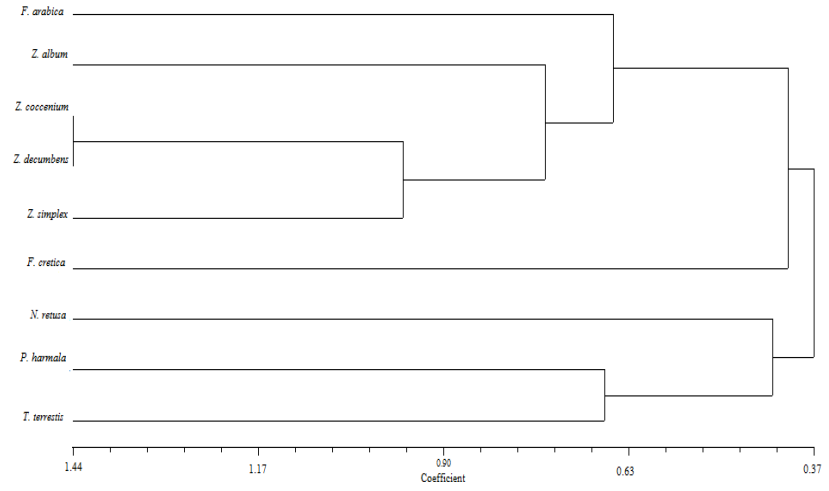


Fig. 3. Dendrogram studied species based on pollen.

Discussion

The above results showed that *T. terrestis* is separated completely from the studied taxa belonging Zygophyllaceae family. This may be due to changes in pollen morphology which is affected by environmental stresses and human impact (October 6 City). In this respect, Sailaja *et al*., 2005 who stated that pollen can be shrivelled under the temperature and radiation treatment compared with the pollen from plants grown under control conditions. Similarity, the pollen morphological aberrations observed at high temperature stress (Cross *et al*., 2003) and water deficit stress (Shen and Webster, 1986) might have resulted in abnormal exine with deeply pitted and smooth regions. It is generally considered that for taxonomic purposes the best results are achieved by combining evidence from as many different fields or levels of organization as possible such as morphological, biochemchal and molecular markers. Then, taking into account that the analysis of pollen can offer another tool for the taxonomic characterization of species. Stanley and Linskens (1974) concluded that pollen exine pattern is so genetically stable for the different plant species that it can be used for species identification in recent and in fossil pollens.

The composition of seed proteins is highly stable and affected only slightly by environmental conditions or seasonal fluctuations (Ladizinsky & Hymowitz, 1979). Seed proteins are mainly storage proteins and not likely to be changed in dry mature seed. The use of seed protein electrophoresis is supported by the fact that mature seeds possess the same protein components and this provide valid evidence for relationships of plants (Emre *et al*., 2006; Emre, 2009; Vural *et al*., 2009, Khafagi, 2012, Pragati & Sreenath, 2013 and Anitalakshmi *et al*., 2014).

In the present investigation, no common protein bands were observed between the nine studied taxa while, 29 polymorphic bands were recorded showing 100% polymorphism. The highest band number (14) was scored in the banding profile of *P. harmala*, while the lowest number (5) was found in *Fagonia cretica* indicating high genetic variation between the studied taxa. On the other hand, some species-specific protein bands were recorded characterized some species *e.g*. positive unique bands with molecular weight of 64.6, 60.7 and 36.0 KDa are specific to *N. retusa*. The UPGMA protein dendrogram separated *N. retusa*, *P. harmala* and *T. terrestis* in single cluster indicating that protein analysis confirmed the above results obtained and it is useful markers for the identification at the species level. Similar results obtained with Youssef (1990) who studied the genetic relations among eleven species of genus *Vicia* and four varieties of *Vicia faba* using SDS-PAGE. The results should some similarities among the examined varieties of *V. faba* and each of the different species showed distinguishable protein profiles. Some species- specific protein bands were recorded in certain studied. Pragati & Sreenath (2013) showed that seed protein profile among nine species of *Ipomoea* revealed some bands that are characteristic and constant markers for each species.

In this study, the UPGMA dendograms obtained from pollen grain or protein agreed more or less with combined dendrogram resulted from RAPD and ISSR analysis on the same nine studied species (EL-Atroush *et al*., 2014). The results of this study is agreement with Hussein *et al*., (2009) who found that the macro- and micro-morphological as well as phytochemical criteria encourage the separation of *N. retusa* from Zygophyllaceae.

Conclusion

The present data which is achieved by combining taxonomic tools from pollen grain, molecular (RAPD and ISSR) and total seed protein analysis agreed with Hussein *et al.* (2009) and Kadry (2012) who separated each *Nitraria retusa* and *Peganum harmala* into a distinct family respectively besides Sheahan and Chase (1996 & 2000) separated *Nitraria* and *Peganum* from Zygophyllaceae.

It agreed also with Sheahan and Cutler (1993) and Khalkuziev (1990) who separated *Tribulus* from Zygophyllaceae and placed in Tribulaceae. The current study recommend to separate the studied species from Zygophyllaceae family except *Zygophyllum* sp.

Fagonia sp. and *Peganum harmala* are separated together in a family called Peganiaceae preceding Zygophyllaceae in evolutionary trends.

Nitraria retusa is separated in a family called Nitrariaceae which can be placed between Peganiaceae and Zygophyllaceae.

Finally, *Tribulus* sp. is placed in a family called Tribulaceae which can be placed after Zygophyllaceae.

