*Al Azhar Bulletin of Science Vol. 29, No. 1 (June) 2018, : pp. 43-56*

# TAXONOMIC IMPLICATION OF POLLEN MORPHOLOGY AND SEED PROTEIN ELECTROPHORESIS OF SOME SPECIES OF SOLANACEAE IN EGYPT

**Abbas A. El- Ghamery, Azza A. F. Khafagi and Osama G. Ragab**

*Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt*

## ABSTRACT

*The pollen morphology and seed protein electrophoresis of 24 species, representing seven genera of Solanaceae were studied to determine the significance as taxonomic characters. Pollen morphological characters: including type, size, shape and ornamentation were investigated by using light and scanning electron microscopy (SEM). The pollen differed in type, size and shape and also seven types of ornamentation were recorded in the different species in this study. The storage seed protein was determined by using gel electrophoresis. The number of protein bands was detected with different molecular weights and ranged from 25 KDa to 77 KDa. The results of the two criteria were represented in the dendrogram which showed that species grouped into two major clusters, the first cluster (I) contains 12 species, while the second cluster (II) comprises 12 species and which can be divided into two groups. The results indicate that the morphological characteristics of pollen morphology and seed protein electrophoresis appeared to be of significant importance in differentiation and identification of studied taxa of Solanaceae.*

**Keywords:** Pollen morphology, Seed protein electrophoresis, Fruit, SEM, Solanaceae

## INTRODUCTION

The Solanaceae is one of the most important and large families of flowering plants. The family is widely distributed in a diversity range of ecological habitats in both tropical and temperate regions. It consists of about 92-98 genera and 2300-2700 species **(Wettstein 1895, D'Arcy 1991, Hunziker 2001 and Olmstead and Bohs 2007)**.

In Egypt Solanaceae is a well-represented family, about 30-33 wild species belonging to eight genera according to **(Tãckholm 1974 and Boulos 1995, 2002 and 2009)**. Moreover **(Hepper 1998)** reported that the family is represented by 25 genera and about 91 wild and cultivated species.

The first systematic classification of the Solanaceae was proposed by **(Dunal 1852)** and consisted of a division of the 61 genera known at the time, into two tribes and 11 subtribes, while (**Wettstein 1895)** divided the Solanaceae into two series A and B which comprises three and two tribes respectively. Later on **Hunziker (2001)** classified the family into 6 subfamilies and about 21 tribes.

The described of the pollen morphology of various members of the family Solanaceae has been studied by different workers from time to time, such as (**Natarjan 1957, Murray and Eshbaug 1979, Sharma 1974, Anderson and Gensel 1976, Palri and Koche 1976, Anderson 1977, Srivastava 1977, Edmonds 1984, Gentry 1986, Bernardello and Luján 1997, Plowman 1998, Persson *et al.*, 1999, Perveen and Qaiser 2007, Hayrapetyan 2008, Al-Quran 2004 and 2008, Adedeji and Akinniyi 2015, Dhanya and Devipriya 2016 and Khosromehr *et al.* 2017)** by light microscope and scanning electron microscope.

Moreover, recently **AL-Wadi and Lashin (2007), Lashin (2011)**, **Anil Kumar *et al.* (2015) and Anil Kumar *et al.* (2016)** described the pollen morphology of some species of *Solanum* by light, scanning electron and transmission microscopes.

On the other hand, **Sharma *et al.* (2017)** discussed the palynological characters of 9 species belonging to 4 genera of the family Solanaceae. **Song *et al.* (2018)** studied of the pollen grains of 38 species and one variety of 17 genera in the family Solanaceae were

studied using light microscopy (LM) and scanning electron microscopy (SEM).

Although many studies has been done on the electrophoretic of storage seed protein. Some works has been carried out on the storage seed protein and the contribution of PAGE profiles to the classification and phylogenetic relationships in the Solanaceae. **Edmonds and Glidewell (1977), Yousaf *et al.* (2006) and Ahmed and Fadl (2015)** investigated the protein profile of some species of *Solanum* were analyzed by SDS-PAGE. In addition **Khalifa *et al.* (1998)** used SDS-PAGE to reassess review the taxonomic relationships of 45 species belonging to 15 genera and 8 tribes of the Solanaceae.

**Sheidai *et al.* (2000) and Nejadhabibvash *et al.* (2014)** studied the seed storage protein analysis of some *Hyoscyamus* species by using the Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method.

Moreover the seed protein profile of 42 accessions belonging to 7 species of 4 different genera (*Datura, Hyoscyamus*, *Withania* and *Atropa*) from the family Solanaceae was investigated through Poly Acrylamide Gel Electrophoresis by **(Yousaf *et al.* 2008)**.

**Bhat and Kudesia (2011)** investigated the seed protein pattern of 5 species *Solanum melongena, S. xanthocarpum, Datura alba, Lycopersicon esculentum* and *Capsicum annum* by using SDS-PAGE.

The present study described the diagnostic characters of pollen morphology and seed protein electrophoresis of the studied species as important tools in the delimitation of clad within Solanaceae and in turn helps in extracting vital taxonomic criteria and utilize in determining tribal affinities.

## MATERIALS AND METHODS

The present study included 24 species of Solanaceae belonging to two subfamilies and seven genera and five tribes according to the classification of **Hunziker (2001)**. The fresh collections of the studied species from different localities in Egypt are shown in **Table 1.**

The specimens studied were identified by means of comparison with specimens kept in the Herbarium of the Agricultural Museum (CAIM) and Herbarium of the Desert Research Center (CAIH). In addition, keys of **Tãckholm (1974)** and **Boulos (2002).** Reference herbarium specimens of studied species were prepared and kept in the herbarium of Botany and Microbiology Department, Faculty of Science (Boys branch) Al-Azhar University, Cairo, Egypt.

### Pollen morphology

The naturally dried pollen grains were prepared for light microscopy (LM) by acetolysis **(Erdtman 1969)**, then mounted in glycerin jelly.

For the scanning electron microscopy (SEM) study pollen grains were mounted on metal stubs, coated, golden, examined and photographed by JEOL Scanning electron microscope at the accelerating voltage of 18 kv, at the Electron Microscope at The Regional Center for Mycology and Biotechnology, Al Azhar University, Cairo, Egypt.

Palynological terminology was referenced from previous works (**Erdtman 1952, Wang *et al.* 1997, Punt *et al.* 2007, Hayrapetyan 2008 and Hesse *et al.* 2009)**. Pollen size was characterised according to the method of **Erdtman (1952)**.

### Seed protein electrophoresis

The Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was prepared according to the method of **Laemmli (1970).** Total bands for each species were scored and their molecular weight (Mol. Wt.) calculated using the protein marker as standard. The gel scanning was done on Helena Jonior 24 photo scanner and the data were integrated using the scanner software.

All the examined species were used as operational taxonomic units (OUT's). The 39 character states which resulted from pollen morphological characters and seed protein data

were analyzed by means of Hierarchical Cluster analysis used distance and similarity measure Bray-Curtis Sorensen.

The relationships between the studied species of Solanaceae have been demonstrated as dendrograms **(Fig. 2)** by using statistical programs PC-ORD (Software, version 5) and PRIMER (Software, version 6.0). A total of 39 comparative pollen morphological characters and seed protein for studied species were scored and coded for creating data matrix used for numerical analysis.

