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Description of seed and pollen micromorphology and their taxonomic impact in some *Solanum* L. species

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Abstract

Seed micromorphology, of 17 species and pollen micro-morphology of 16 species of *Solanum* L. (Solanaceae) were examined using both light and scanning electron microscopy (SEM) to determine the significance of seed coat features and pollen variations as taxonomic characters. The species, used in this study, represent three subgenera and 11 sections of *Solanum* in Egypt. Macro and micro-morphological characters including seed shape, size, colour, surface; hilum shape, size, position, level; seed coat ornamentation, cell wall; shape of pollen in both polar and equatorial views, colpus membrane and apices beside exine ornamentation have been described and their taxonomic impacts have been outlined. Seed shape was either obovate or reniform and their sizes range between 1.22 ± 0.19 and 3.05 ± 0.91 mm in length and 1.14 ± 0.21 to 3.28 ±0.63 mm in diameter. Four types of seed coat appearance have been described: reticulate, cerebelloid, striate and without conspicuous cell lumen. Palynological study revealed six types of pollen shape within the studied species: prolate, subprolate, spheroidal, prolate-spheroidal, oblate-spheroidal and perprolate. Pollen length in polar view ranged from 15 ± 0.50 µm to 34 ± 2 µm but in the equatorial view it ranged from 9.06 ± 1.94 µm to 25 ± 2 µm. Four types of exine ornamentation have been recognized: scabrate, psilate, granulate and verrucate. Nineteen characters of seed and pollen micromorphological features gave useful tools in the construction of a dichotomus indented systematic key for the examined species. The major remarks are the differentiation of by their reniform seed shape from the remaining species which have ovoid seed shape. Seed coat sculpture and pollen grain feature were useful traits for the identification of the other nine taxa.

***Keywords*:** Pollen grains, seed morphology, SEM, *Solanum* species, taxonomy

Introduction

Seeds provide major morphological macro and micro-structures for the distinction between various plant species and higher taxonomic ranks (Barthlott 1981 & 1984). Studies on the Solanaceae have shown that the seed micro-morphology and epidermal patterns are of considerable systematic importance at tribal level in Hyoscyameae (Zhang *et al*. 2005) and subtribal level in Hyoscyamine (Lu & Zhang 1986); sectional level (Edmonds 1983) and generic and specific levels. Characters of the seed coat were used to distinguish species in the genera *Physalis* L., *Capsicum* L., *Tubacapsicum* (Wettst.) Makino (Zhang & Wen 1996; Zhang & Lu 1999), and in *Nicotiana* (Bahadur & Farooqui 1986), *Schwenckia* (Carvalho *et al*. 1999), *Withania somnifera* (Ghimire *et al*. 2011)) and cultivars of *Lycopersicon esculentum* (Chakrabarti *et al*. 2003). The taxonomic importance of seed characters of *Solanum* has been emphasized by a number of authors, e.g. Lester (1991), and Junlakitjawat *et al*. (2010). Khafagy *et al*. (2018) used 20 fruit and seed characters for the construction of a dichotomous key for 24 species of the family Solanaceae. Ahmed & Fadl (2016) used seed coat morphology for the investigation of the relationships among seven *Solanum* L. species collected from Taif highlands in Saudi Arabia. Scanning electron microscope (SEM) investigation of seed coat sculpturing showed three basic patterns, namely; regulate, reticulate and lavigate.

Pollen grain morphology has been found of fundamental value in the recognition and identification of pollen grains found in various conditions (Arora & Modi 2008). The pollen wall has been a subject of considerable attention, especially in attempts to establish the evolutionary history of angiosperms (Singh, 2006). The pollen morphology of *Solanaceae* had been a subject of interest for various researchers from time to time (Perveen & Qaiser, 2007). Bradley (1960) examined the

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pollen surfaces of *Solanum nigrum* and *S. dulcamara* by SEM, *S. nigrum* pollen was described as regularly papillate while *S. dulcamara* was irregularly papillate with non-apertural exine in both taxa. A light and SEM study of the pollen of 16 taxa of *Solanum*, section Basarthrum by Anderson & Gensel (1976) revealed differences in exine characters and size amounting to taxa distinctions. Edmonds (1984) confirmed the spheroidal to subprolate shape of pollen morphology in *Solanum* section Solanum using SEM, their tricolporate shape and granular surface sculpturing, typical of *Solanum* pollen, but failed to demonstrate the occurrence of exine patterns which could be of practical taxonomic use in differentiating the species in section Solanum. More investigations on the palynomorphs of genus *Solanum* were made by Sharma (1974) and Lashin (2012). However, El-Ghamery *et al.* (2018) confirmed a homogeneous morphological pattern to the pollen grains of the genus *Solanum*.

The genus *Solanum* comprises about 1500 species, widely distributed in the temperate and tropical regions of the world (Jennifer & James, 1997). Several works dealt with the species of *Solanum* in Egypt the number of species were 33 in Hepper (1998) and nine in Boulos (2002). The aim of this work is to analyse the seed coat and pollen macro and micro-morphological features as revealed by light microscopy (LM) and scanning electron microscopy (SEM) to evaluate the taxonomic impact of their features on the systematic of 17 species of *Solanum* growing in Egypt.

Material and Methods

The present investigation is based on some freshly collected seeds and pollen, of most species or taken from herbarium collections kept at Cairo University Herbarium (CAI). Localities and collectors of samples are given in Table 1. Fresh mature seeds and flowers were collected of *S. abutiloides, S. diphyllum, S. elaeagnifolium, S. laciniatum, S. lycopersicum, S. macrocarpon, S. melongena, S. nigrum, S. sinaicum, S. umbellatum* and *S. villosum* during 2016-2017 while seeds and pollen of *S. incanum, S. schimperianum, S. coagulans, S. forskalii, S. seaforthianum* and *S. virginianum* were taken from herbarium collections kept in Cairo University Herbarium (CAI).

Seed preparation for SEM was done by removing seeds from fruit pulp prior to cleaning in running tap water and air drying. The dried seeds were carefully fixed to labeled double-sided adhesive tape. Each sample was coated with gold in a Polaron E 5000 and examined by JEOL JSM scanning electron microscopy accelerated by a voltage of 15 kv at SEM Microscopy unit, Assiut University, Egypt. Ten seeds were used in the morphological description. The terminology for seed characteristics follows Barthlott (1981), Anil Kumar *et al*. (2014).