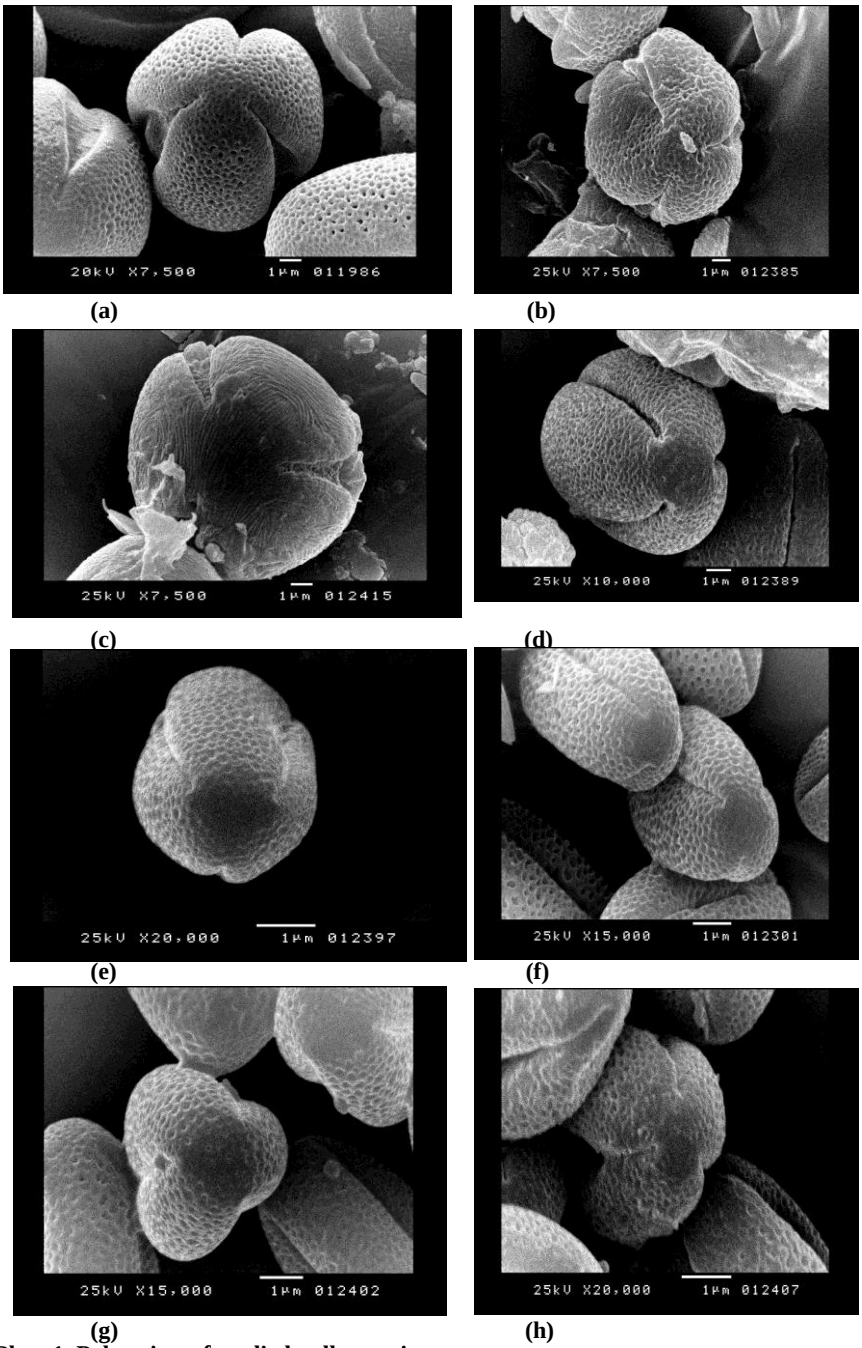


Plate 1. Polar view of studied pollen grains
a- ***F. arabica,*** **b-** ***F. cretica,*** **c-** ***N. retusa*** **d-** ***P. harmala***
e- ***Z. album,*** **f-** ***Z. coccenium,*** **g-** ***Z. decumbens*** **h-** ***Z. simplex***

