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Pollen morphology of family Solanaceae and its taxonomic significance

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Abstract: The pollen micro-morphology of family Solanaceae from the different phytogeographical region of Pakistan has been assessed. In this study, thirteen species belonging to ten genera of Solanaceae have been studied using light and scanning electron microscopy for both qualitative and quantitative features. Solanaceae is a eurypalynous family and a significant variation was observed in pollen size, shape, polarity and exine sculpturing. Examined plant species includes, Brugmansia suaveolens, Capsicum annuum, Cestrum parqui, Datura innoxia, Solanum lycopersicum, Nicotiana plumbaginifolia, Petunia hybrida, Physalis minima, Solanum americanum, Solanum erianthum, Solanum melongena, Solanum surattense and Withania somnifera. The prominent pollen type is tricolporate and shed as a monad. High pollen fertility reflects that observed taxa are well-known in the study area. Based on the observed pollen traits a taxonomic key was developed for the accurate and quick identification of species. Principal Component Analysis was performed that shows some morphological features are the main characters in the identification. Cluster Analysis was performed that separate the plant species in a cluster. The findings highlight the importance of Palyno-morphological features in the characterization and identification of Solanaceous taxa. It is concluded that both LM and SEM significantly play a key role in correct identification of taxa studied.

Key words: Solanaceae, pollen morphology, principal component analysis, taxonomic significance.

INTRODUCTION

Solanaceae is a eurypalynous family including 94 genera and about 2950 species, distribution is sub cosmopolitan and more prominent in tropical America. In Pakistan, it was represented by 14 genera and 50 species. Some leading genera of the family Solanaceae are *Solanum, Atropa, Datura, Capsicum, Nicotiana, Lycium, Hyoscyamus, Lycopersicon* and *Petunia* (Perveen & Qaiser 2007). Solanaceae is one of the important family having unique floristic, phytochemistry, economic and ethnobotanical importance (Ahmed et al. 2019, Ashfaq et al. 2019b, Adedeji & Akinniyi 2015, Bahadur et al. 2020a, Waheed et al 2020). Palynological studies are essential to explore some aspects related to pollen class and shape, P/E ratio, polar and equatorial measurements, and pollen outline because morphological evidence sometimes to depend upon alone is not adequate to demarcate plant species. Pollen grains morphology is important for the identification of plants found in numerous conditions. Palyno-morphological data provide valuable validation about closely related genera and species (Arora & Modi 2008). Morphology of pollen is highly diverse and have

unique taxonomic importance (Tellería & Daners 2003).

Morphology of pollen grains of the family Solanaceae has been studied by different researchers (Persson et al. 1999, Plowman & Knapp 1998, Gentry 1986). Pollen morphology of the genus *Solanum* was investigated by Du et al. (2018) using light microscopy and scanning electron microscopy and demonstrated sufficient pollinic heterogeneity in their shapes, aperture feature and sexine ornamentation to enable their palynological characterization. Pollen morpho diversity of some genera viz., *Capsicum, Datura, Petunia* and *Solanum* of the family Solanaceae were investigated by Rajurkar (2017) and observed that pollen grains vary from sub-oblate, oblate-spheroidal to prolate spheroidal and striate-reticulate, striate, perforate, scrabate, psilate and granulate exine sculpturing. Murry et al (1971) reported the pollen grains of Solanaceous genera using light microscopy and statistical analysis.

Pollen morphology of seven genera viz., *Datura, Lycium, Hyoscymus, Nicotiana, Physalis, Solanum* and *Withania* of the Solanaceae from Pakistan have been investigated by using light and scanning electron microscope (SEM) and reported that pollen grains were isopolar, radially symmetrical, prolate-spheroidal to oblate-spheroidal and sub-oblate to sub-prolate, tricolporate, tectal surface commonly scabrate to verrucate (Perveen & Qaisar 2007). Anil et al. 2015 investigated pollen morphology of genus Solanum and analyzed the palynological and evolutionary relationship shared with both wild and cultivated species.

There is no comprehensive study for the unique palyno-morphological characteristics of family Solanaceae in Pakistan. Therefore, this research project was design and conducted with the aim (1) to evaluate the taxonomic significance of pollen features of Solanaceae taxa, (2) to enlighten the morphology of pollen grains, pollen types and their relationship within the family using light and scanning electron microscopy and (3) to develop a taxonomic key for the accurate and quick identification of Solanaceae taxa.

MATERIALS AND METHODS

Collection and identification

Thirteen Solanaceous species were collected in different flowering seasons during the period from April 2016 to May 2017. Five samples were collected from each locality randomly (Table I and Figure 1). The collected plant species were dried, preserved, poisoned and mounted on herbarium sheet following the previously published protocol of Sufyan et al. (2018). The International Plant Name Index (IPNI) was used to get the accurate botanical names authorization for taxonomic validation. The voucher specimens were submitted in the Herbarium of Pakistan (ISL), Quaid-i-Azam University Islamabad, Pakistan.

Exploration of taxonomic characters using light microscopy

According to the previously published protocol of Ashfaq et al. (2018) anthers from the flowers where separated and they were dipped into 1-2 drops of acetic acid. Finally, they were crushed and mounted on a slide and with glycerin jelly staining was done. With the help of Leica Dialup 20 light microscope, pollen morphology and several parameters were measured. The quantitative measurements were conducted by using the SPSS 16.0 software for observing minimum, mean, standard error and a maximum of each quantitative character is denoted as 37.50(38 ±0.30)38.75 and given in (Table II).

Table I. Locality and voucher numbers of the taxa studied.