**Table 1: List of the collected species for the present study and their taxonomic position as assigned according to Hunziker, 209**

| | Species | Subfamily | Tribe | Collection date |
|---|---|---|---|---|
| 1- | *Datura innoxia* Mill. | Solanoideae | Datureae | Osama G., Al-Azhar University Cairo, 11/2016 |
| 2- | *Datura stramonium* L. | | | Osama G., Al-Azhar University Cairo, 5/2017 and Kafr El-Dwar, 6/2017 |
| 3- | *Hyoscyamus albus* L. | | Hyoscyameae | Ghaly O.N, Burg El Arab- El Hammam coastal road, 5/2017 |
| 4- | *Hyoscyamus boveanus* (Dunal) Asch. & Schweinf. | | | Ghaly O.N., Wadi El Messerdy, Saint Katherine, South Sinai (CAIH), 2/5/2010 |
| 5- | *Hyoscyamus desertorum* (Asch. & Boiss) Täckh. | | | L. Boulos, Wadi El-Arish; (CIA), 14/9 1965 |
| 6- | *Hyoscyamus muitcus* L. | | | Osama G., Cairo-Alexandria road, 100 km, 12/2016 and Ghaly O.N, Shalateen-Abu Ramad road,60 km, 7/ 4/2017 |
| 7- | *Hyoscyamus pusillus* L. | | | H. Shabana, Wadi Tiniya, Saint Katherine Protectorate, 20/4/2008 |
| 8- | *Lycium europaeum* L. | | Lycieae | Osama G., Alexandria- Matruh Coastal road, 237 km, 3/2017 |
| 9- | *Lycium schweinfurthii* (Dammer) Feinbrun var. *aschersonii* | | | Osama G., Alkom Alakhdar island, El Brullus Protectorate, 3/2017 |
| 10- | *Lycium shawii* Roem & Schult. | | | Osama G., Wadi Hagul, 12/2017 |
| 11- | *Physalis angulata* L. | | Solaneae | Osama G., Pajour, Minufiyah, 11/2017 |
| 12- | *Physalis ixocarpa* Brot. ex Hornem. | | | Osama G., Kafr El-Dwar, 3/2017 |
| 13- | *Physalis peruviana* L. | | | Osama G., Nasr City- Cairo, 3/2018 |
| 14- | *Solanum coagulans* Forssk. | | | Iman Al- Gohary, Wadi Darweena, Gabel Elba (CAIH), 1/4/ 2000 |
| 15- | *Solanum elaeagnifolium* Cav. | | | Ghaly O.N., Burg El Arab City, 15/5/2017 |
| 16- | *Solanum forsskaolii* Dunal. | | | Ghaly O.N., wadi Maarafaii,Gebel Elba, 8/4/2017 |
| 17- | *Solanum incanum* L. | | | Ghaly O.N, wadi Kanthesrob, Gebel Elba, 9/4/2017 |
| 18- | *Solanum nigrum* L. var. *nigrum* | | | Osama G., Kafr El-Dwar in 10/2016 and Pajour Minufiyah in 11/2017 |
| 19- | *Solanum sinaicum* Boiss. | | | Osama G, from Agriculture Museum Garden, Giza, 10/1/2018 cultivated |
| 20- | *Solanum villosum* Mill. subsp. *villosum* | | | Osama G., Minufiyah in 4/2017 and Burg El Arab City, 15/5/2017 |
| 21- | *Withania obtusifolia* Täckh. | | | Ghaly O.N. and M. Abutaha, Wadi Akaw, Gebel Elba(CAIH) , 26/4/2013 |
| 22- | *Withania somnifera* (L.) Dunal | | | Osama G., Cairo University, 4/2017; Minufiyah, 4/2017 and Nasr City-Cairo, 4/2018 |
| 23- | *Nicotiana glauca* R.C. Graham | Cestroideae | Nicotianeae | Osama. G, Alexandria-Matrouh road, 237 km, 3/2017 |
| 24- | *Nicotiana rustica* L. | | | S. Khalifa, Agriculture Museum Garden (CAIH), 4/1988 (cultivated) |

**RESULTS AND DISCUSSION**

**1. Pollen morphology**

Pollen grains are very variable in type, shape, size, polar/equatorial ratio and ornamentation as shown in **(Table 2 and Plates 1, 2 and 3)**. The Pollen class type of varied from tricolpate in *Datura innoxia* and *Datura stramonium*, tricolporate and tetracolporate in *Nicotiana rustica* and tricolporate in the rest.

The pollen shape of the studied species ranged between prolate spheroidal in most studied species, suboblate in *Solanum elaeagnifolium* and *Solanum forsskaolii*, oblate-spheroidal in *Datura stramonium*, *Lycium europaeum*, *Nicotiana glauca*, *Nicotiana rustica*, *Withania obtusifolia* and *Withania somnifera* and subprolate in *Lycium shawii* and *Physalis angulata*.

The morphometric data of the pollen grains referring to the values of the arithmetic averages of polar (P) and equatorial (E) axis were as follows: Polar measurements ranged between 21.7-27.6 µm in (8 species) *Hyoscyamus pusillus*, *Lycium schweinfurthii* var. *aschersonii*, *Lycium shawii*, *Nicotiana glauca*, *Nicotiana rustica*, *Physalis angulata*, *Physalis ixocarpa* and *Physalis peruviana*, 30.2-40.5 µm in (6 species) *Datura innoxia*, *Datura stramonium*, *Hyoscyamus albus*, *Hyoscyamus boveanus*, *Hyoscyamus desertorum* and *Hyoscyamus muitcus* and 16.9-20.8 µm in the rest. Equatorial measurements ranged between 16.2-23.2 µm in most studied species, 23.3-25.88 µm in (5 species) *Hyoscyamus pusillus*, *Lycium schweinfurthii* var. *aschersonii*, *Nicotiana glauca*, *Physalis ixocarpa* and *Physalis peruviana* and 30.2-40.5 µm in (7 species) *Datura innoxia*, *Datura stramonium*, *Hyoscyamus albus*, *Hyoscyamus boveanus*, *Hyoscyamus desertorum*, *Hyoscyamus muitcus* and *Nicotiana rustica*.

Polar / Equatorial ratio ranged between 0.84-0.98 in *Datura stramonium*, *Lycium europaeum*, *Nicotiana glauca*, *Nicotiana rustica*, *Solanum elaeagnifolium*, *Solanum forsskaolii*, *Withania obtusifolia* and *Withania somnifera* and between 1.01-1.33 in rest.

The pollen size ranged between small (10-24 µm) in most studied species and medium (25-49 µm) in *Datura innoxia*, *Datura stramonium*, *Hyoscyamus albus*, *Hyoscyamus boveanus*, *Hyoscyamus desertorum*, *Hyoscyamus muitcus*, *Hyoscyamus pusillus*, *Nicotiana rustica*, *Physalis ixocarpa* and *Physalis peruviana*.