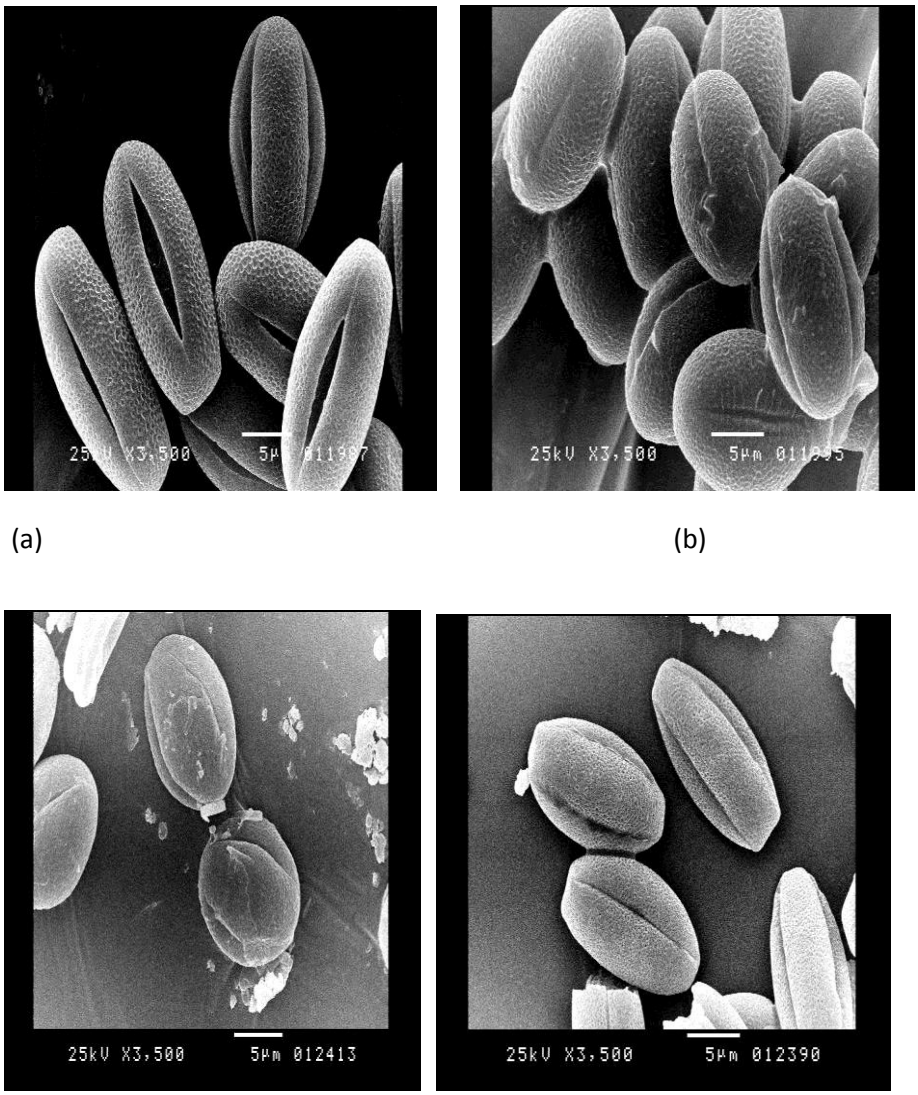
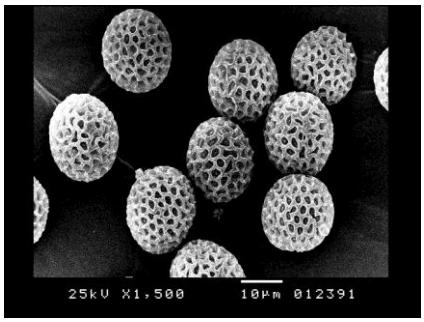
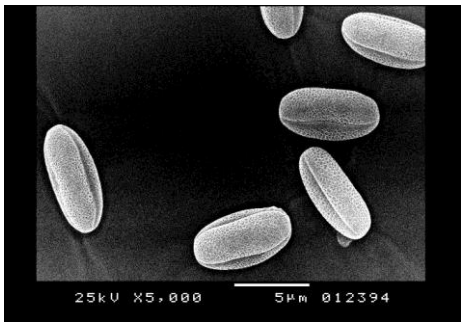


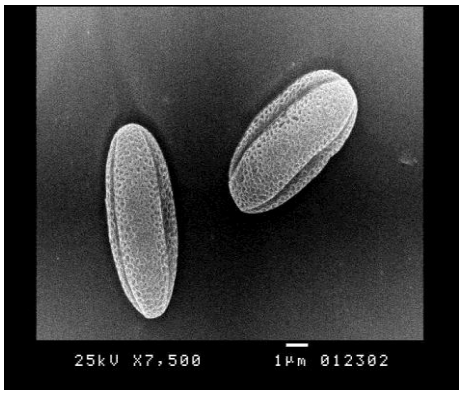
Plate 2. Equatorial view of studied pollen grains (a- *F. arabica*, b- *F. cretica*, c- *N. retusa* and d- *P. harmala*).



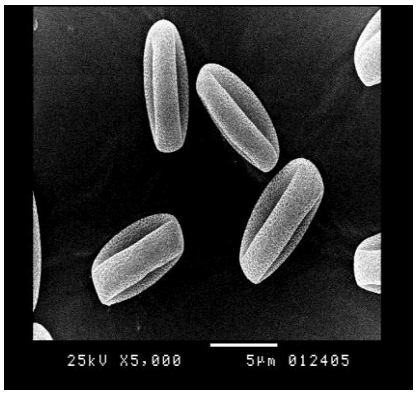
(e)



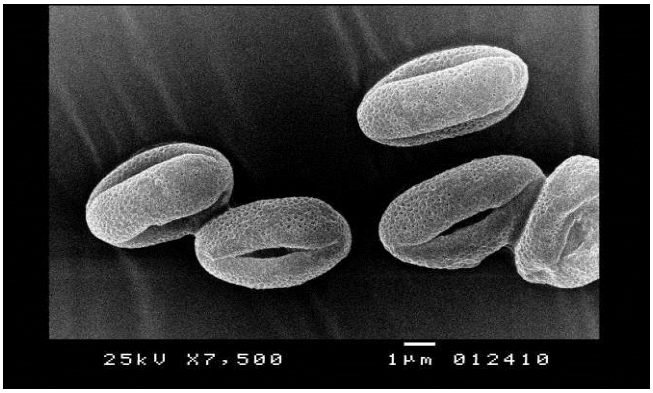
(f)



(g)