Species name	Voucher No.	Locality	Altitudes (m)
Brugmansia suaveolens (Humb. and Bonpl. ex Willd.) ***Sweet***	QAU-SA-501	Attock	2758
Capsicum annuum L	QAU-SA-702	Jhelum	234
Cestrum parqui (Lam.) L'Hér.	QAU-SA-143	Rawalpindi	508
Datura innoxia Mill	QAU-SA-434	Bhakkar	159
Solanum lycopersicum Mill.	QAU-SA-310	Jhelum	234
Nicotiana plumbaginifolia Viv.	QAU-SA-500	Attock	2758
*Petunia hybrida*M.C.Ferguson and Ottley	QAU-SA-121	Rawalpindi	508
Physalis minima L.	QAU-SA-117	Jhelum	234
Solanum americanum Mill.	QAU-SA-300	Rawalpindi	508
Solanum erianthum D. Don	QAU-SA-119	Bhakkar	159
Solanum melongena L.	QAU-SA-302	Attock	2758
Solanum surattense Burm. f.	QAU-SA-332	Attock	2758
Withania somnifera (L.) Dunal	QAU-SA-314	Mianwali	210

Scanning electron microscopy: a tool for identification

For scanning electron microscopy (SEM) the anthers were crushed in few drops of acetic acid following the method of Bahadur et al. (2018) and directly transferred to double-sided tape fixed to a stub with a gold-coated sputter and fine pipette to 150 A. The specimens were scanned and photographed with Scanning Electron Microscope (Model JEOL JSM- 5910) installed in the Central Resource Library (CRL), Department of Physics University of Peshawar, Pakistan.

Statistical analysis

Cluster analysis

To show the relationship between the species, Cluster analysis was performed based on Euclidean Distances by using the MVSP version 3.22 statistical software.

Principal component analysis

According to (Harman 1976) Principal Component Analysis (PCA) was performed with the help of statistical software XLSTAT. Five metric variables for the PCA were used i.e polar diameter, equatorial diameter, colpi length, colpi width, and exine thickness.

RESULTS

Different palynological characters of thirteen Solanaceae taxa were examined. Pollen was measured both qualitatively and quantitatively and shown in Table II and III. Solanaceous taxa exhibited a wide range of variation in sculpturing and size showing their potential taxonomic value (Figure 2 to 5). The maximum size of the pollen was observed in *Brugmansia suaveolens* (38) µm and *Datura innoxia* (37.25) µm in polar views and minimum in *Solanum erianthum* (14.3) µm. While rest of the species are intermediate in size.