On the other hand, the ornamentation types varied in different species and were **granulate** in most studied species, **striate** in (7 species) *Datura innoxia*, *Hyoscyamus albus*, *Hyoscyamus boveanus*, *Hyoscyamus muitcus*, *Hyoscyamus pusillus*, *Lycium europaeum* and *Lycium shawii*, **irregular striate** in (3 species) *Datura stramonium*, *Hyoscyamus desertorum* and *Lycium schweinfurthii* var. *aschersonii*, **striate rugose** in (one species) *Nicotiana rustica*, **irregular reticulate** in (two species) *Withania obtusifolia* and *Withania somnifera*, **tuberculate** in (one species) *Nicotiana glauca* and **verrucate** in (one species)*Solanum incanum*.

Colpus length of short (9.98-10.6) µm in *Datura innoxia* and *Datura stramonium* while very long 2(1.47-30.1) µm in *Hyoscyamus albus*, *Hyoscyamus boveanus*, *Hyoscyamus*

*desertorum, Hyoscyamus muitcus, Hyoscyamus pusillus, Nicotiana glauca, Nicotiana rustica, Physalis ixocarpa* and *Physalis peruviana* and long (15.5-19.39) µm in the rest.

**2. Seed protein electrophoresis**

The results of the electrophoresis pattern analysis of the seed proteins of the 24 species of Solanaceae are presented in (**Table 3**) and (**Fig. 1**).

The bands were detected with different molecular weights ranged from 25 KDa to 77 KDa. The total number of bands is 30 varied from plant to another, ranging between ( 4 - 1 1 ) bands for each species. The highest number of protein bands (11) was found in *Hyoscyamus albus, Hyoscyamus boveanus, Hyoscyamus muitcus, Hyoscyamus pusillus* and *Nicotiana glauca* while the lowest number (4) was recorded in *Physalis angulata, Solanum coagulans, Solanum forsskaolii, Solanum nigrum* var. *nigrum, Solanum sinaicum* and *Solanum villosum* subsp. *villosum.*

Some of the examined species had one a specific band as *Lycium shawii, Withania obtusifolia, Physalis ixocarpa* and *Datura stramonium* (mol. wt. 70, 59, 42 and 33 KDa) respectively.

From the result in (Table 3) there are five bands found in two species only. The bands of (mol. wt. 75 and 36 KDa) presents in *Nicotiana glauca* and *Nicotiana rustica* while the band with (mol. wt. 56 KDa) recorded in *Hyoscyamus muitcus* and *Hyoscyamus pusillus* only but the band of (mol. wt. 49 KDa) found in *Hyoscyamus pusillus* and *Solanum villosum* subsp. *villosum* and the band having (mol. wt. 41 KDa) found in *Hyoscyamus albus* and *Hyoscyamus boveanus*.

Also, the result showed there are six bands found in three species. The bands of (mol. wt. 64 KDa) presents in *Nicotiana glauca, Withania obtusifolia* and *Withania somnifera*, the band with (mol. wt. 61 KDa) recorded in *Physalis ixocarpa, Physalis peruviana* and *Withania obtusifolia,* the band of (mol. wt. 54 KDa) found in *Lycium europaeum, Lycium schweinfurthii* var. *aschersonii* and *Lycium shawii,* the band having (mol. wt. 52 KDa) found in *Hyoscyamus albus, Hyoscyamus muitcus* and *Hyoscyamus pusillus* while the bands of (mol. wt. 48KDa) presents in *Solanum elaeagnifolium, Solanum nigrum* var. *nigrum* and *Solanum sinaicum* and the bands of (mol. wt. 40 KDa) presents in *Solanum incanum, Withania obtusifolia* and *Withania somnifera*

On the other hand, the results in (Table 3) showed that the band with (mol. wt. 50 KDa) was found in all studied species except only *Solanum forsskaolii*. In this concern, the bands with (mol. wt. 28 and 27 KDa) were found in most of the studied species. The band of (mol. wt. 28 KDa) was not detected in *Datura innoxia, Datura stramonium, Solanum coagulans, Solanum elaeagnifolium, Solanum nigrum* var. *nigrum, Solanum sinaicum* and *Solanum villosum* subsp. *Villosum,* while the band of (mol. wt. 27 KDa) was not detected in *Hyoscyamus albus, Hyoscyamus boveanus, Hyoscyamus desertorum, Hyoscyamus muitcus, Lycium europaeum, Lycium shawii, Solanum nigrum* var. *nigrum, Solanum sinaicum* and *Solanum villosum* subsp. *villosum.*

**Table 2: Morphological characters of pollen grains**

| Character / Species | Pollen class type | Size | | | P / E | Pollen size | Ornamentation | Colpus | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pollen shape | Polar diameter µm | Equatorial diameter µm | | | | Length µm | Width µm | Ratio length / width | Colpus |
| ***Datura innoxia*** | 3 | 3 | 39.3-40.5 | 34.5-36.3 | 1.13 | 2 | 1 | 10.6 | 3.45 | 3.07 | 1 |
| ***Datura stramonium*** | 3 | 2 | 35.2-35.4 | 33.1-39-3 | 0.98 | 2 | 2 | 9.98 | 2.45 | 4.07 | 1 |
| ***Hyoscyamus albus*** | 1 | 3 | 32.6-34.6 | 33.2-28.5 | 1.05 | 2 | 1 | 23.1 | 5.9 | 3.91 | 3 |
| ***Hyoscyamus boveanus*** | 1 | 3 | 30.2-31.5 | 28.6-30.7 | 1.04 | 2 | 1 | 22.98 | 4.7 | 4.91 | 3 |
| ***Hyoscyamus desertorum*** | 1 | 3 | 30.7-35.4 | 26.5-31.9 | 1.13 | 2 | 2 | 24.9 | 3.62 | 6.87 | 3 |
| ***Hyoscyamus muitcus*** | 1 | 3 | 31.9-33.9 | 31.8-32.8 | 1.02 | 2 | 1 | 30.1 | 4.89 | 6.15 | 3 |
| ***Hyoscyamus pusillus*** | 1 | 3 | 25-27 | 24.4-25.88 | 1.03 | 2 | 1 | 21.56 | 3.21 | 6.71 | 3 |
| ***Lycium europaeum*** | 1 | 2 | 16.9-17.8 | 17.9-18.1 | 0.96 | 1 | 1 | 15.5 | 3.5 | 4.42 | 2 |
| ***Lycium schweinfurthii* var. *aschersonii*** | 1 | 3 | 23.9-24.3 | 23.7-24.2 | 1.01 | 1 | 2 | 17.8 | 3.76 | 4.7 | 2 |
| ***Lycium shawii*** | 1 | 4 | 21.7-22.8 | 17.9-18.1 | 1.24 | 1 | 1 | 19.39 | 3.46 | 5.6 | 2 |
| ***Nicotiana glauca*** | 1 | 2 | 22.3-23.6 | 23.3-24.4 | 0.96 | 1 | 6 | 21.47 | 3.16 | 6.79 | 3 |
| ***Nicotiana rustica*** | 2 | 2 | 26-27.6 | 26.9-28.6 | 0.97 | 2 | 3 | 22.8 | 3.4 | 6.7 | 3 |
| ***Physalis angulata*** | 1 | 4 | 24-24.5 | 20.1-20.9 | 1.18 | 1 | 5 | 19.11 | 2.22 | 8.6 | 2 |
| ***Physalis ixocarpa*** | 1 | 3 | 26.7-27.6 | 24-24.6 | 1.12 | 2 | 5 | 22.4 | 2.7 | 8.29 | 3 |
| ***Physalis peruviana*** | 1 | 3 | 25-25.9 | 24.4-25.6 | 1.016 | 2 | 5 | 22.85 | 2.5 | 9.14 | 3 |
| ***Solanum coagulans*** | 1 | 3 | 17.1-18.5 | 16.2-16.5 | 1.08 | 1 | 5 | 17.2 | 2.19 | 7.85 | 2 |
| ***Solanum elaeagnifolium*** | 1 | 1 | 19.4-19.7 | 22.6-22.8 | 0.86 | 1 | 5 | 17.54 | 1.48 | 11.85 | 2 |
| ***Solanum forsskaolii*** | 1 | 1 | 17.7-18.9 | 20-23.2 | 0.84 | 1 | 5 | 17.14 | 1.32 | 12.98 | 2 |
| ***Solanum incanum*** | 1 | 3 | 19.4-20.2 | 18.3-18.7 | 1.07 | 1 | 7 | 16.8 | 1.5 | 11.2 | 2 |
| ***Solanum nigrum* var. *nigrum*** | 1 | 3 | 19.5-20.4 | 17.3-20.5 | 1.05 | 1 | 5 | 17.49 | 2.43 | 7.19 | 2 |
| ***Solanum sinaicum*** | 1 | 3 | 20.4-20.8 | 18.8-20.2 | 1.05 | 1 | 5 | 18.49 | 2.8 | 7 | 2 |
| ***Solanum villosum* subsp. *villosum*** | 1 | 3 | 18.6-19.4 | 18.1-18.9 | 1.027 | 1 | 5 | 17.22 | 2.33 | 7.39 | 2 |
| ***Withania obtusifolia*** | 1 | 2 | 18.5-19.7 | 19.1-22.7 | 0.93 | 1 | 4 | 17.75 | 2.25 | 7.88 | 2 |
| ***Withania somnifera*** | 1 | 2 | 20.3-20.5 | 20.6-20.9 | 0.98 | 1 | 4 | 17.9 | 2.45 | 7.3 | 2 |