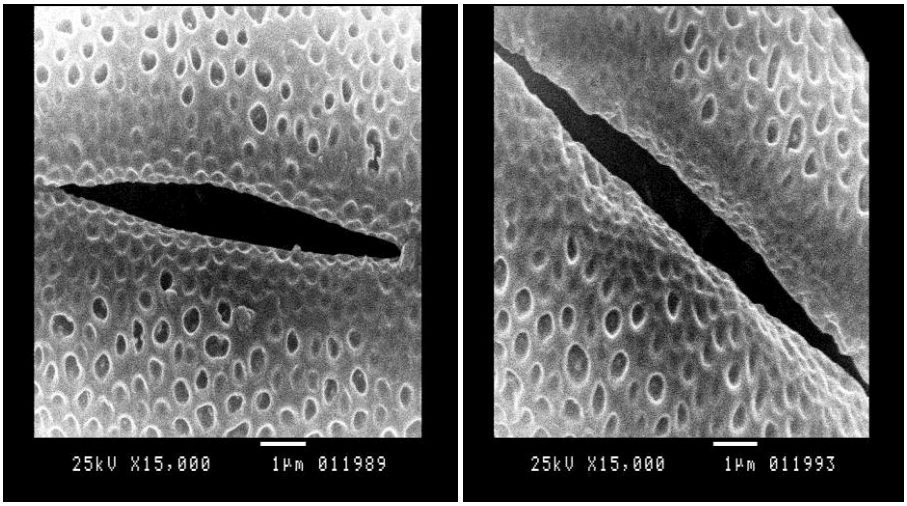


(h)

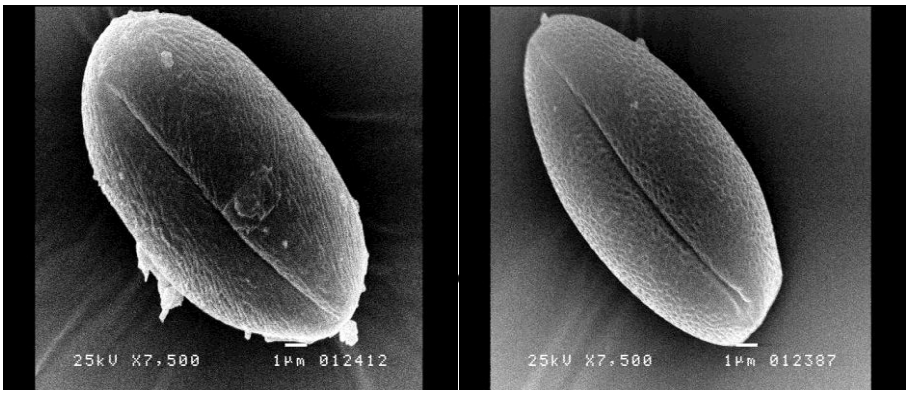


(i)

Plate 2. Cont. Equatorial view of studied pollen grains (e- *T. terrestis*, f- *Z. album*, g- *Z. coccenium*, h- *Z. decumbens* and i- *Z. simplex*).

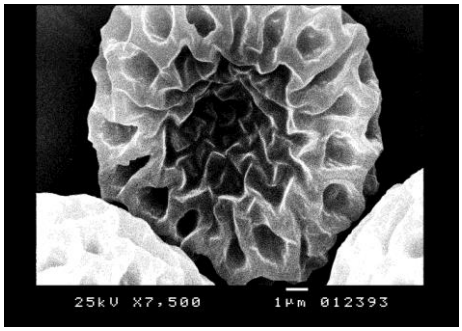


(a) **(b)**

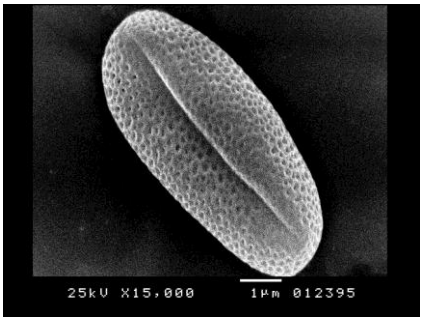


(c) **(d)**

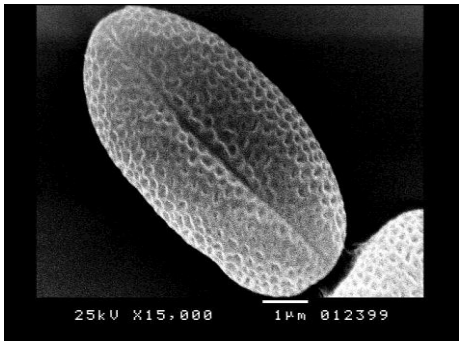
Plate 3. Aperture of studied pollen grains (a- *F. arabica*, b- *F. cretica*, c- *N. retusa* and d- *P. harmala*).