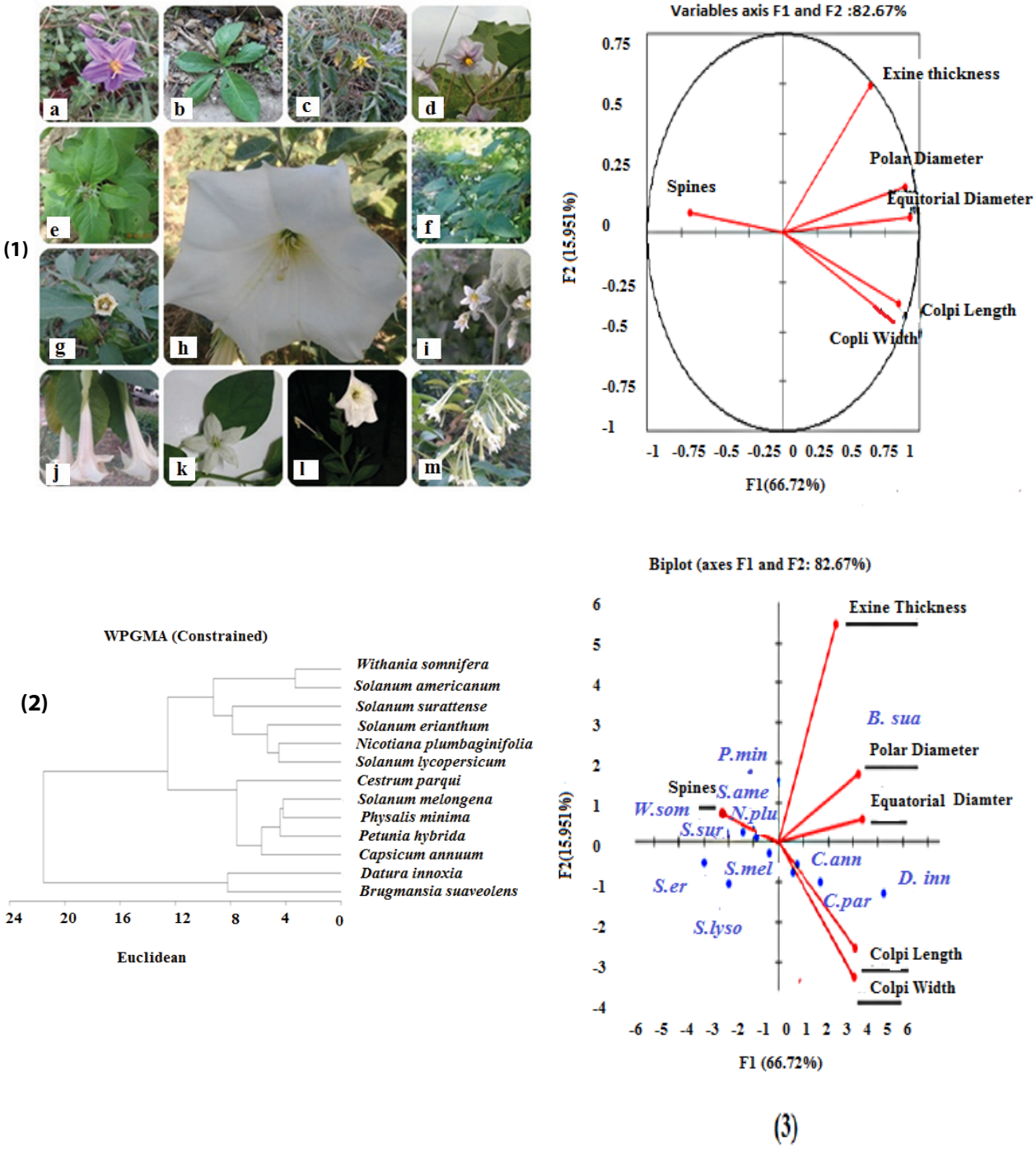

Figure 1. (1) Studied taxa of family Solanaceae (A) Solanum surattense (B) Nicotiana plumbaginigolia (C) Solanum lycopersicum(D) Solanum melongena (E) Withania somnifera (F) Solanum americanum (G) Physalis minima (H) Datura innoxia (I) Solanum erianthum (J) Brugmansia suaveolens (K) Capsicum annuum (L) Petunia hybrida (M) Cestrum parqui (2) Cluster analyses showing the relationship among the studied Solanaceous species based on the palynological characters. (3) Principal Component Analysis (PCA) among the studied Solanaceous species based on palynological characters. B.sua: Brugmansia suaveolens, C.ann: Capsicum annuum, C.par: Cestrum parqui, D.inn: Datura innoxia, L.esc: Solanum lycopersicum, N.plu: Nicotiana plumbaginifolia, P.alb: Petunia hybrida, P.min: Physalis minima, S.ame: Solanum americanum, S.eri: Solanum erianthum, S.mel: Solanum melongena, S.sur: Solanum surattense , W.som: Withania somnifera.

Table II. Quantitative data for the pollen of Solanaceous species.

Species name	Polar axis (µm)	Equatorial diameter(µm)	P/E ratio	No of Colpi	Colpi		Exine thickness (µm)
					Length (µm)	Width(µm)	
Brugmansia suaveolens	37.50(38 ±0.30) 38.75	40(40.75±0.50) 42.50	1.14	3	5(5.25±0.25) 6.25	10(10.25±0.25) 11.25	1.5-2
Capsicum annuum	22.50(23±0.30) 23.75	30(30.75±0.50) 32.50	0.83	3	5(5.25±0.25) 6.25	10(10.25±0.25) 11.25	0.8-1
Cestrum parqui	25(25.75±0.5) 27.50	32.50(34.75±0.61) 36.25	0.83	3	5(5.25±0.25) 6.25	13.75(14.50±0.30) 15	0.8-1
Datura innoxia	36.25(37.25±0.25) 37.50	40(42±0.63) 43.75	0.09	3	8.75(9.25±0.30) 10	16.25(17.25±0.25) 17.50	1-1.1
Lycopersicon esculantum	15(15.55±0.33) 16.50	17.50(17.75±0.25) 18.75	1.05	3	2.50(3.45±0.24) 3.75	7.50(7.75±0.25) 8.75	0.5-0.6
Nicotiana plumbaginifolia	17.50(17.75±0.25) 18.75	20(21.25±0.55) 22.50	0.91	3-4	5(5.1±0.1) 5.50	6.25(7.15±0.24) 7.50	0.8-1
Petunia hybrida	27.50(28±0.30) 28.75	27.50(29.25±0.75) 31.25	1.2	3	5(6±0.46) 7.50	7.50(10.75±1.08) 12.50	0.7-0.8
Physalis minima	27.50(27.75±0.25) 28.75	27.50(28±0.30) 28.75	1.08	3	2.50(3±0.3) 3.75	7.50(7.6±0.1) 8	1-1.2
Solanum americanum	22.50(23±0.30) 23.75	22.50(22.75±0.25) 23.75	1.6	3	2.50(2.90±0.25) 3.75	6.50(7.15±0.21) 7.50	1-1.1
Solanum erianthum	13.50(14.3±0.31) 15	21.25(22.25±0.25) 22.50	1.64	3	1(1.2±0.5) 1.25	6.25(6.65±0.25) 7.50	0.4-0.5
Solanum melongena	25(26±0.46) 27.50	25(25.75±0.50) 27.50	1.28	3	5(5.4±0.27) 6.25	8.75(9.5±0.30) 10	0.8-0.9
Solanum surattense	16.25(17.25±0.25) 17.50	27.50(28.10±0.37) 29.25	0.78	3	2.50(2.75±0.25) 3.75	7.50(7.75±0.25) 8.75	0.9-1
Withania somnifera	25(25.45±0.27) 25.25	20(22±0.5) 22.50	1.57	3	2.50(2.75±0.15) 3.25	8.75(9.25±0.3) 10	0.8-0.9

The polar and equatorial relationship (P/E ratio) was also noted. *Datura innoxia* is characterized by a low P/E ratio 0.09, while that of *Solanum erianthum* is high 1.64. The exine thickness was highest in *Brugmansia suaveolens* (1.5-2) µm, *Datura innoxia, Solanum americanum* (1-1.1) µm and *Physalis minima* (1-1.2) µm. Tricolporate pollen was noted as a prominent feature among the Solanaceous flora. Subspheroidal pollen shape was observed in *Brugmansia suaveolens, Capsicum annuum , Cestrum parqui, Datura innoxia, Lycopersicon esculantum, Petunia hybrida, Physalis minima, Solanum melongena,* and *Solanum surattense.* Prolate shape pollen was noted in *Solanum americanum , Solanum erianthum,* and *Withania somnifera,* while peroblate shape was observed in *Nicotiana plumbaginifolia* (Table III).

Large size colpi length was noted in *Datura innoxia* (9.25) µm and width is (17.25) µm. While small size colpi were observed in *Solanum erianthum* (1.2) µm and width are (6.65) µm respectively. Pollen fertility percentage was also noted for the Solanaceous taxa. Fertility levels show the highest percentage of fertility in *Withania somnifera* 90% and lowest in *Capsicum annuum* 69% (Table IV).