Pollen preparations for SEM were done by mounting dry pollen grains directly onto clean stubs using double-side cello tape and silver best. Each sample was coated with gold in a Polaron E 5000. The pollens were examined by JEOL JSM scanning electron microscopy accelerated by a voltage of 15 kv at SEM Microscopy unit, Assiut University, Egypt. The terminology for pollen characteristics follows Erdtman (1952) and Punt *et al.*, (2007).

Results and Discussion

Description of seed characteristics

Seed shape of the investigated species was obovate in *S. abutiloides, S. coagulans, S. elaeagnifolium, S. laciniatum, S. lycopersicum, S. nigrum, S. seaforthianum, S. sinaicum, S. umbellatum, S. villosum* and *S. virginianum* (Plate 1, Figs. 1,2); and reniform in the remaining taxa (Plate 1, Fig. 3). Seeds dimension vary greatly among the examined taxa; their size ranged from 3.05 ± 0.91 in *S. lycopersicum* x 3.28 ± 0.63 in *S. diphyllum to* 1.22 ± 0.19 x 1.14 ± 0.21 in *S. abutiloides*. The colour of the seeds in the studied species were yellow, reddish brown, dark brown and black. Seeds are black in *S. coagulans*, reddish brown in *S. laciniatum*, dark brown in *S. forskalii, S. incanum, S. seaforthianum, S. sinaicum* and *S. virginianum* and yellow in the remaining investigated taxa. Seed surface is of two main categories; smooth or hairy; they are hairy in *S. nigrum, S. lycopersicum, S. seaforthianum* and *S. villosum* but smooth in the remaining species.

Table 1. List of Solanum species used in SEM studies on seed and pollen and their subgeneric and sectional delimitation and their source and/or site of collection

Taxa	Subgenus	Section	Source and/or site
1. ***Solanum abutiloides*** (Griseb.) Bitter & Lillo	Brevantherum	Brevantherum	Zagazig: Botanic Garden, Faculty of Pharmacy, Zagazig University, 17.5.2017s.n.; Z. El-Sayed s.n, (CAI)
2. ***S. diphyllum*** L.	Solanum	geminata	Giza: Research institute of vegetables and aromatic plants, Dokki, 20.4.2017; M. Mahdy s.n. (Helwan University herbarium)
3. ***S. laciniatum*** Aiton	Solanum	Archaeosolanum	Faiyum, 30.9.1971; Imam et al. s.n. (CAI)
4. ***S. nigrum*** L.	Solanum	Solanum	18 km north of Senbellaein, Tal El-Ruba, 21-24.7.1977; A. El-Gazzar s.n. (CAI)
5. ***S. sinaicum*** Boiss.	Solanum	Solanum	Sinai: outside St. Catherine monastery, 23.4.1961; Vivi Täckholm *et al*. s.n. (CAI)
6. ***S. umbellatum*** Mill.	Brevantherum	Brevantherum	Giza: Baragile, 25.10.2016; Hamdy s.n. (CAI)
7. ***S. villosum*** Mill.	Solanum	Solanum	Giza: Faculty of Pharmacy Garden, Boulaq El-Dakrour, 10.5.2015; M. Mahdy s.n. (Helwan University herbarium).
8. ***S. coagulans*** Forssk.	Leptostemonum	Monodolichopus	Gebel Elba: Mersa Halaib, 21.1.1929; Gunnar Täckholm s.n. (CAI)
9. ***S. elaeagnifolium*** Cav.	Leptostemonum	Leprophora	Shibin el Qanatir: 6.8.1974; Mahdi Mohamed s.n. (CAI)
10. ***S. forskalii*** Dunal	Leptostemonum	Oliganthes	Gebel Elba: upstream part of Wadi Saremtai, 29.1.1962; Vivi Täckholm s.n. (CAI); 23-27.1.1929; G, Täckholm s.n. (CAI)
11. ***S. incanum*** L.	Leptostemonum	Melongena	Gebel Elba: Khor across Gebel Shallal, 24.1.1962; Vivi Täckholm s.n. (CAI)
12. ***S. macrocarpon*** L.	Leptostemonum	Melongena	Zagazig: Botanic Garden, Faculty of Pharmacy, Zagazig University, 17.5.2017; Z. El-Sayed s.n, (CAI)
13. ***S. melongena*** L.	Leptostemonum	Melongena	Eastern Desert: St. Paul Monastery Garden, 11.3.1954; L. Boulos s.n. (CAI); N Qostul, 29.12.1963; L. Boulos s.n. (CAI)
14. ***S. virginianum*** L.	Leptostemonum	Melongena	Umm Gayli, Sennaar, 19.3.1938; Vivi Täckholm 691 (CAI)
15. ***S. schimperianum*** Hochst ex Rich.	Leptostemonum	Giganteiformia	Red Sea: Jebel Wad Nubao, 23.12.1966; V. Täckholm 997 (CAI)
16. ***S. lycopersicum*** L.	Potatoe	Lycopersicum	Helwan: gardens of Helwan University, 14.1.2016; M. Mahdy s.n. (Helwan University Herbarium)
17. ***S. seaforthianum*** Andrews	Potatoe	Jasminosolanum	Giza: Orman garden, 12.4.1928; M. Hassib s.n. (CAI)