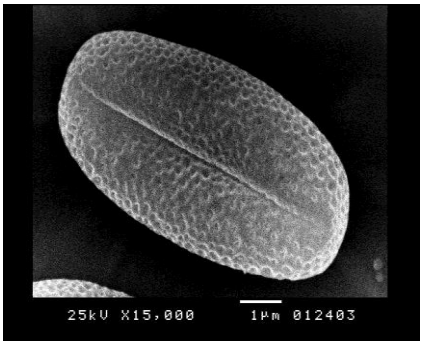
(e)



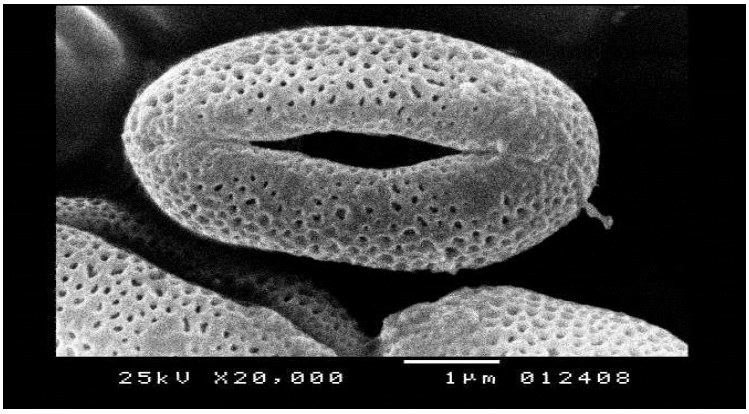
(f)



(g)

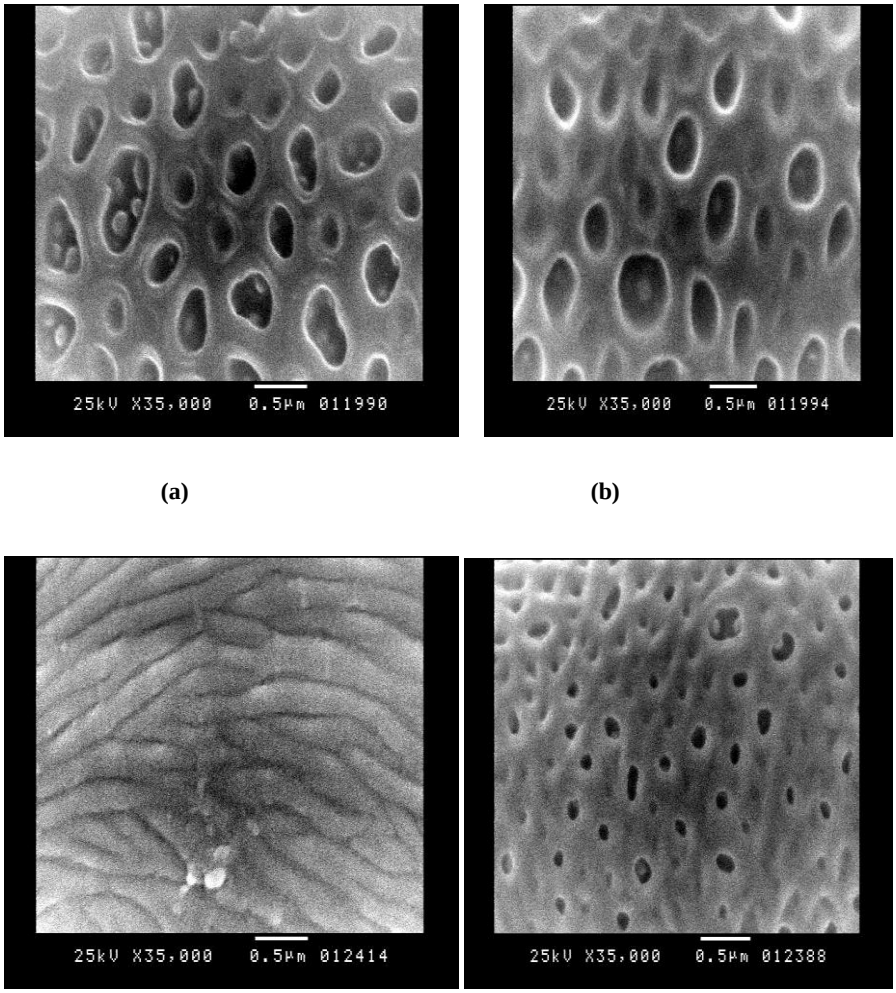


(h)



(i)

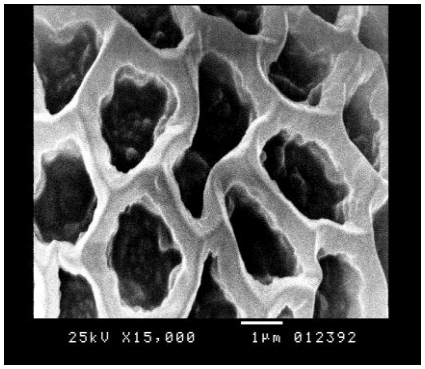
Plate 3. Cont. Aperture of studied pollen grains (e- *T. terrestis*, f- *Z. album*, g- *Z. coccenium*, h- *Z. decumbens* and i- *Z. simplex*).



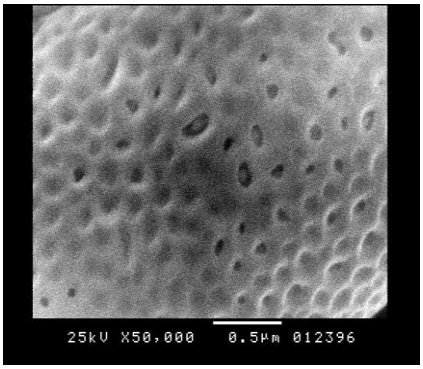
(a) **(b)**

(c) **(d)**

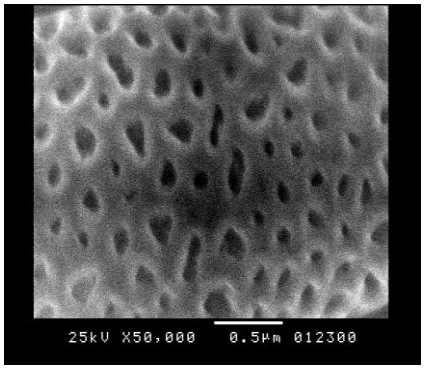
Plate 4. Sculpturing view of studied pollen grains (a- *F. arabica*, b- *F. cretica*, c- *N. retusa* and d- *P. harmala*).