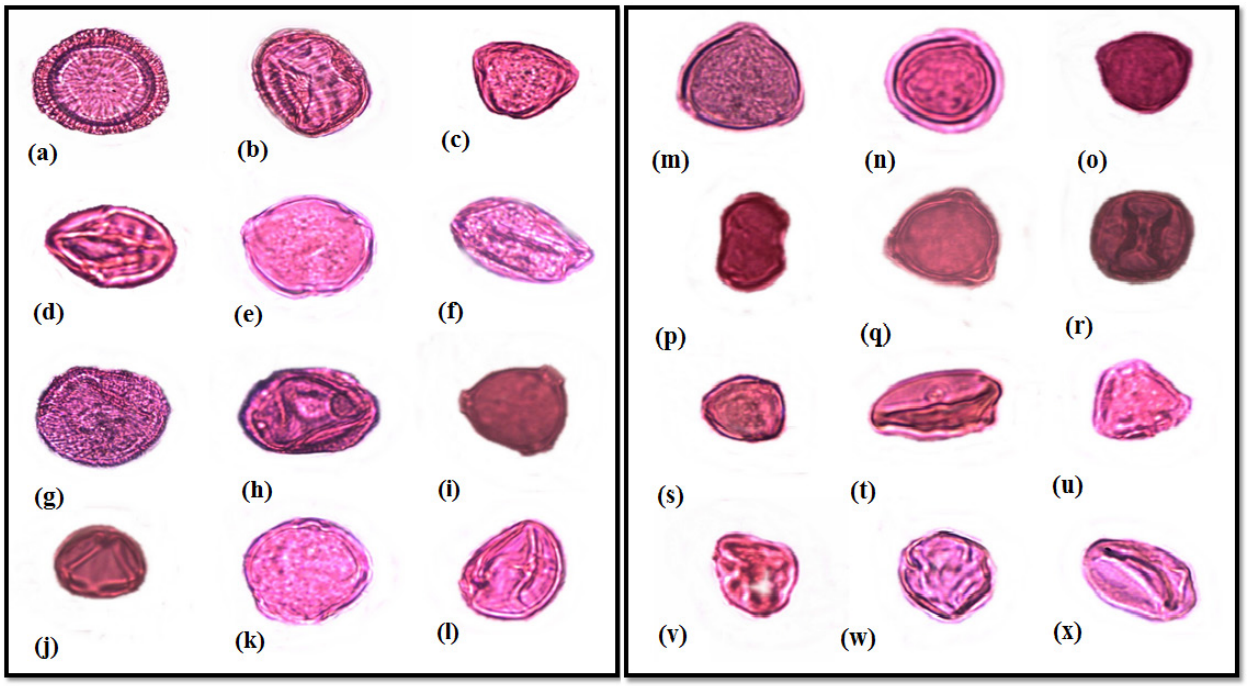

Figure 2. Light microscope photomicrographs of Solanaceae pollen grains. PV = polar view; EV =equatorial view (a) Brugmansia suaveolens (PV), (b) Brugmansia suaveolens (EV), (c) Capsicum annuum (PV) general view (d) Capsicum annuum (EV), (e) Cestrum parqui (PV), (f) Cestrum parqui (EV), (g) Datura innoxia (PV), (h) Datura innoxia (EV), (i) Solanum lycopersicum(PV), (j) Solanum lycopersicum (EV), (k) Physalis minima (PV), (l) Physalis minima (EV), (m) Solanum americanum (PV), (n) Solanum americanum (EV), (o) Solanum erianthum (PV), (p) Solanum erianthum (EV), (q) Solanum melongena (PV), (r) Solanum melongena (EV), (s) Solanum surattense (PV), (t) Solanum surattense (EV), (u) Withania somnifera (PV), (v) Withania somnifera (EV), (w) Nicotiana Plumbaginigolia (PV), (x) Petunia× alba (EV).

The Solanaceous taxa studied were separated by using cluster analysis WPGMA. Cluster analysis represents the similarity of species based on anatomical characters (Figure 1).

The dendrogram distributed the species into three major clusters. C1 (cluster 1) includes species *Datura innoxia* and *Brugmansia suaveolens* which entirely distinct from other species. C2 (cluster II) contained *Cestrum parqui, Solanum melongena, Physalis minima, Petunia hybrida, Capsicum annuum* whereas the third cluster retains *Withania somnifera, Solanum americanum, Solanum surattense, Solanum erianthum, Nicotiana plumbaginifolia, Solanum lycopersicum.* The highest similarities were observed among *Solanum melongena, Physalis minima* in C2 (cluster 2), *Nicotiana plumbaginifolia* and *Solanum lycopersicum* in C3 (cluster 3).

Principal Component Analysis (PCA) reveals the maximum contribution to the total variability at each axis (Figure 1). The Eigen values characterize the total number of variable which is often used to as certain that how many factors to hold. In the current study, three PC's limited more than one eigen value and 93.10% of total variation (Table V). Moreover, PC1 demonstrated 66.721 % of variability which had high positive loading component of the polar axis, equatorial diameter, colpi length, colpi width and exine thickness whereas negative loading of species. Second PC (PC-II) accounted 15.951% of the variation and strongly positive associated with species, polar axis, and equatorial diameter