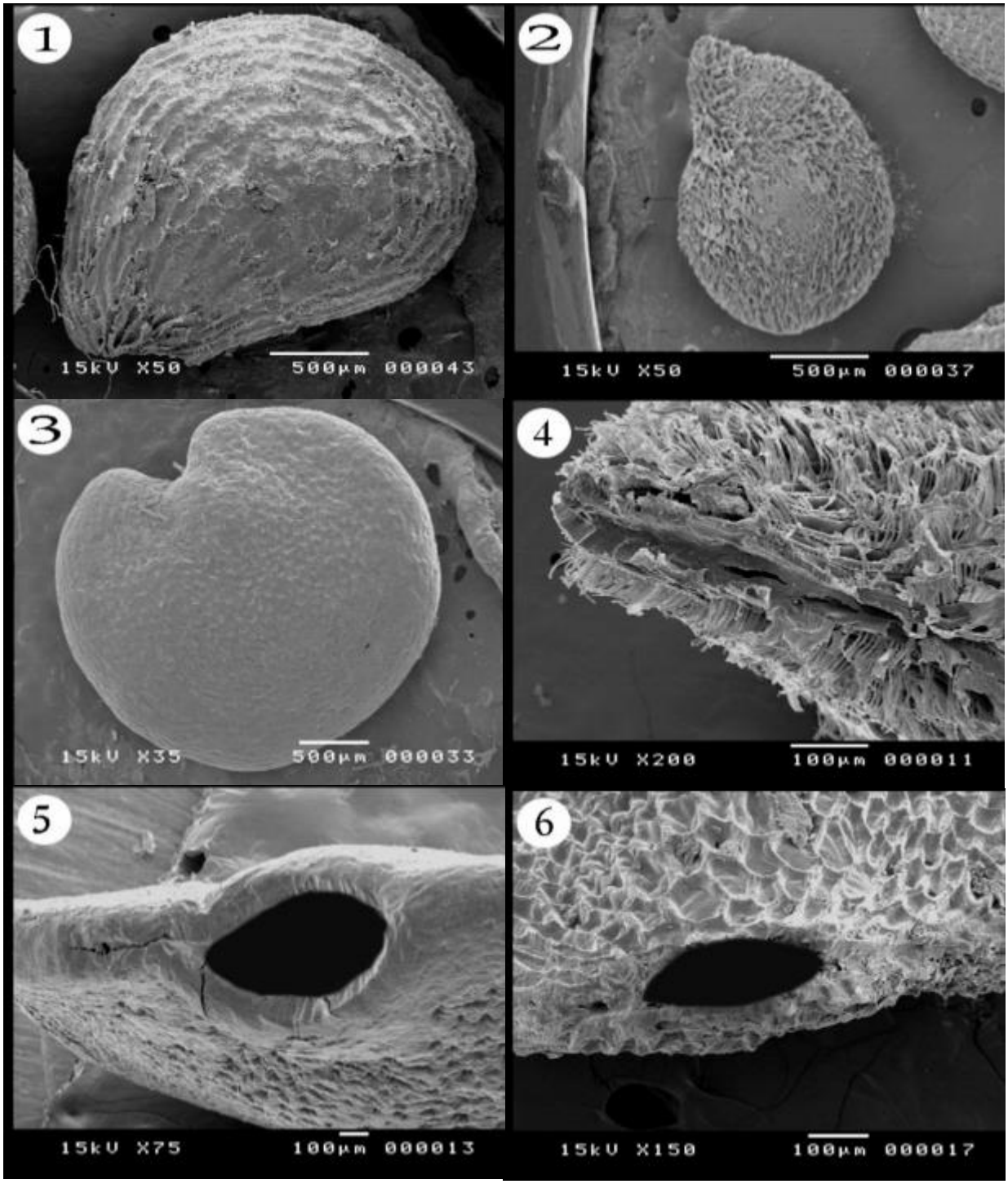

Plate I: Figs. 1-3. SEM micrographs of seed shape: Obovate **Figs. 1-2** (*S. abutiloides, S. coagulans*, *S. elaeagnifolium*, *S. incanum*, *S. laciniatum*, *S. lycopersicum, S. macrocarpon*, *S. nigrum*, *S. seaforthianum*, *S. sinaicum*, *S. umbellatum*, *S. villosum* and *S. virginianum*): **Fig.3** Reniform (*S. diphyllum*, *S. forskalii*, *S. melongena* and *S. schimperianum*; **Figs. 4-6**. SEM micrographs of hilum shape **Fig.4.** Slit like (*S. diphyllum*, *S. nigrum* and *S. villosum*), **Fig.5**. Rounded (*S. abutiloides*, *S. laciniatum*, *S. schimperianum* and *S. umbellatum*), **Fig.6**. Elliptical (*S. coagulans, S. elaeagnifolium, S. forskalii, S. incanum, S. lycopersicum, S. macrocarpon, S. melongena, S. seaforthianum, S. sianicum* and *S. virginianum*).

Hilum shape varies from slit-like in *S. villosum*, *S. diphyllum* and *S. nigrum* (Plate 1, Fig. 4); round in *S. abutiloides, S. umbellatum, S. laciniatum and S. schimperianum* (Plate 1, Fig. 5); and elliptical in the remaining taxa (Plate 1, Fig. 6). Hilum dimension varies among the examined taxa; the largest hilum size (1.953 mm × 0.578 mm) was found in *S. lycopersicum* and the smallest hilum (0.16 mm × 0.065 mm) in *S. abutiloides*. Hilum position is median in *S. incanum, S. nigrum, S. sinaicum* and *S. virginianum*; submedian-basal in *S. abutiloides*, *S. laciniatum* and *S. lycopersicum* but median in the remaining taxa. Hilum level varies from sunken in S. *forskalii, S. laciniatum, S. lycopersicum, S. macrocarpon, S. melongena, S. seaforthianum* and *S. virginianum* but slightly sunken in the remaining species.

Four different shapes of seed coat were recognized in the investigated taxa. It is cerebelloid in *S. coagulans* (Plate II, Fig. 2), *S. incanum* (Plate II, Fig. 6), *S. macrocarpon* (Plate II, Fig. 9), *S. melongena* (Plate II, Fig. 10), *S. umbellatum* (Plate II, Fig. 15) and *S. virginianum* (Plate II, Fig. 17); striated in *S. laciniatum* (Plate II, Fig. 7); without conspicuous cell in *S. elaeagnifolium* (Plate II, Fig. 4), *S. lycopersicum* (Plate II, Fig. 8), *S. nigrum* (Plate II, Fig. 11) and *S. seaforthianum* (Plate II, Fig. 13); and reticulate in the remaining taxa. Cell size varies from small, large, extremely large or not distinct between taxa. The cells are almost regular or irregular in shape; they are usually 4-7 gonal or 7-9 gonal. The cell pattern is not isodiametric in *S. abutiloides* (Plate II, Fig. 1), *S. coagulans* (Plate II, Fig. 2), *S. diphyllum* (Plate II, Fig. 3), *S. forskalii* (Plate II, Fig. 5), *S. incanum* (Plate II, Fig. 6), *S. macrocarpon* (Plate II, Fig. 9) and *S. sinaicum* (Plate II, Fig. 14); not isodiametric & elongate in one direction in *S. laciniatum*(Plate 2, Fig. 7), *S. nigrum* (Plate II, Fig. 11) and *S. villosum* (Plate II, Fig. 16); nearly isodiametric in *S. lycopersicum* (Plate II, Fig. 8), *S. macrocarpon* (Plate II, Fig. 9), *S. melongena* (Plate III, Fig. 10), *S. schimperianum* (Plate II, Fig. 12), *S. umbellatum* (Plate II, Fig. 15) and *S. virginianum* (Plate II, Fig. 17); or conjugate in *S. elaeagnifolium* (Plate II, Fig. 4) and *S. seaforthianum* (Plate II, Fig. 13).