Pollen class: 1=tricolporate /2= tricolporate and tetracolporate /3=tricolpate

Pollen shape: 1=suboblate 0.76-0.86 /2= oblate-spheroidal 0.89-0.99 / 3= prolate – spheroidal 1.01-1.14 / 4= subprolate 1.15-1.33

Pollen size: 1= small (10-24) µm /2= medium (25-49) µm

Ornamentation: 1= striate/2=irregular striate /3= striate rugose/ 4= irregular reticulate /5= granulate /6=tuberculate /7= verrucate

Colpus: 1= short (9.98-10.6) µm/2=long (15.5-19.39) µm/3= very long (21.4-30.1) µm

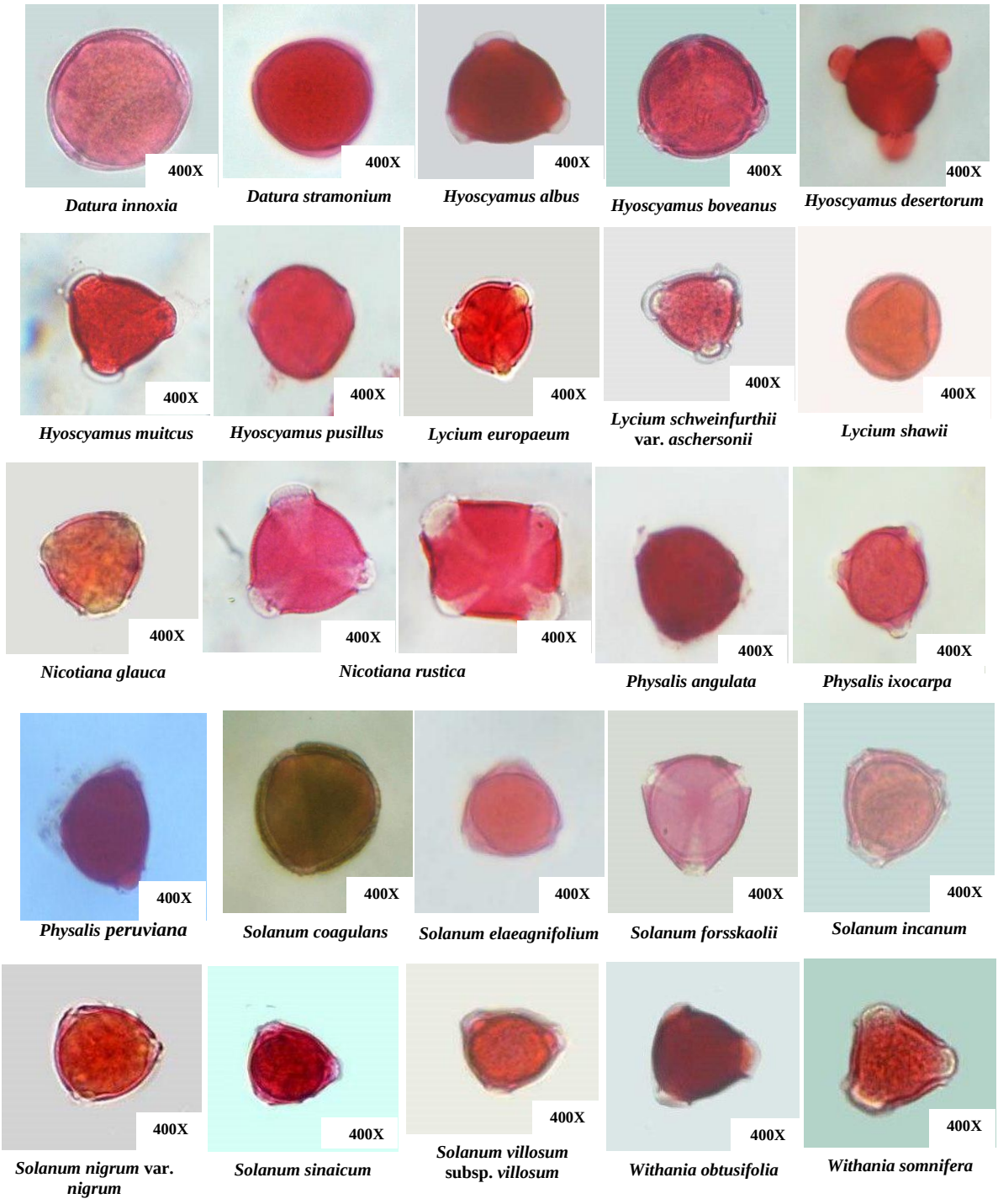
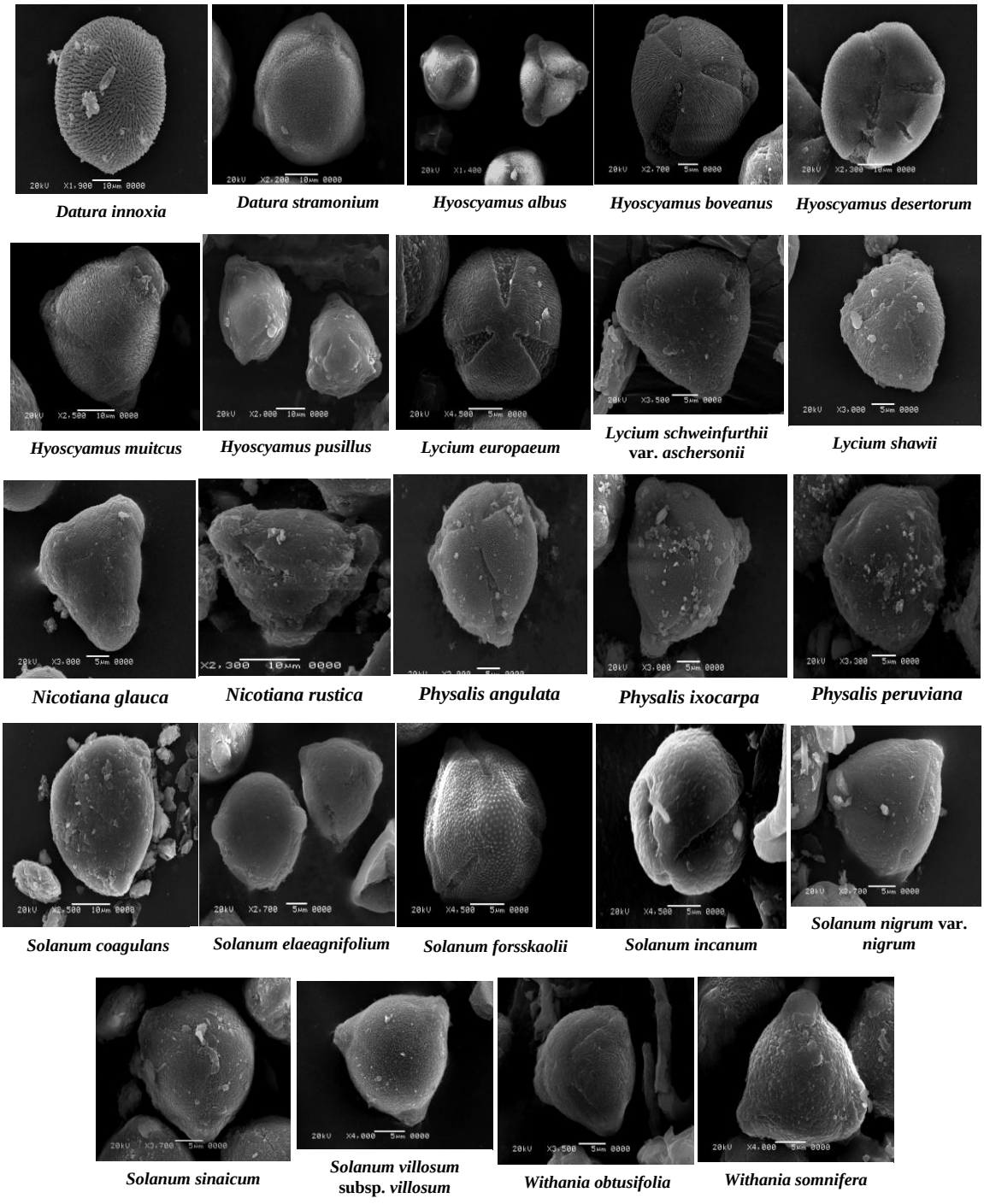