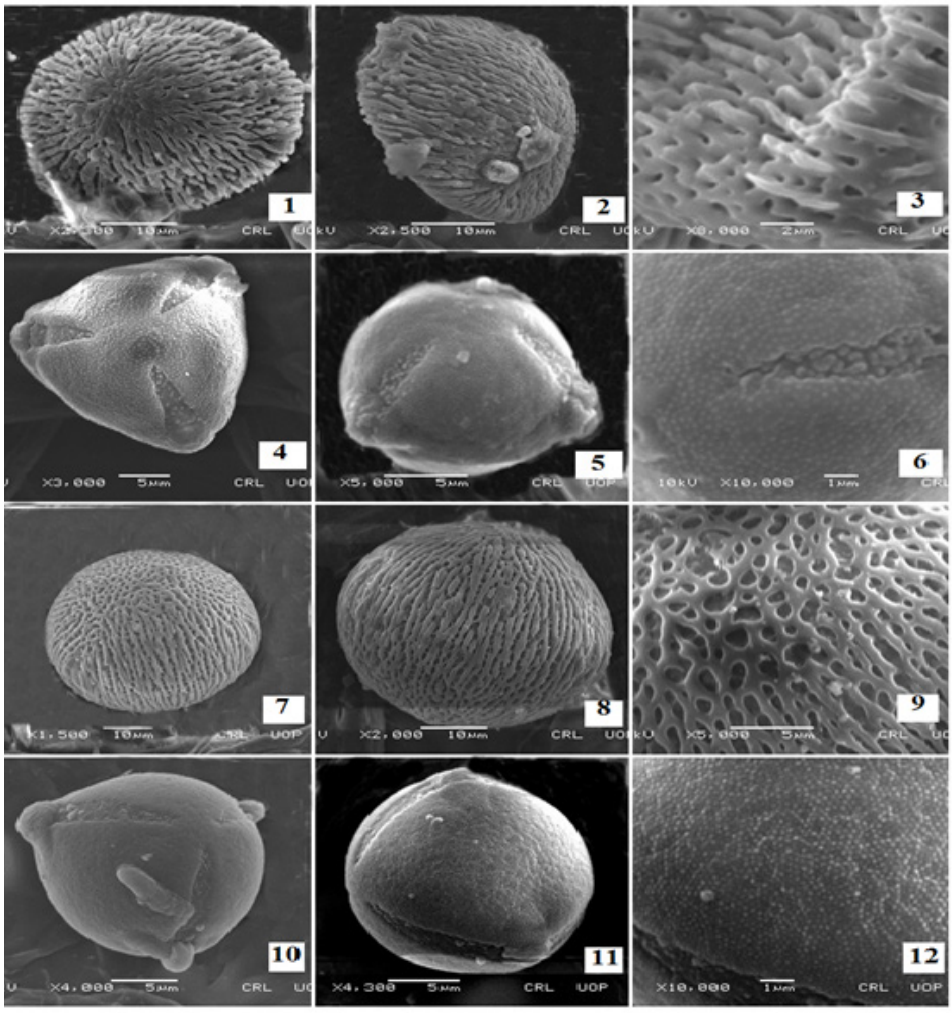

Figure 3. S Scanning electron microscope photomicrographs (SEM) of Solanaceous pollen grains. The scale bar represents 5 and 10µm for whole pollen grains and 1µm for detailed exine ornamentation. PV = polar view; EV =equatorial view; ES= exine sculpture. (1) Brugmansia suaveolens (PV), (2)Brugmansia suaveolens (EV), (3) Brugmansia suaveolens (ES). (4) Capsicum annuum (PV), (5) Capsicum annuum (EV), (6) Capsicum annuum (ES). (7) Datura innoxia (PV), (8)Datura innoxia (EV), (9) Datura innoxia (ES). (10)Solanum lycopersicum(PV), (11) Solanum lycopersicum(EV), (12) Solanum lycopersicum(ES).

and exine thickness whereas the third PC (PCIII) contained 10.433% variability in which species, polar axis, equatorial diameter, colpi length, and width were important.

Taxonomic key based on pollen micro-morphological features

1. Striate-reticulate exine
2. Pollen small, circular, oval to elliptic shaped, P/E ratio is 1.14..... *Brugmansia suaveolens*

1'. Rugulate and gemmate exine

2'. Pollen small, triangular shaped, P/E ratio is 1.57..... *Withania somnifera*

3. Micro-echinate and perforate exine
4. Pollen medium size, elliptic shaped ..... *Capsicum annuum*

3'. Perforate, verrucate, and psilate exine

4'. Pollen medium size, circular shaped..... *Cestrum parqui*

5. Exine striate and reticulate, reticule arrange in series to form long striation
6. P/E ratio is 0.09.....*Datura innoxia*

5'. Rugulate and perforate exine

6'. P/E ratio is 0.91.....*Nicotiana plumbaginifolia*

7. Exine striate and reticulate
8. Pollen trilobate shaped, P/E ratio is 1.2 ..... *Petunia hybrida*

7'. Scabrate and micro-echinate exine

Table III. Qualitative characters for pollens of Solanaceous species from arid zone of Pakistan.

Species name	Pollen Size	Pollen Shapes	amb	Pollen type	Pollen description
Brugmansia suaveolens	Medium	Subspheroidal	Circular and oval to elliptic shaped	Tricolporate	Striate-reticulate exine. Each striae are long, broad and arranged in series to form a network like structure. having psilate and perforate lumina. It was sparsely scabrate at the apocolpium and lobed at the mesocolpium
Capsicum annuum	Medium	Subspheroidal	Circular, triangular, elliptic	Tricolporate	Pollen shed as monad, exine micro-echinate, scabrate and perforate. Pollen outline lobate in polar view. Aperture are sunken having scabrate and gemmate surface. whereas surface of the oriented lobe at the mesocolpium was psilate to sparsely scabrate.
Cestrum parqui	Medium	Subspheroidal	Circular in polar view	Tricolporate	Pollen shed as monad, exine perforate, verrucate, and psilate.
Datura innoxia	Medium	Subspheroidal	Circular	Tricolporate	Pollen shed as monad, exine striate-reticulate, eache striae are narrow and arrange in series to form a network like structure. However it was reticulate at the polar area. Surface of the colpus was scabrate to sparsely gemmate
Lycopersicon esculantum	Small	Subspheroidal	Circular, lobate, elliptic shaped.	Tricolporate	Exine scabrate, and sparsely gemmate. colpus was long having pointed tip. Surface of the colpus was scabrate to verrucate while oriented lobe have psilate surface at the mesocolpium
Nicotiana plumbaginifolia	Small	Peroblate	Circular to lobate	Tri and tetra-colporate	Pollen unit monad. Exine rugulate, perforate. Surface of the colpus verrucate, and surface of the lobe at the mesocolpium was psilate to scabrate.
Petunia hybrida	Medium	Subspheroidal	Elliptic shaped in equatorial view, circular and trilobate in polar view.	Tricolporate	Pollen shed as monad and lobate outline. Exine striate, reticulate. Each striae are long and narrow. It was rugulate along the colpus area and psilate, rugulate at the apocolpium. Surface of the colpus was verrucate, gemmate and scabrate. While surface of the lobe at the mesocolpium was psilate to regulate.