Three main categories of elevation of cell boundaries are recognized on the examined taxa. It is raised in *S. nigrum* (Plate II, Fig. 11), *S. schimperianum* (Plate II, Fig. 12), *S. seaforthianum* (Plate III, Fig. 13) and *S. villosum* (Plate II, Fig. 16) but slightly raised in the remaining taxa. The degree of sinuosity of cell boundaries varies from straight to sinous; it is sinous in *S. coagulans* (Plate II, Fig. 2), *S. incanum* (Plate II, Fig. 6), *S. laciniatum* (Plate II, Fig. 7), *S. lycopersicum* (Plate II, Fig. 8), *S. macrocarpon* (Plate II, Fig. 9), *S. seaforthianum* (Plate II, Fig. 13), *S. villosum* (Plate II, Fig. 16) and *S. virginianum* (Plate II, Fig. 17) and straight to - slightly sinous in the remaining species. Sculpture of cell boundaries showed great variation among taxa; it is smooth to - obscurely papillate in *S. abutiloides* (Plate II, Fig. 1); smooth in *S. incanum* (Plate II, Fig. 6); papillate in *S. laciniatum* (Plate II, Fig. 7) and *S. schimperianum* (Plate II, Fig. 12); pusticulated in *S. sinaicum* (Plate II, Fig. 14); striated in *S. coagulans* (Plate II, Fig. 2), *S. elaeagnifolium* (Plate II, Fig. 4)and *S. umbellatum* (Plate II, Fig. 15); but not distinct in the remaining species.

Distal appendages on the anticlinal wall may be present or absent If present, it may ribbon-like, fibrils. The ribbon-like is divided into six shapes: A- ribbon-like in *S. abutiloides* (Plate II, Fig. 1) and *S. forskalii* (Plate II, Fig. 5); B- very thin ribbon-like deeply divided forming loops in *S. melongena* (Plate 3, Fig. 10); C- thin ribbon-like forming loops in *S. incanum* (Plate II, Fig. 6) and *S. virginianum* (Plate II, Fig. 17); D- thick ribbon-like forming loops in *S. macrocarpon* (Plate II, Fig. 9); E- thin ribbon-like in a depression in *S. umbellatum* (Plate II, Fig. 15); F- minute ribbon-like, flap-like with finger like laciniations in *S. diphyllum* (Plate II, Fig. 3). The fibril is of two main types either radiate in *S. lycopersicum* (Plate II, Fig. 8) or palaceous in *S. nigrum* (Plate II, Fig. 11), *S. seaforthianum* (Plate II, Fig. 13) and *S. villosum* (Plate II, Fig. 16).

Two types of periclinal cell wall elevation were recorded, the outer periclinal cell wall is concave in *S. forskalii* (Plate II, Fig. 5), *S. nigrum* (Plate II, Fig. 11), *S. villosum* (Plate II, Fig. 16), *S. virginianum* (Plate II, Fig. 17) and *S. umbellatum* (Plate II, Fig. 15) or flat to slightly concave in the remaining taxa. The