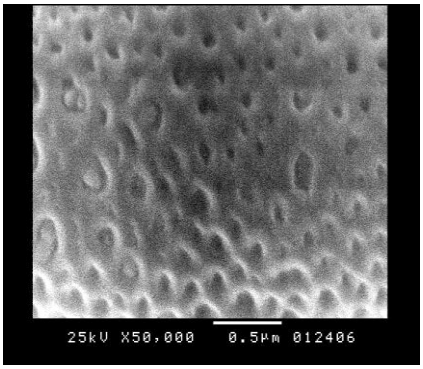
(e)



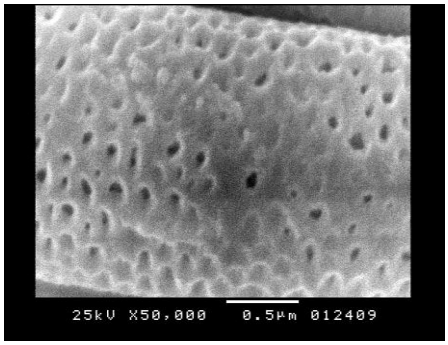
(f)



(g)



(h)



(i)

Plate 4. Cont. Sculpturing view of studied pollen grains (e- *T. terrestis*, f- *Z. album*, g- *Z. coccenium*, h- *Z. decumbens* and i- *Z. simplex*).

References

Abd El-khalik, K. (2012) A numerical taxonomic study of the family Zygophyllaceae from Egypt. *Acta Botanica Brasilica.*, **26** (1): 165.

Anitalakshmi, V., Rame, G., Sathisha, C.S. and Shailaja, H. (2014) Characterization of cultivars based on electrophoretic analysis of seed proteins, isozymes and DNA markers in rice (*Oryza sativa* L.). *Indian J. Plant Sci.*, 3 (2): 2319.

Badr, A. (1995) Electrophoretic studies of seed protein in relation to the chromosomal criteria and relationships of some taxa of *Trifolium*. *Taxon*, 44: 183-191.

Badr, A., El-Shazly, H.H., and Abou-El-Enain, M. (2000) Seed protein diversity and its implications on the relationships in the genus *Lathyrus* L. (Fabaceae).1st *int. Conf. Biol. Sci*. (ICBS) Fac. Sci. Tanta Univ. (May 7-8) 1: 1-15.

Barbara, L., Vincenzo, M. and Nicola, M. (1995) Olive pollen ultrastructure: Characterization of exine pattern through image analysis-Scanning electron microscopy. *Scientia Hort.*, **65** (4): 283-294.

Blackmore, S. (1981) Palynology and inter-generic relationships in sub-tribe Hyoseridine (Compositae: Lactuaceae). *Bot. J. Linn. Soc.*, 82: 1-13.

Boulos, L. (1999) " *Flora of Egypt*", Vol. 1. *Azollaceae -oxalidaceae.* Al Hadara Publishing, Cairo, 417pp.

Boulos, L. (2000) "*Flora of Egypt".* Alhadara Publishing, Egypt Vol. 2. Cairo,: 12-18.

Cross, R.H., McKay, S.A., McHughen, A.G. and Bonham-Smith, P.C. (2003) Heat-stress effects on reproduction and seed set in *Linum usitatissimum* L. (flax). *Plant Cell Environ.*, 26: 1013–1020.

Dahlgren, G. (1989) The last dahlgrenogram. System of classification of the dicotyledons. "*The Davis and Hedge Festschrift".* Edinburgh University Press, Edinburgh, pp 249–260 Tan, K. (Ed.)..

Dahlgren, R. (1980) A revised system of classification of the angiosperms. *Bot. J. Linn. Soc.*, 80: 91–124.

Dahlgren, R. (1983) General aspects of angiosperm evolution and macrosystematics. *Nord. J. Bot.*, **3**: 119–149.

Echlin, P. (1968) The use of the scanning reflection electron microscope in the study of plant and microbial material. *J. Roy. Microscop. Soc*., 88: 407-418.

El-Atroush H., Barakat H.M., Tantawy M.A. and EL-Shabasy A.E. (2014) Molecular characterization of Nitraria retusa and selected taxa of Zygophyllaceae using RAPD and ISSR markers**.** *Egyptian. J. of Genet. and Cytology* (under press).

El-Ghamery, A.A., Abdel-Azeem, E. A. and Kasem, A. M. (2003) Electrophoretic seed protein, isozyme patterns and nucleic acids content in some Egyptian taxa of the genus *Zygophyllum*. *Egypt J. Biotechnol.*, **13**: 268–83.

Emre, I., Turgut-Balik, D., Genc, H. and Sahin, A. (2006) The use of seed proteins revealed by SDS-PAGE in taxonomy of some *Lathyrus* L. species grown in Turkey. *Pak. J. Bio. Sci.*, **9** (12): 2358-2361.