Table III. (continuation)

Species name	Pollen Size	Pollen Shapes	amb	Pollen type	Pollen description
Physalis minima	Medium	Subspheroidal	Circular shape in polar view and elliptic shape in equatorial.	Tricolporate	Pollen unit monad, exine microechinate, scabrate. Surface of the colpus psilate and sparsely scabrate while tip of the colpus obtuse.
Solanum americanum	Small	Prolate	Circular shape in polar view and elliptic shape in equatorial view	Tricolporate	Pollen outline lobate and shed as monad. Exine scabrate. Colpus was long and wide having pointed tip. surface of the colpus densely gemmate to verrucate while surface of the lobe was psilate to gemmate.
Solanum erianthum	Small	Prolate	Circular	Tricolporate	Pollen unit monad, exine micro-echinate, scabrate and verrucate. Colpus was long and narrow having pointed tip. While surface of the colpus was gemmate, verrucate and scabrate.
Solanum melongena	Small	Subspheroidal	Circular, rectangular and elliptic shaped	Tricolporate	Exine thin, scabrate and perforate. Colpus was long and wide having obtuse end. while well oriented lobed at mesocolpium having densly verrucate and gemmate surface.
Solanum surattense	Medium	Subspheroidal	Circular shaped in polar view elliptic shaped in equatorial view	Tricolporate	Pollen shed as monad and exine scabrate to psilate. Colpus was long, narrow, sunken and lobed at mesocolpicum.
Withania somnifera	Small	Prolate	Triangular in polar view	Tricolporate	Exine rugulate and gemmate. Colpus was long and very wide with well develop lobe at mesocolpium.

8'. Pollen circular to elliptic shaped, P/E ratio is 1.08..... *Physalis minima*

9. Micro-echinate and verrucate exine

10. Pollen circular shaped, P/E ratio is 1.64..... *Solanum erianthum*

9'. Scabrate exine

10'. Pollen elliptic shaped, P/E ratio is 1.6 ..... *Solanum americanum*

11. Lobe depressed having scabrate to psilate surface

12. Pollen elliptic shaped, P/E ratio is 0.78 ..... *Solanum surattense*

11'. Lobe well oriented having densely verrucate and gemmate surface

12'. Pollen rectangular shaped, P/E ratio is 1.28 ..... *Solanum melongena*

13. Exine gemmate..... *Solanum lycopersicum*

DISCUSSION

Palyno-morphological characters show heterogeneous features. Tricolporate pollen type is a prominent feature in the family Solanaceae,

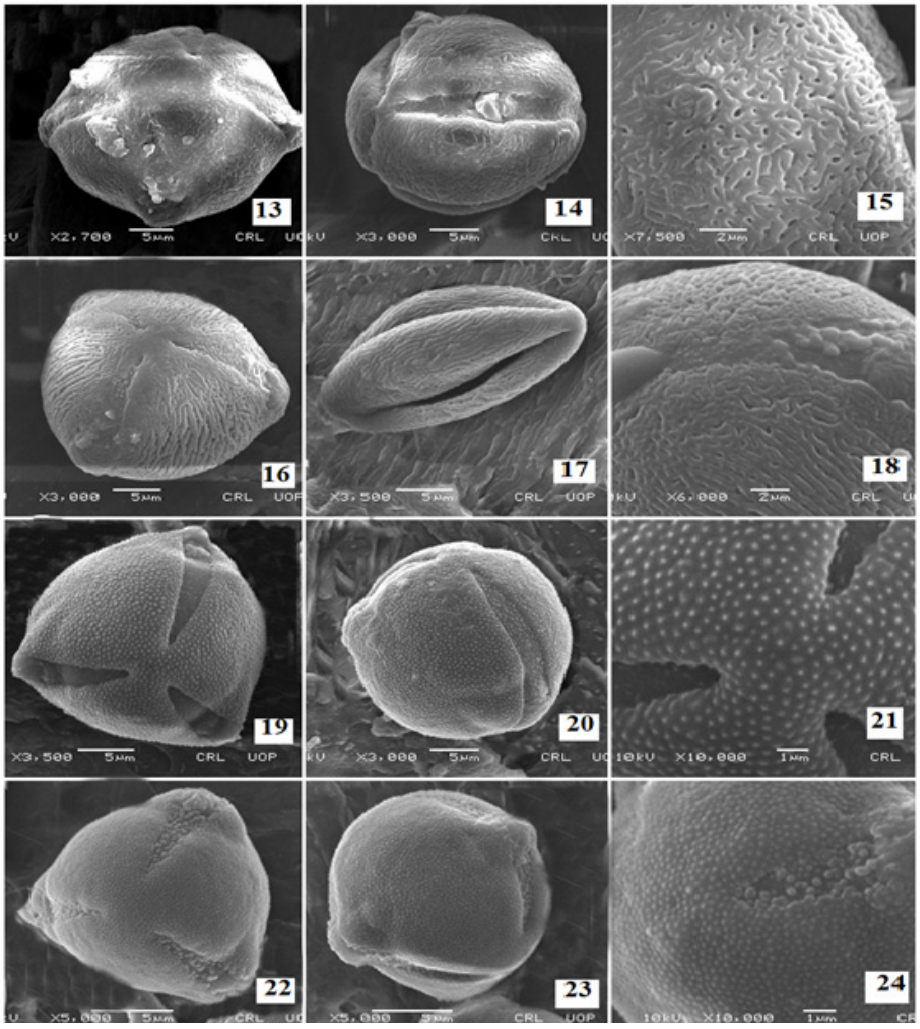

Figure 4. Scanning electron microscope photomicrographs of Solanaceous pollen grains. The scale bar represents 5 and 10µm for whole pollen grains and 1µm for detailed exine ornamentation. PV = polar view; EV =equatorial view; ES= exine sculpture. (13) Nicotiana Plumbaginigolia (PV), (14)Nicotiana Plumbaginigolia (EV), (15)Nicotiana Plumbaginigolia (ES). (16) Petunia× alba (PV), (17) Petunia× alba (EV), (18) Petunia× alba (ES). Fig 19. Physalis minima (PV), (20) Physalis minima (EV), (21) Physalis minima (ES).(22); Solanum americanum (PV), (23) Solanum americanum (EV), (24) Solanum americanum (ES).