Description of seed and pollen micromorphology

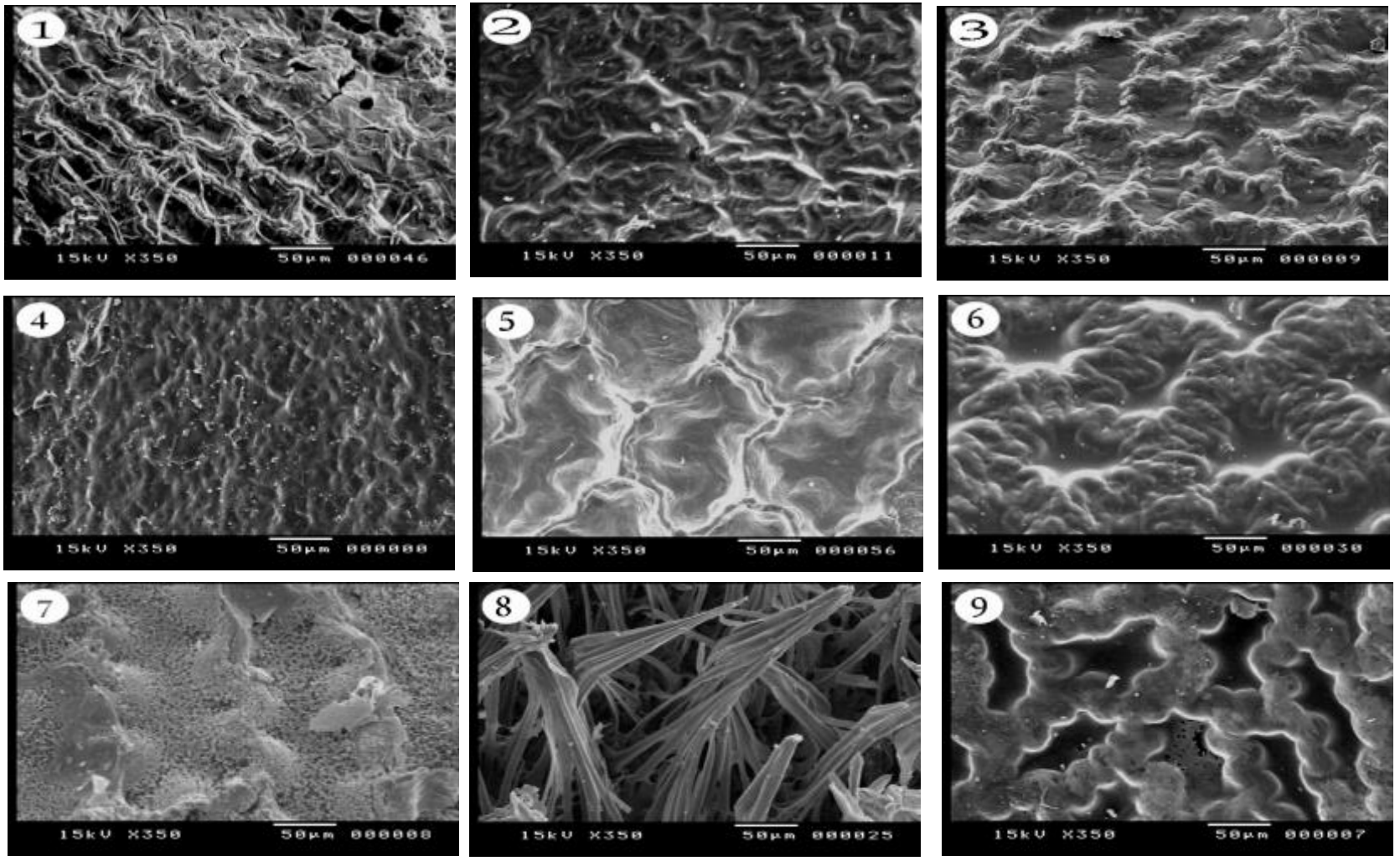

Plate II: SEM micrographs of seed coat morphology: **Fig.1.** *S. abutiloides*, **Fig.2**. *S. coagulans*, **Fig.3**. *S. diphyllum*, **Fig.4.** *S. elaeagnifloium*, **Fig.5**. *S. forskalii*, **Fig.6.** *S. incanum*, **Fig.7**. *S. laciniatum*, **Fig.8**. *S. lycopersicum*; **Fig.9.** *S. macrocarpon*.

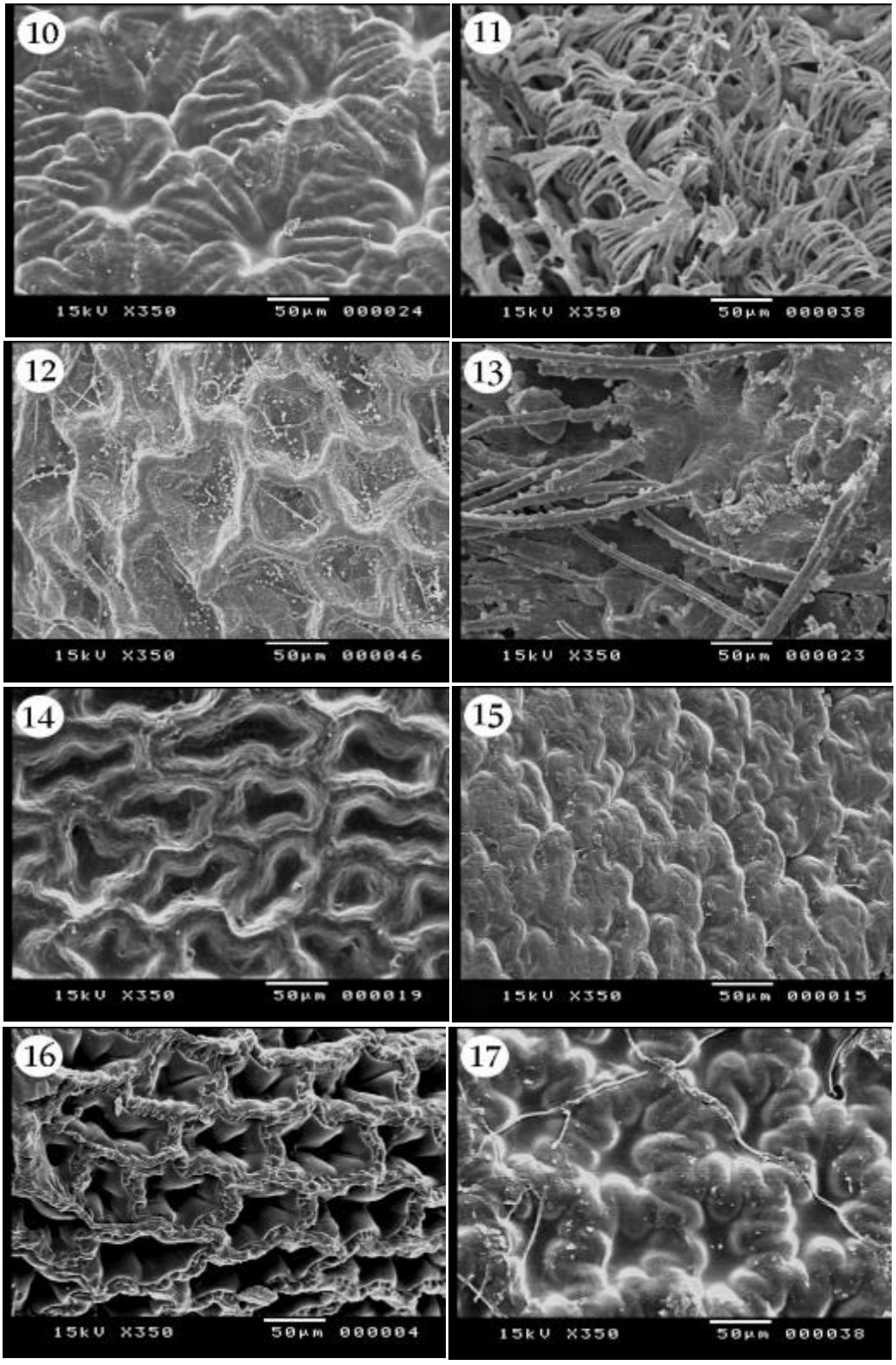

Plate II (cont.): SEM micrographs of seed coat morphology, **Fig.10**. *S. melongena*, **Fig.11**. *S. nigrum*, **Fig.12**. *S. schimperianum*, **Fig.13.** *S. seaforthianum*, **Fig.14.** *S. sinaicum*, **Fig.15**. *S. umbellatum*, **Fig.16**. *S. villosum*, **Fig.17**. *S. virginianum*.

Description of seed and pollen micromorphology

sculpture of periclinal cell wall showed great variation among the investigated species. It ranges from smooth in *S. melongena* (Plate II, Fig. 10); smooth-papillate in *S. macrocarpon* (Plate II, Fig. 9); papillate in *S. forskalii* (Plate II, Fig. 5), *S. lycopersicum* (Plate II, Fig. 8), *S. nigrum* (Plate III, Fig. 11), *S. schimperianum* (Plate II, Fig. 12), *S. sinaicum* (Plate II, Fig. 14); smooth- verrucate in *S. abutiloides* (Plate II, Fig. 1); reticulate- foveate in *S. laciniatum* (Plate II, Fig. 7); perforate-microreticulate in *S. seaforthianum* (Plate II, Fig. 13); but not prominent due to inconspicuous cell lumen in the remaining species.