**Plate 1: LM micrographs of the pollen morphology in studied species**



*Datura innoxia* | *Datura stramonium* | *Hyoscyamus albus* | *Hyoscyamus boveanus* | *Hyoscyamus desertorum*

*Hyoscyamus muitcus* | *Hyoscyamus pusillus* | *Lycium europaeum* | *Lycium schweinfurthii* var. *aschersonii* | *Lycium shawii*

*Nicotiana glauca* | *Nicotiana rustica* | *Physalis angulata* | *Physalis ixocarpa* | *Physalis peruviana*

*Solanum coagulans* | *Solanum elaeagnifolium* | *Solanum forsskaolii* | *Solanum incanum* | *Solanum nigrum* var. *nigrum*

*Solanum sinaicum* | *Solanum villosum* subsp. *villosum* | *Withania obtusifolia* | *Withania somnifera*

**Plate 2: SEM micrographs of the pollen morphology in studied species**

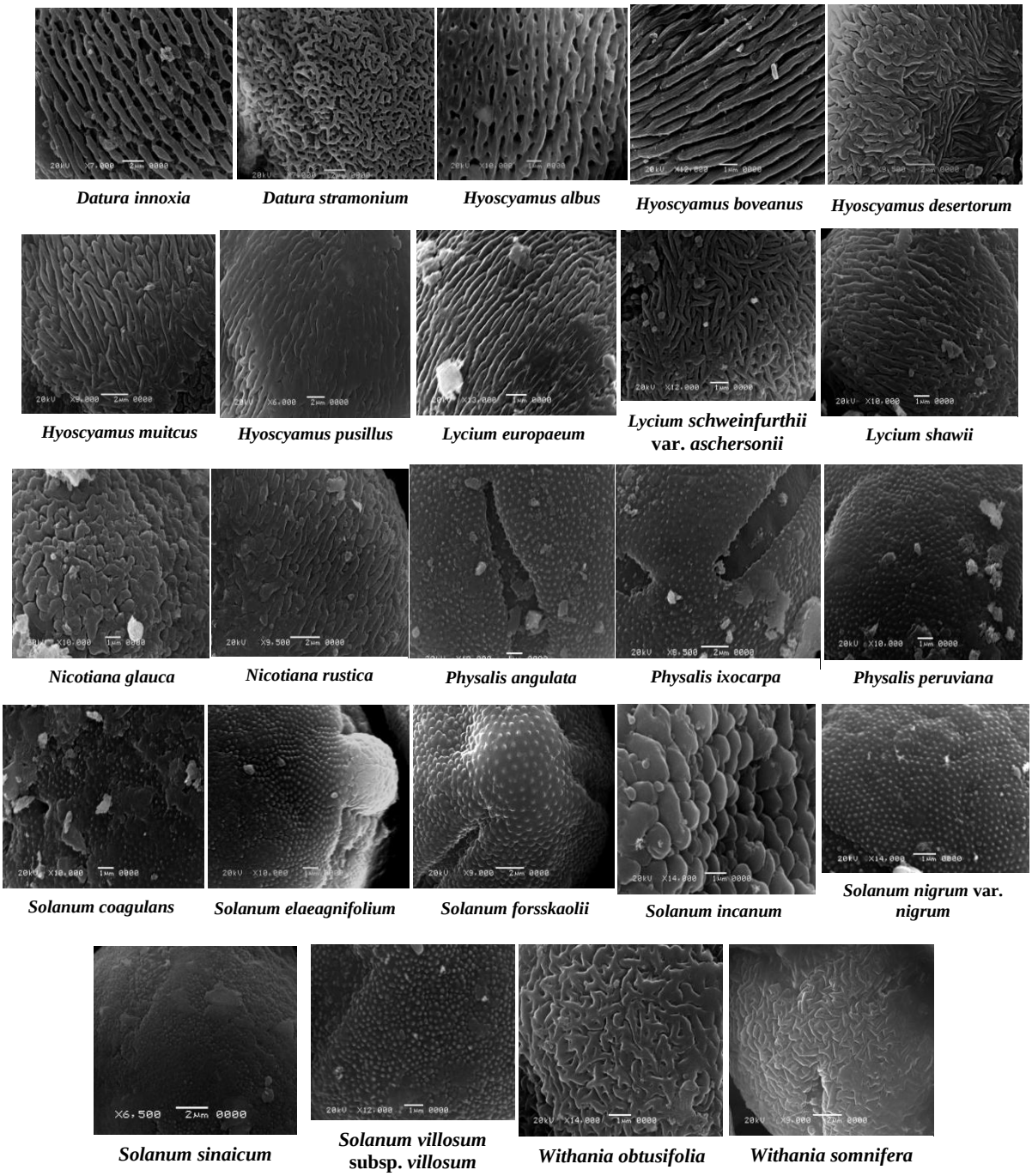



**Plate 3: SEM micrographs of pollen ornamentation in studied species**

## 3. Numerical analysis

### Cluster analysis:

The 39 (9 pollen morphological and 30 seed protein electrophoresis) characters used in cluster analysis are included in data matrix occupy in operational taxonomic units OUT's to analyse the relationships between the 24 studied species by means of Hierarchical Cluster analysis using the PC-ORD software, version 5 and PRIMER software, version 6.0 analysis used similarity and distance measure Bray Curtis.

In the dendrogram produced from using PC-ORD program used distance measure Bray Curtis showed that twenty four species were grouped into two major clusters.

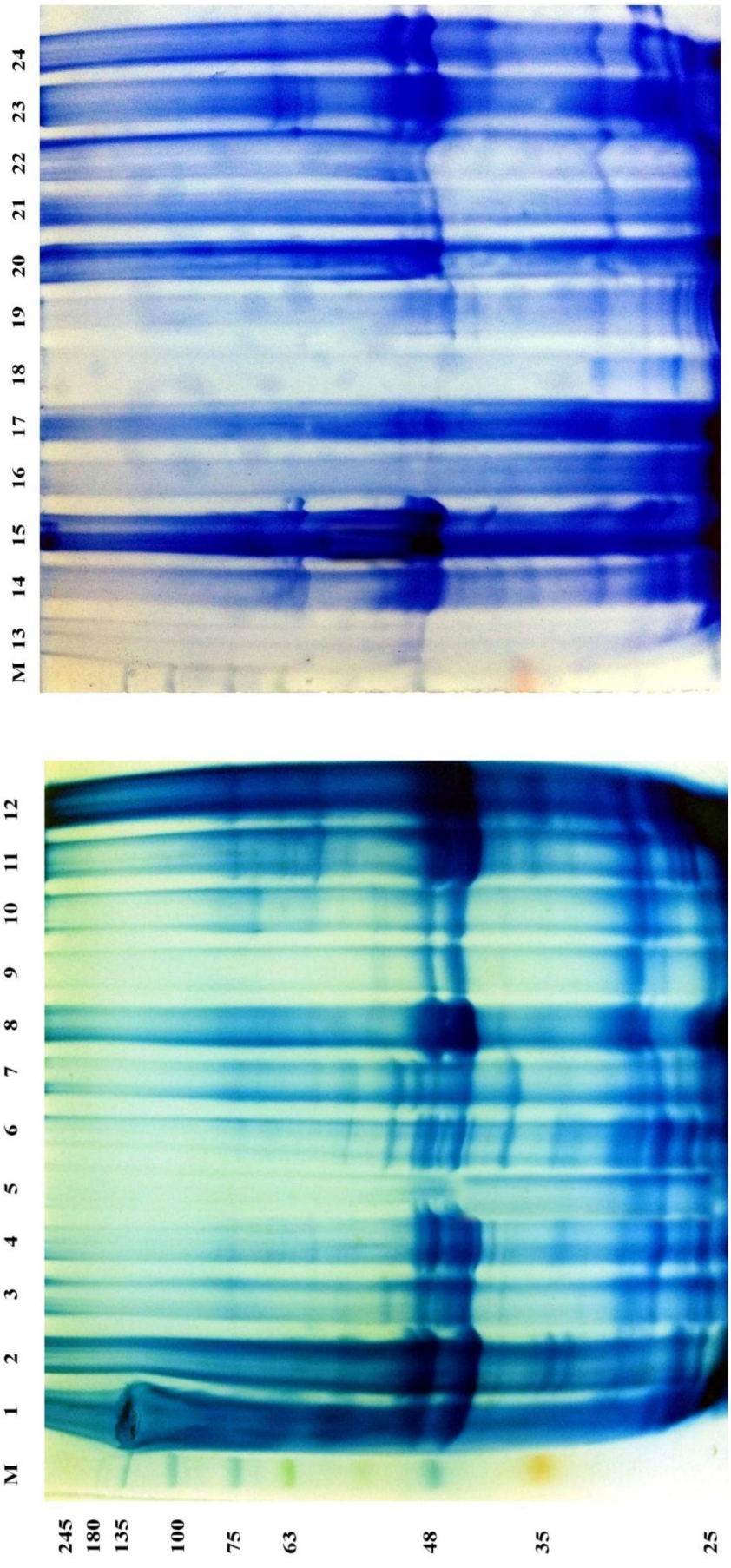
The first cluster (I) consisted of (12 species) *Physalis angulata, Physalis ixocarpa, Physalis peruviana, Solanum coagulans, Solanum incanum, Solanum sinaicum, Solanum nigrum* var. *nigrum, Solanum villosum* subsp. *villosum, Solanum elaeagnifolium, Solanum forsskaolii, Withania obtusifolia* and *Withania somnifera*.

The second cluster (II) comprises the remainders (12 species) which divided into two groups: group "1" consisted of five species, and subdivided into two subgroups: subgroup "a" contains three species *Lycium europaeum, Lycium schweinfurthii* var. *aschersonii* and L*ycium shawii*. At the same time the subgroup "b" consists of two species *Nicotiana glauca* and *Nicotiana rustica*. The group "2" consisted of seven species and subdivided into two subgroups: subgroup "a" contains five species *Hyoscyamus albus, Hyoscyamus desertorum, Hyoscyamus boveanus, Hyoscyamus pusillus* and *Hyoscyamus muitcus* and subgroup "b" comprises two species *Datura innoxia* and *Datura stramonium.*