Emre, L. (2009) Electrophoretic analysis of some *Lathyrus* L. species based on seed storage proteins. Genet. Resour. Crop Evol., **56**: 31-38.

Engler, A. (1931) Zygophyllaceae. In: *Die Natürliche Pfl Anzenfamilien*, Engler, A., Prantl, K. Eds. 2nd vol. 19a, section **2**: 144-184. Leipzig, Engelmann.

Erdtman, G. (1943) "*An Introduction to Pollen Analysis". Waltham Mass*., pp. 239.

Erdtman, G. (1952) "*Pollen Morphology and Plant Taxonomy*". Angiosperms. Waltham. *Chronica Botanica,* pp. 222.

Faegri, K. (1956) Recent trends in palynology. *Bot. Rev*., **22**: 639-664.

Ghazanfar, S.A. (1994) "*Hand Book of Arabian Medicinal Plants*". CRC Press, Boca Raton Ann Arbor, London, Tokyo, pp. 587.

Godwin, H., Echlin, P. and Chapman, B. (1967) The development of the pollen grain wall in *Ipomoea purpurea* (L.) Roth. *Review of Palaeobotany and Palynology*, **3**: 181-195.

Goldberg, A. (1986) Classification, evolution and phylogeny of the families of dicotyledons. *Smithson Contrib. Bot.*, **58**:1–314.

Haidar, A.S., Walid, M.F., Mohamed, A. S. and Mohamed, A. B. (2013) Variability of morphological characters, protein patterns and random amplified polymorphic DNA (RAPD) markers in some *Pisum* genotypes. *Afr. J. Agric. Res.*, **8** (17): 1608-1616.

Hess, M. (1981a) The fine structure of the exine in relation to the stickness of Angiosperm pollen. *Rev. Palaeobot. Palynol.*, **35**: 81-92.

Hess, M. (1981b) Pollenkitt and viscin threads: their role in cementing pollen grains. *Grana*, **20**: 145-152.

Hesse, M. (1986) Nature, form and function of pollen–connecting threads in angiosperms, "*Pollen and Spores*". Blackmore and I.K. Ferguson (Eds.), Academic Press, London. pp.109–18.

Hussein, S.R., Tantawy, M.E., Kawashty, S.A. and Sale, N.A. (2009) Chemosystematic studies of *Nitraria retusa* and selected taxa of Zygophyllaceae in Egypt. *Plant Syst. Evol.*, 277: 251–264.

Hutchinson, J. (1967) "*The Genera of the Flowering Plants*". Clarendon Press, Oxford. pp. 34.

Khafagi, A.A.F. (2012) The taxonomic significance of seed proteins in *Zygophyllum* species (Zygophyllaceae) in Egypt. *Egypt. J. Biotechnol.*,**15**: 1.

Khafagi, A.A. (2003) The significance of seed proteins in *Zygophyllum* species (Zygophyllaceae) in Egypt. *Egypt J. Biotechnol.*, **15**: 186–94.

Khalkuziev, P. (1990) Orodstvennykh svyazyahk nekotorykh semeistv rastenii pustynnykh oblastei. *Dissertation*, Tashkent. pp. 114.

Konarev, V. G. (1983) Belki rastenii kak geneticheskie marker *Plant Proteins As Genetic Markers*" , Moscow: *Nauka*., pp. 14.

Kremp, G.O.W. (1968) *Morphologic Encyclopaedia of "Palynology*. 2nd ed., Univ. Arizona Press, Tucson. pp. 263.

Kress, W. J. and Stone, D. E. (1982) Natural of the sporoderm in monocotyledons with special reference to the pollen grains *Canna* and *Heliconia*. *Grana*, **21**: 129-148.

Kress, W.J. and Stone, D. E. (1983) Morphology and phylogenetic significance of exine-less pollen of *Heliconia* (Heliconiaceae). *Syst. Bot*., **8**: 149-167.

Ladizinisky, G. and Hymowitz, T. (1979) Seed protein electrophoresis in taxonomic and evolutionary studies. *Theor*. *Appl. Genet.*, **45**: 145–51.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **122**: 680–5.

Martens, J. and Fretz, T. A. (1980) Identification of eight crab apple by pollen surface sculpture. *J. Amer. Hort. Sci*., **105** (2): 257-263.

Mohamed, E. Tantawy, Sayed, F. Khalifa; Karima, A. Hamed and Heba, M. Elazab (2005) Palynological study on some taxa of mimosoideae (Leguminosae). *Int. J. Agri. Biol.* **7** (6): 857.

Mohmed, H. M. (2006) Taxonomic significance of seed proteins and iso-enzymes in *Tribulus* (Zygophyllaceae). *Int. J. of Agri. & Biol.*, **8** (5): 573–575.

Moradpour, K., Najaphy, A., Mansoorifar, S. and Mostafaie, A. (2014) Evaluation of leaf protein pattern in wheat genotypes under drought stress. *Int. J. Adv. Biol. Biom. Res.* **2** (3): 840-846.

Pragati, V.G. P. and Sreenath, K. P. (2013) Phylogenetic relationship of some *Ipomoea* seed proteins by SDS-PAGE. *Int. Res. J. Biol. Sci.*, **2** (10):64.

Punt, W., Blackmore, S., Nilson, S. and LeThomas, A. (1994) "*Glossary of Pollen and Spore Terminology*". Found. Ultrecht., pp.71.

Raj, B. (1983) A contribution to the pollen morphology of Verbenaceae. *Paleobot. Palynol.*, **39**: 343-422.