however, pollen grains are typically isopolar, radially symmetrical, prolate- spheroidal to sub prolate. Generally, tricolporate pollen grains having a coarsely granulated colpal membrane. Different type of tectum was found i.e reticulate, regulate, verrucate and striate. In Solanaceous taxa, scabrate tectum with tricolporate pollen was observed. However, conspicuous variation was noted in pollen shape, class, type, aperture and tectal membrane. Smallest pollen size of pollen grains supports to the fact that pollens are wind pollinated rather than flowers pollinated (Barbola et al. 2006).

Significant variation was observed in micromorphology of the pollen. Pollen morphology of Solanaceae is obscure. Sometimes different tribes or subtribes have a similar type of pollen or vice versa genera referred to same tribe or subtribe may have a different type of pollen (Erdtman 1952). For instance, two species of the genus *Withania* i.e., *W. coagulans* and *W. somnifera*, have somewhat dissimilar pollen grains and fall under different types. *Withania* (*W. coagulans* and *W. somnifera*) similarity affinities with the other genera than with each other. Pollen of *W. coagulans* is very similar to grains of genus *Solanum*, while *W. somnifera*, pollen is closely related to *Lycium* grains. Eurypalynous nature of subfamily Solanoideae

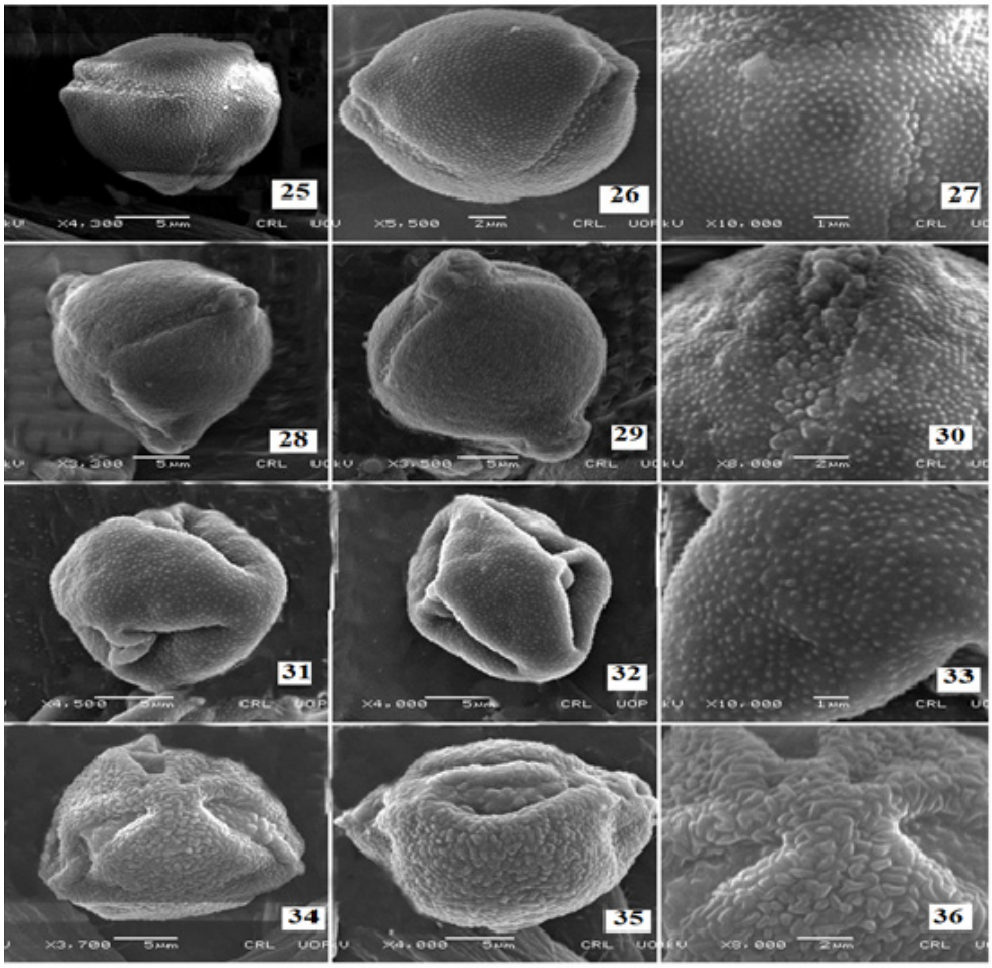

Figure 5. Scanning electron microscope photomicrographs of Solanaceous pollen grains. The scale bar represents 5 and 10µm for whole pollen grains and 1µm for detailed exine ornamentation. PV = polar view; EV =equatorial view; ES= exine sculpture. (25) Solanum erianthum (PV), (26) Solanum erianthum (EV), (27) Solanum erianthum (ES).(28) Solanum melongena (PV), (29) Solanum melongena (EV), (30) Solanum melongena (ES).(31) Solanum surattense (PV), (32) Solanum surattense (EV), (33) Solanum surattense (ES).(34) Withania somnifera (PV), (35) Withania somnifera (EV), (36) Withania somnifera (ES).

suggests its polyphyletic origin that supports (D'Arcy 1978, 1991).