Identification Key based on seed features

1a. seed coat without conspicuous cell lumen……..……..……..…...2
1b. Seed coat with conspicuous cell lumen…….....……...5
2a. Seeds broadly ovate……..……..……..3
2b. Seeds obovate-reniform……..……..…….4
3a. Anticlinal wall sinuate……..……*S. seaforthianum*
3b. Anticlinal wall straight- slightly sinuate…….….*S. elaeagnifolium*
4a. Epidermal cells irregular, 5–7– gonal; elongate in one direction…...……. *S. nigrum*
4b. Epidermal cells nearly isodiametric……...…….….. *S. lycopersicum*
5a. Seed small, 1–2 x 1–2 mm…….…….....6
5b. Seed large, 2.4– 4 x 1–3 mm…….…….…….....…….……...…...9
6a. Seed coat cerebelloid ……...……*S. virginianum*
6b. Seed coat reticulate……..…….……7
7a. Periclinal wall verrucate……...……...…..…*S. abutiloides*
7b. Periclinal wall papillate…….……8
8a. Seed glabrous; hilum shape elliptic, 0.3 x 0.1 mm……...…….……*S. sinaicum*
8b. Seed surface hairy; hilum slit- like, 0.5 X 0.1 mm……..……...*S. villosum*
9a. Seed coat striate, disconnected longitudinal furrowed……..…. *S. laciniatum*
9b. Seed coat cerebelloid or reticulate……...……..……..10
10a. Seed coat reticulate……..……..11
10b. Seed coat cerebelloid …….…..……..…13
11a. Hilum ± rounded, 0.3 x0.2 mm…….……..…..… *S. schimperianum*
11b. Hilum slit-like or elliptic, 0.3 x 0.1–0.2 mm……..……...……12
12a. Hilum slit-like, anticlinal wall straight- slightly sinuate….……... *S. diphyllum*
12b. Hilum elliptic, anticlinal wall slightly sinuate…….…… *S. forskalii*
13a. Epidermal cells 5–7- gonal…….....…….14
13b. Epidermal cells 5–9– gonal …….....15
14a. Seeds 2–3 x 2–3 mm; epidermal cells irregular…….....*S. incanum*
14b. Seeds 3–3.5 x 2–3 mm; epidermal cells regular……...….……...…...*S. umbellatum*
15a. Periclinal wall striate; seeds black…….……..……... *S. coagulans*
15b. Periclinal wall smooth; seeds yellowish or pale brown…….…….…..16
16a. Anticlinal wall sinuate, hilum elliptic….……..…..…….... *S. macrocarpon*
16b. Anticlinal wall straight – slightly sinuate, hilum rounded……..…..…*S. melongena*

Description of pollen characteristics

Pollen grain size in polar view ranges from 15 ± 0.50 µ in *S. seaforthianum* to 34 ± 2 µm in *S. laciniatum* but in the equatorial view it ranges from 9.06 ± 1.94 µm in *S. diphyllum* to 25 ± 2 µm in *S. macrocarpon*. Four different shapes of pollen were recognized in the polar view; trilobed in *S. nigrum* and *S. seaforthianum*; elliptical in *S. laciniatum*, *S. lycopersicum*, *S. sinaicum* and *S. villosum*; circular in *S. forskalii*, *S. macrocarpon* and *S. melongena*; and triangular in the remaining taxa. There are also four different shapes of pollen in the equatorial view; broad elliptic in *S. elaeagnifolium*; rhombic-elliptic in *S. schimperianum*; circular in *S. forskalii*, *S. incanum* and *S. macrocarpon*; and elliptic in the remaining taxa.

Six distinct types of pollen shapes were differentiated; oblate-spheroidal in *S. seaforthianum* (Plate III, Figs. 9,10); spherical in *S. incanum* (Plate III, Figs. 5,6); subprolate in *S. elaeagnifolium, S. forskalii*, *S. melongena* and S. *schimperianum* (Plate III, Figs. 3,4); perprolate in *S. lycopersicum* and *S. sinaicum* (Plate III, Figs. 11,12); prolate-spheroidal in *S. macrocarpon* (Plate III, Figs. 7,8); and prolate in the remaining species (Plate III, Figs. 1,2). The pollen grains have tricolporate aperture in all studied species.

The aperture type in all the investigated species are tricolporate, with very small bridged pores. Colpus size ranges from 13.8 µm in *S. diphyllum* to 30 µm in *S. laciniatum*. Colpus apex may be obtuse in *S. elaeagnifolium, S. forskalii, S. incanum, S. melongena*, *S. nigrum* and *S. schimperianum* or acute in the remaining taxa. The colpi are syncolpate in *S. abutiloides, S. coagulans, S. eleaegnifolium, S. forskalii, S. incanum, S. macrocarpon, S. melongena, S. schimperianum* and *S. seaforthianum*, while they are apocolpate in the rest of the species. There are four different exine ornamentation in the investigated taxa; verrucate in *S. coagulans* (Plate IV, Fig. 1); scabrate in *S. abutiloides, S. incanum* and *S. nigrum* (Plate IV, Fig. 2); granulate in *S. laciniatum, S. macrocarpon, S. melongena*, *S. schimperianum* and *S. villosum* (Plate IV, Fig. 3); and psilate in the remaining taxa (Plate IV, Fig. 4).

Taxonomic impact of seed and pollen characteristics

Seed and pollen morphological and microscopic characters were helpful in distinguishing the majority of the examined species (Table 2 & 3). These characters were also useful in preparing an identification key for the studied species of *Solanum*. However, the pollen and seed traits do not confirm the sectional classification of some species of the studied taxa as proposed by Lester *et al.* (2011) as for *S. diphyllum, S. coagulans, S. elaeagifolium, S. lycopersicum* and *S. seaforthianum*. The identification key divided the species into two groups based on the seed shape. The *S. lycopersicum* and *S. seaforthianum* which are delimited in subgenus Potatoe due to their morphological characters but based on seed characters they are similar to the species of subgenus Solanum because the seed surface is hairy. *Solanum diphyllum* belongs to subgenus Solanum but in resemblance with the species of subgenus Leptostemonum due to its seed shape is reinform and presence of appendages on anticlinal cell wall which is minute ribbon flap like with finger like projection. Seed colour was found useful to distinguish *S. coagulans* from the other species which have seed colour black.