Ronse Decraene, L.P. and Smets, E.F. (1991) Morphological studies in Zygophyllaceae. I. The floral development and vascular anatomy of *Nitraria retusa*. *Amer. J. Bot.*, **78** (10): 1438–1448.

Ronse Decraene, L.P. De Laet, J. and Smets, E. F. (1996) Morphological studies in Zygophyllaceae. II. The floral development and vascular anatomy of *Peganum harmala*. *Amer. J. Bot.*, **83** (2): 201–215.

Sailaja, K.K., Raja, R.K., Reddy, V. R., Kakani, V. G. and Duli, Z. (2005). Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths. *J. of Experimental Bot.*, **56** (412): 725–736.

Savithiry S N. (2014) Analysis of Soybean Seed Proteins Using Proteomics. *J Data Mining Genomics Proteomics*, **5** (1): 2

Sheahan, M. C. and Chase, M. W. (1996) A phylogenetic analysis of Zygophyllaceae based on morphological, anatomical and *rbc*L DNA sequence data. *Botanical J. Linnean Society*, **122**: 279-300.

Sheahan, M. C. and Cutler, D. F. (1993) Contributions of vegetative anatomy to the systematics of Zygophyllaceae R. Br. *Botanical J. of the Linnean Society*, **113**: 227-262.

Shen, X.Y. and Webster, B.D. (1986) Effects of water stress on pollen of *Phaseolus vulgaris* L. *J. Am. Soc. Hort. Sci.*, **111**: 807–810.

Skvarla, J.J. (1966) Techniques of pollen and spores electron microscopy. Part I. Staining, dehydration and embedding. *Okla. Geol*., **26**: 179-186.

Stanley, R. G. and Linskens, H. F. (1974) "*Pollen in Biochemistry Management. Biology"*, Springer-Verlag, Berlin and New York. pp.99.

Täckholm, V. (1974) "*Student's Flora of Egypt*". Cairo: Cairo University Press. pp. 888.

Thanikaimoni, G. (1986) "*Pollen Apertures: Form and Function*". Academic Press, London, Blackmore and I.K. Ferguson (Eds.), pp. 119-136.

Van Campo, M. (1966) Pollen et phylogenie. Les bréviaxes. *Pollen et Spores*, **8**: 57-73.

Van Campo, M. (1967) Pollen et classification. *Palaeobot. Palynol.*, **3**: 65-71.

Vural, C., Äzcan, S. and Akbulut, M. (2009) New combination *Veronica* (Scrophulariaceae) based on morphological characters and the seed storage protein polymorphism. *J. Syst. Evol*., **47** (2): 168-172.

Walker, J. W. and Doyle, J. A. (1975) The bases of Angiosperm phylogeny: Palynology. *Annals of the Missouri Botanical Garden*, **62**: 664–723.

Youseesf, S.S. (1990) Protein profiles as a tool to detect genetic variability among *Vicia* species. *Assiut J. Agri. Sci.*, **21**: 303-317.

Zohary, M. (1972) "*Flora Palaestina"* (Part II: Text). Jerusalem: The Israel Academy of Sciences and Humanities, Jerusalem, pp. 489.

Zohary, M. (1987) "*Flora Palaestina"*, part II. The Israel Academy for Science and Humanities, Jerusalem, pp. 244–257.

(*Received* 17/5 / 2015;
accepted 19 /5 /2015)

دراسة الشكل الظاهري لحبوب اللقاح ونمط البروتين لنبات النتراريا ريتيوزا وبعض الأنواع المختارة من الفصيلة الزيجوفيليه في مصر

هالة محمد الأطروش*، أحمد عيسى الشباسى، محمد السيد طنطاوي*، هدى صبحى بركات***
*قسم النبات – كلية العلوم – جامعة عين شمس و ** قسم النبات – كلية العلوم – جامعة ٦ أكتوبر – مصر .

تم دراسة تسعة أنواع مختارة من الفصيلة الزيجوفيليه وذلك بعمل تحليلات على أشكال حبوب اللقاح وتحليلات بيوكيميائية بعمل نمط البروتين الذائب الكلي في بذور النباتات قيد الدراسة من الفصيلة الزيجوفيليه وهي كالآتى:
Fagonia arabica, F. cretica, Peganum harmala, Tribulus terrestis, Zygophyllum album, Z. coccenium, Z. decumbens and *Z. simplex*
وقد اعتمدت الدراسات التصنيفية على دراسة الأشكال المختلفة لحبوب اللقاح وتركيبها بإستخدام المجهر الإلكتروني الماسح (SEM). وقد تم إجراء بعض القياسات وتم وضعها في صورة (+) موجود أو (-) غائب.
كما أوضحت نتائج التحليل الإحصائي كل من شجرة القرابة للأنواع وعلاقة الأنواع ببعضها برسم ثلاثى الأبعاد PCA.
كما اعتمدت الدراسات التصنيفية على دراسة نمط البروتين الذائب الكلي المخزن بالبذور بإستخدام تقنية SDS-PAGE. أوضحت نتائج الإحصاء كل من شجرة القرابة للأنواع وعلاقة الأنواع ببعضها.
كما تم إنشاء شجرة قرابة مجمعه وأوصلت بفصل كل من *Nitraria retusa* و*Tribulus terrestis* من الفصيلة Zygophyllaceae ووضع كل منهما في فصيلة مستقلة.