According to Hunziker (1979), Solanaceae is divided into 2 sub families, Solanoideae and Cestoideae. However, the more recent study of subfamily Solanoideae by (Olmstead et al. 1999, Knapp 2002) indicates that the Solanoideae is monophyletic and derived from the Cestoideae. Only a single genus *Nicotiana* of subfamily Cestoideae having different mesocolpium and apocolpium pattern but not as diverse as of *Datura*. Subpsilate apocolpium and fossulate to the rugulose mesocolpium region are found in *Nicotiana*. Reported highest fertility percentage shows that the species is well adopted in the area. Perveen & Qaiser (2007) investigated seven genera of family Solanaceae including *Datura Capsicum*, *Solanum* and *Petunia* species. The investigated pollen was medium size oblate spheroidal, radially symmetrical, prolate- spheroidal. Which are quite like our present findings. Vijayakumari & Vilasini (2005) expose a trizonocolporate and granulate exine ornamentation in *Solanum melongena*. In present findings, *Solanum melongena* shows thin exine, scabrate and perforate. Colpus was long and wide having an obtuse end, while well oriented lobed at mesocolpium having densely verrucate and gemmate surface.

Table IV. Pollen fertility percentages for Solanaceous species from arid zone of Pakistan.

Species name	Fertile Pollen	Sterile Pollen	Fertility (%)
Brugmansia suaveolens	70	15	82
Capsicum annuum	90	40	69
Cestrum parqui	77	10	88
Datura innoxia	99	12	89
Solanum lycopersicum	88	22	80
Nicotiana plumbaginifolia	55	9	86
Petunia hybrida	70	13	84
Physalis minima	72	10	88
Solanum americanum	87	20	81
Solanum erianthum	80	17	82
Solanum melongena	54	11	83
Solanum surattense	90	21	81
Withania somnifera	86	9	90

Table V. Correlation coefficients for metric variables of principal component analysis (PCA). Ordination in for Solanaceous species.

	PC1	PC2	PC3	PC4	PC5	PC6
Species	-0.690	0.102	0.712	0.031	-0.081	-0.011
Polar diameter	0.895	0.232	0.266	0.038	0.269	-0.030
Equitorial diameter	0.938	0.079	0.069	-0.275	-0.126	-0.134
Colpi Length	0.852	-0.360	0.055	0.359	-0.095	-0.059
Colpi Width	0.840	-0.456	0.199	-0.147	-0.024	0.159
Exine thicknes	0.645	0.741	-0.039	0.097	-0.121	0.094
Eigenvalue	4.003	0.957	0.626	0.238	0.119	0.057
Variability (%)	66.721	15.951	10.433	3.966	1.986	0.943
Cumulative %	66.721	82.671	93.104	97.071	99.057	100.000

Cluster analysis presented the similarities of species based on their palynological characters. Attained cluster also supported the taxonomic key. Cluster analysis helps to identify the distinctness and similarities among different species which can be useful to determine the phylogeny. Cluster analysis showed the similarity of species within the family based on their palynological characters. In our results, the similarities were observed between *Withania somnifera*, *Solanum americanum*, *Nicotiana plumbaginifolia*, *Solanum lycopersicum*, *Solanum melongena* and *Physalis minima* while distinctness was observed in *Datura innoxia* and *Brugmansia suaveolens*. Similarly, Shah et al. (2018) characterized Dryopteridaceae Family into five clusters based on foliar morphology and stomatal traits.

According to Sharma & Paliwal (2007), Principal Component Analysis (PCA) reflects the maximum contribution to the total variability at each axis. The Eigen values represent the total number of variable which is often used to as certain that how many factors to retain. PCA was based on both the qualitative and quantitative data analysis, which shows a highly dominant variation of 66% to 82%. Both PCA and Cluster analysis used as a statistical method for the separation of species and genera (Shah et al. 2018). In the present study, we used the PCA and Cluster analysis of Pollen Morphology of Solanaceous species. A similar analysis was done on leaf anatomy of many plants group (Ozcan 2017, De Luna et al. 2017).

The observed pollen micromorphological features are taxonomically significant examined under SEM to identify Solanaceae taxa at the micro level. Similarly, Amina et al. 2020, Arshad et al. 2019, Ashfaq et al. 2018, 2019a, Bahadur et al. 2018a, 2018b, 2020b, Bano et al. 2018, Gul et al. 2019a, b, Kosenko 1999, Naz et al. 2019, Nazish et al. 2019, Qureshi et al. 2019, Rashid et al. 2018, Sufyan et al. 2018 and Ullah et al. 2018 investigated pollen of various taxa, using light and scanning electron microscopy techniques and reported that the pollen traits were of important characters for the taxonomic identifications of species and genera in different plant families. Furthermore, phytochemical, antimicrobial, and molecular studies are recommended to strengthen the knowledge about the family Solanaceae in detail (Azam et al. 2020, Hussain et al. 2019, Naeem et al. 2020, Rubab et al. 2020, Sajad et al. 2020, Sarah et al. 2019).

CONCLUSION

Pollen micromorphology of thirteen Solanaceous species provides significant taxonomic information about the qualitative and quantitative characteristics. A significant various was observed in pollen morphological features. Palyno-morphological characteristics considered to be a very supportive tool at taxonomic level. Based on the diagnostic characteristics of the pollen a taxonomic key was developed that is considered supportive in the delimitation of Solanaceous taxa. The observed pollen features through LM and SEM is a good source of taxonomic information that can help the species and genera delimitation, identification and strengthen their systematic position. The main purposes of the study are to expand the pollen morphological study thus, this study contributes to taxonomic aspects and subsequent knowledge of the family Solanaceae from Pakistan. So far, micromorphological description of other Pakistani Solanaceae taxa is recommended to use these characters in a broader taxonomic and evolutionary context. It is further suggested that chemotaxonomically and DNA characteristics of Solanaceous taxa will be more useful to develop monograph to solve the taxonomic problem for the correct identification of the species.

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