**Table 3: SDS-PAGE of total seed protein bandsof investigated species**

| Bands No. | M.W(KDa) | 1-*Datura innoxia* | 2-*Datura stramonium* | 3-*Hyoscyamus albus* | 4-*Hyoscyamus boveanus* | 5-*Hyoscyamus desertorum* | 6-*Hyoscyamus muitcus* | 7-*Hyoscyamus pusillus* | 8-*Lycium europaeum* | 9-*Lycium schweinfurthii* var. *aschersonii* | 10-*Lycium shawii* | 11-*Nicotiana glauca* | 12-*Nicotiana rustica* | 13-*Physalis angulata* | 14-*Physalis ixocarpa* | 15-*Physalis peruviana* | 16-*Solanum coagulans* | 17-*Solanum elaeagnifolium* | 18-*Solanum forsskaolii* | 19-*Solanum incanum* | 20-*Solanum nigrum* var. *nigrum* | 21-*Solanum sinaicum* | 22-*Solanum villosum* subsp. *villosum* | 23-*Withania obtusifolia* | 24-*Withania somnifera* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 77 | 2 | 2 | 1 | 1 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | 75 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 3 | 70 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 4 | 64 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 |
| 5 | 61 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 |
| 6 | 60 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 7 | 59 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 |
| 8 | 56 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 9 | 54 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 10 | 52 | 2 | 2 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 11 | 50 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| 12 | 49 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 |
| 13 | 48 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 2 |
| 14 | 47 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 15 | 45 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 |
| 16 | 42 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 17 | 41 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 18 | 40 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 1 |
| 19 | 39 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 20 | 38 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 21 | 36 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 22 | 34 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 23 | 33 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 24 | 31 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 |
| 25 | 30 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 |
| 26 | 29 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 27 | 28 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 1 | 1 |
| 28 | 27 | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 1 | 1 |
| 29 | 26 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 1 |
| 30 | 25 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 |
| Total No. of bands | **30** | **5** | **7** | **11** | **11** | **6** | **11** | **11** | **6** | **8** | **9** | **11** | **10** | **4** | **10** | **7** | **4** | **5** | **4** | **9** | **4** | **4** | **4** | **10** | **8** |