Junlakitjawat *et al.*, (2010) noted the presence of fibrils or hairs on *S. nigrum* seeds of the paleaceous type growing out from the muri and on *S. lycopersicum* with thicker base and finely tapering tips; this observation agrees with our results. On the other hand, Lashin (2011) observed that the pollen shape of *S. nigrum* and *S. villosum* was prolate-spheroidal but our observation indicated that the pollen shape in these two species is prolate. He regarded pollen shape of *S. incanum* is prolate which is spherical in the examined material we examined of this species. Pollen shape for *S. incanum*, *S. nigrum* and *S. villosum* agree with the differences in morphology and seed characters.

The observed differences in pollen morphology between the studied species agree with the observations of Gbile & Sowunmi (1979) that conducted a palynological study in *Solanum* species and found highly significant differences in pollen size and shape within the studied groups (Bonnefille & Riollet; 1980 & El-Ghazali,1993). Perveen and Qaiser (2007) reported that pollen shape of *S. seaforthianum* was prolate which is inconsistent with our result indicating that it is oblate-spheroidal but agree with them in *S. nigrum* ornamentation which scabrate. Anil Kumar *et al.* (2015) described pollen shape for *S. seaforthianum* as spherical which conflicts with our records of oblate-spheroidal and also for *S. melongena* that was regarded as prolate-spheroidal but in our material, it is subprolate. However, our results agree with the above authors for pollen shape of *S. macrocarpon* which was prolate-spheroidal.

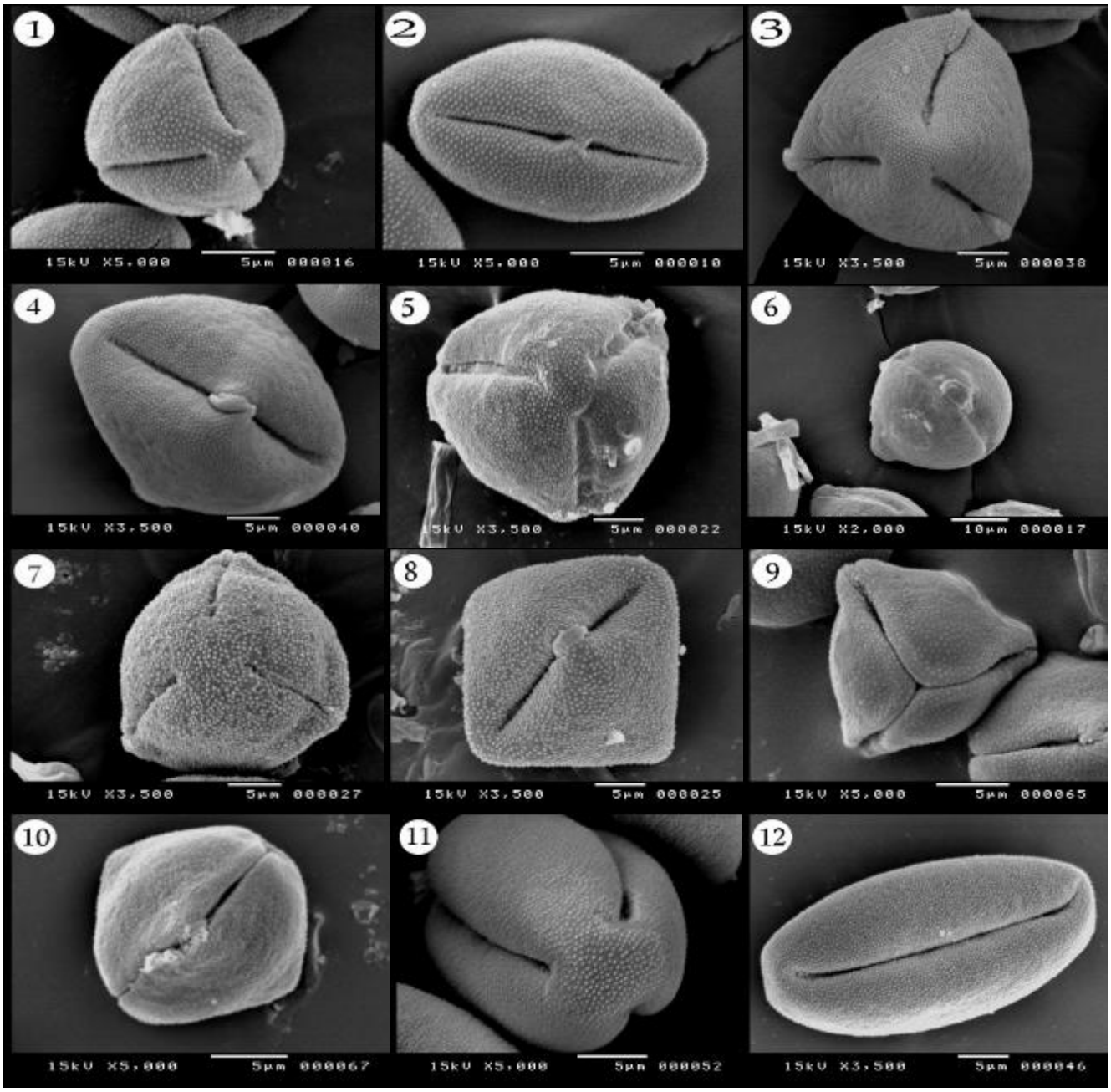

Plate III: SEM micrographs of pollen shapes common in the examined *Solanum* species. **Fig.1.** Prolate- Polar view, **Fig.2**. Prolate- Equatorial view (*S. abutiloides*, *S. coagulans*, *S. diphyllum*, *S. laciniatum*, *S. nigrum*, *S. umbellatum* and *S. villosum*), **Fig.3.** Sub-prolate- Polar view, **Fig.4**.Sub-prolate- Equatorial view (*S. elaeagnifolium*, *S. forskalii*, *S. melongena* and *S. schimperianum*), **Fig.5**. Spherical- Polar view, **Fig.6.** Spherical- Equatorial view (*S. incanum*), **Fig.7**. Prolate-spheroidal- Polar view, **Fig.8.** Prolate-spheroidal- Equatorial view (*S. macrocarpon*), **Fig.9**. Oblate-spheroidal- Polar view, **Fig.10**. Oblate-spheroidal- Equatorial view (*S. seaforthianum*), **Fig.11**. Perprolate- Polar view, **Fig.12**. Perprolate- Equatorial view (*S. lycopersicum* and *S. sinaicum*).