1 = present 2 = absent


M 13 14 15 16 17 18 19 20 21 22 23 24
M 1 2 3 4 5 6 7 8 9 10 11 12
245 180 135 100 75 63 48 35 25

## CONCLUSION

The results of the present study revealed a number of significant features with some characters which could be of taxonomic and diagnostic values. Pollen class types, shape, polar / equatorial ratio and size indicates close interrelationship between certain species in this family. Also the length and size of colpus and the pollen ornamentation could provide evidence for taxonomic conclusions of the family.

The studied species of the tribe Solaneae are grouped in one cluster, which characterized by a specific band (mol. wt. 47) and pollen ornamentation are reticulate, granulate or verrucate. While the second cluster including the rest of studied species characterized by a specific band (mol. wt. 45) and pollen ornamentation are striate to tuberculate.

Finally, this result agree and supports the view of the classification of **Hunziker, 2001**.

The pollen morphology and seed protein electrophoresis in the present study are considered diagnostic at the generic and specific level of Solanaceae.

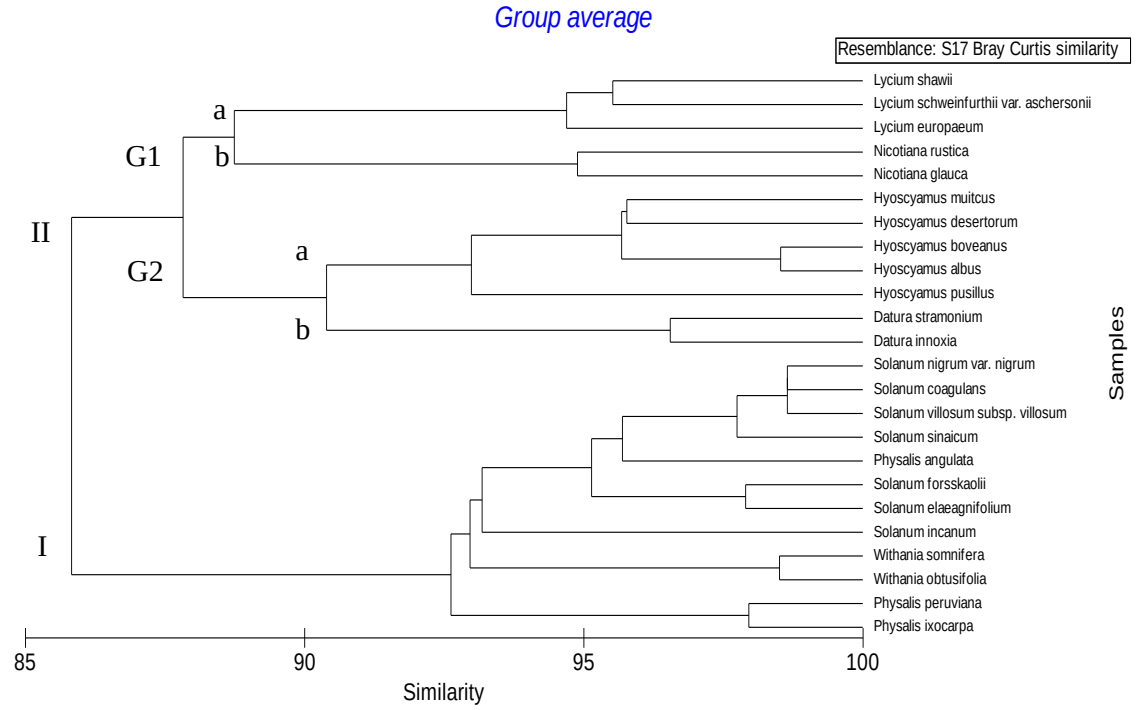



**Fig. 2: Dendrogram showing the interrelationships between 24 species of Solanaceae based on pollen morphology and seed protein electrophoresis characters by using PRIMER Program.**

## REFERENCES


**Adedeji, O. and Akinniyi, T.A. (2015):** Pollen morphology of some species in the family Solanaceae. J. Adv. Lab. Res. Biol., 6 (4): 124-128.

**Ahmed, S.M. and Fadl, M.A. (2015)**: RAPD based genetic diversity analysis within the genus *Solanum*. Egypt. J. Bot., 55(2):175 – 185.

**Al-Quran, S. (2004):** Pollen morphology of Solanaceae in Jorden. Pak. J. Biol. Sci., 7(9): 1586-1593.

**Al-Quran, S. (2008):** Pollen morphology of *Hyoscyamus* L. (Solanaceae) attracts hymenoptera species as pollen visitors in Jordan. Pak. Entomol., 30(1): 83-92.

**AL-Wadi, H. M. and Lashin, G.M.A. (2007):** Palynological and cytological characters of three species of genus *Solanum* (family: Solanaceae) from Saudi Arabia. J. Biol. Sci., 7(4): 626-631.

**Anderson, G.I. and Gensel, P.G. (1976):** Pollen morphology and the systematics of *Solanum* section Basarthrum. Pollen et Spores, 18: 533-552.

**Anderson, R.J.F. (1977):** Notes on *Solanum* (Solanceae) in Australia. Austrobaileya, I: 13-22.

**Anil Kumar, V.S.; Maya Nair C. and Murugan, K. (2015):** Pollen morphology of selected taxa of the genus *Solanum* from Southern Western Ghats, Kerala, India. Rheedea, 25(2):128-145.

**Anil kumar, V.S.A.; Sunila, A.V. and Murugan, K. (2016):** Micromorphological and ftir spectral analysis of *Solanum trilobatum* l. (Solanaceae) – A medicinally valued thorny creeper from South India. IJAR., 4(1): 1249-1257.

**Bernardello, L. and Luján, M.C. (1997):** Pollen morphology of tribe Lycieae: *Grabowskia, lyceum, phrodus* (Solanaceae). Review of palaeobotany and palynology, 96: 305-315.

**Bhat, T.M. and Kudesia, R. (2011)**: Evaluation of genetic diversity in five different species of family Solanaceae using cytological characters and protein profiling. GEBJ., 20: 1-8.

**Boulos, L. (1995):** Flora of Egypt, Checklist. Al-Hadara Publishing, Cairo, 283 pages.

**Boulos, L. (2002):** Flora of Egypt. Verbenaceae-Compositae. Al-Hadara Publishing, Cairo, 310 pages.

**Boulos, L. (2009):** Flora of Egypt: Checklist Revised annotated edition. Al -Hadara Publishing, Cairo, Egypt, 410 pages.

**D'Arcy, W.G. (1991):** The Solanaceae since 1976, with a review of its biogeography. In: J.G. Hawkes, R. N. Lester, M. Nee, and N. Estrada-R, (eds.), Solanaceae III: taxonomy, chemistry, evolution. Royal Botanic Gardens, Kew, UK, pp.: 75-137.

**Dhanya, C. and Devipriya, V. (2016):** Pollen morphological studies on two solanaceous genera *Brugmansia* Pers. and *Datura* L. Int. J. Adv. Res., 4(9): 1879-1887.

**Dunal, M.F. (1852):** Solanaceae. In: A.P.De Candolle, Prodromus Systematis Naturalis Regni Vegetabilis. Victoris Masson, Paris, 13(1): 1-690.

**Edmonds, J.M. (1984):** Pollen morphology of *Solanum* L., section *Solanum*. Bot. J. Linn. Soc., 88: 237-251.

**Edmonds, J.M. and Glidewell, S.M. (1977):** Acrylamide gel electrophoresis of seed proteins from some *Solanum* (section *Solanum*) species. Plant Syst. Evol., 127: 277-291.

**Erdtman, G. (1952):** Pollen Morphology and Plant Taxonomy-Angiosperms. Stockholm, Almquist and Wiksell.

**Erdtman, G. (1969):** Handbook of Palynology. An Introduction to The Study of Pollen Grains and Spores. Copenhagen (Denmark), Munksgaard.

**Gentry, J. L. Jr. (1986):** Pollen studies in the Cestreae (Solanaceae). In: Solanaceae: Biology and Systematics. (Eds.): W.G. D'Arcy Colombia University Press, New York, pp. 137-158.

**Hayrapetyan, A.M. (2008):** Features of the exine ornamentation of pollen grains in the family *Solanaceae* Juss. I. The simple types of ornamentation. Nat. Sci., 2(11): 46-50.

**Hepper, F.N. (1998):** Flora of Egypt. Family 159: Solanaceae. Tãeckholmia Additional Series, 6: 1-168.

**Hesse, M.; Halbritter, H.; Zetter, R.; Weber, M.; Buchner, R.; Frosch-Radivo, A. and Ulrich, S. (2009):** Pollen terminology: an illustrated handbook. New York, Springer.

**Hunziker, A.T. (2001):** Genera Solanacearum: The Genera of Solanaceae Illustrated, Arranged According to a New System, Ruggell, Gantner, 500 pages.

**Khalifa, S.F.; Badr, A.; Aboel Atta, A.I. and Abou-El-Enain, M.M. (1998):** Electrophoretic studies of seed proteins and their impact on the taxonomic relationships in the Solanaceae. J. Union Arab Biol., Cairo, 5(B): 171-180.

**Khosromehr, F.; Jafari, A. and Hamdi, M.M. (2017):** Comparative stem anatomical and palynological studies on *Hyoscyamus* L. species (Solanaceae) in northeast of Iran. Bangladesh J. Bot., 46(2): 761-765.

**Laemmli, U.K. (1970):** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

**Lashin, G.M.A. (2011):** Palynology of six species of *Solanum* (Solanaceae). Life Sci. J., 8(4): 687- 697.

**Murray, L.E. and Eshbaug, W.H. (1971):** A palynological study of Solaninae (Solanaceae). Grana, 11: 65-78.

**Natarjan, A.T. (1957):** Studies in the morphology of pollen grains–Tubiflorae. Phyton, 8: 21-42.

**Nejadhabibvash, F.; Gorttapeh, A.H. and Tofigh, S. (2014):** Genetic variation of some Iranian *Hyoscyamus* Landraces based on seed storage protein. EPP., 1 (2): 14-22.

**Olmstead, R.G. and Bohs, L. (2007):** A Summary of Molecular Systematic Research in Solanaceae: 1982-2006. Acta Hort., 745: 255 – 268.

**Palri, G.V. and Koche, S.I. (1976):** Palynological studies of *Solanum xanthocarpum* Schard & Wendl. Bontanique, 7: 125-130.

**Persson, V.; Knapp, S. and Blackmore, S. (1999):** Pollen morphology and the phylogenetic analysis of *Datura* L. and *Brugmansia* Pers. In: Solanaceae IV. (Eds.): M. Nee, D.E. Symon, R.N. Lester, J.P. Jessop. Royal Botanic Gardens, Kew, Richmond, Surrey, pp. 171-187.

**Perveen, A. and Qaiser, M. (2007):** Pollen morphology of family Solanaceae from Pakistan. Pak. J. Bot., 39(7): 2243-2256.

**Plowman, T.C. (1998):** A revision of the South American species of *Brunfelsia* (Solanaceae). Fieldiana, 39: 1-135.

**Punt, W.; Hoen, P.P.; Blackmore, S.; Nilsson, S. and Le Thomas, A. (2007):** Glossary of pollen and spore terminology. Review of Palaeobotany and Palynology, 143: 1–81.

**Sharma, B.D. (1974):** Contribution to the Palynotaxonomy of genus *Solanum* L. I. Palynology, 10: 51-68.

**Sharma, R.; Bharali, P.; Devi, N. and Sarma, G.C. (2017):** Studies on foliar micro-morphology and pollen morphology of some members of Solanaceae from the Jalukbari area of Guwahati in Assam, India. Pleione, 11(1): 125-136.

**Sheidai, M.; Khatamsaz, M. and Mosallanejad, M. (2000):** Numerical taxonomy and seed protein analysis of *Hyoscyamus* species in iran. J. Sci. I. R. Iran, 11(2):83-91.

**Song, Y.; Gu, L. and Liu, J. (2018):** Pollen morphology of selected species from the family Solanaceae. Palynology, https://doi.org/10.1080/01916122.2018.1458663.

**Srivastava, V. (1977):** Pollen dimorphism in the heterostyled *Solanum melongena* L. Curr. Sci. India, 48: 354-355.

**Tãckholm, V. (1974):** Student's Flora of Egypt. Cairo University, 2nd ed. Cairo, pp: 471-483.

**Wang, F.X.; Qian, N.F.; Zhang, Y.L. and Yang, H.Q. (1997):** Pollen flora of China. Science Press, Beijing.

**Wettstein, R. V. (1895):** Solanaceae. In Engler, A. and Prantl, K. (ed.), Die Naturlichen pflanzenfamilien, 4(36): 4-38.

**Yousaf, Z.; Masood, S.; Shinwari, Z. K.; Khan, M.A. and Rabani, A. (2006):** Evaluation of taxonomic status of medicinal species of the genus *Solanum* and *Capsicum* based on Poly Acrylamide Gel Electrophoresis. Pak. J. Bot., 38(1): 99-106.

**Yousaf, Z.; Masood, S.; Shinwari, Z. K.; Khan, M.A. and Rabani, A. (2008):** Evaluation of taxonomic status of medicinal species of the genus *Hyoscyamous*, *Withania*, *Atropa* and *Datura* based on Poly Acrylamide Gel Electrophoresis. Pak. J. Bot., 40(6): 2289-2297.