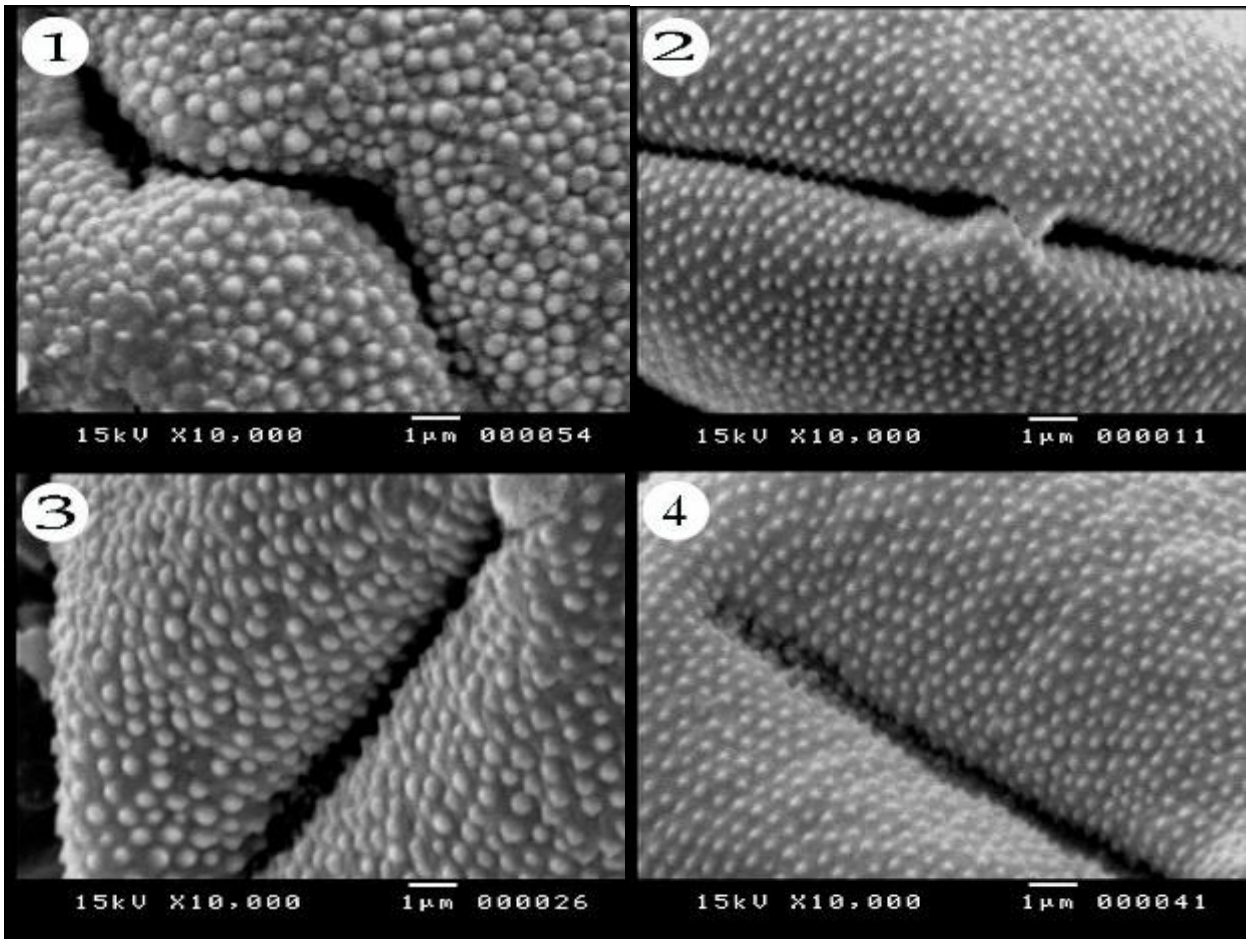

Plate IV: SEM micrographs of pollen sculpture common in the examined *Solanum* species. **Fig**.**1**. Verrucate (*S. coagulans*), **Fig.2**. Scabrate (*S. abutiloides*, *S. incanum*and *S. nigrum*), **Fig.3.** Granulate (*S. laciniatum*, *S. macrocarpon*, *S. melongena*, *S. schimperianum*and *S. villosum*), **Fig.4**. Psilate (*S. diphyllum*, *S. elaeagnifolium*, *S. forskalii*, *S. lycopersicum*, *S. seaforthianum*, *S. sinaicum*and *S. umbellatum*).

Description of seed and pollen micromorphology

Table 2. Seed shape, size and seed coat and hilum characteristics in the studied Solanum taxa

Taxa	Seed shape	Seed size LxW (mm)	Seed colour	Hilum scar		Seed coat appearance	Epidermal cell shape	Anticlinal wall		Periclinal cell wall
				Shape	LxW (mm)			Cell boundaries	Distal appendages	
1. *S. abutiloides*	obovate	1.22 ± 0.19 x 1.14 ± 0.21	yellowish-pale brown	rounded	0.2 x 0.1	reticulate	irregular, 6-7 gonal; small	straight-slightly sinous; smooth-papillate	ribbon- like with papillate surface	slightly concave; smooth- verrucate
2. *S. coagulans*	obovate-± reniform	3 ± 0.24 x 3.14 ± 0.33	dark brown-black	elliptical	0.3 x 0.2	cerebelloid	irregular, 7-9 gonal; extremely large	sinous; striated	absent	slightly concave; striated
3. *S. diphyllum*	reniform	2.94 ± 0.52 x 3.28 ± 0.63	yellowish-pale brown	slit-like	0.3 x 0.1	reticulate	irregular, 4 -7 gonal; small	straight-slightly sinous	minute ribbon, flap-like with finger- like projections	flat- slightly concave and finely striated
4. *S. elaeagnifolium*	broadly ovate- ± reniform	2.54 ± 0.41 x 2.54 ± 0.37	yellowish-pale brown	elliptical	0.6 x 0.3	without conspicuous cell lumen	irregular; conjugate	straight- lightly sinous; striated	absent	slightly concave;
5. *S. forskalii*	reniform	2.9 ± 0.64 x 3.08 ± 1.05	dark brown	elliptical	0.3 x 0.2	reticulate	irregular, 5-7 gonal; large	slightly sinous	ribbon- like	concave; papillate on the foveate surface
6. *S. incanum*	obovate-± reniform	2.61 ± 0.42 x 2.48 ± 0.32	yellowish-brown	elliptical	0.2 x 0.1	cerebelloid	regular, 5-7 gonal; large	sinous; smooth	thin ribbon forming irregular loops	slightly concave
7. S. laciniatum	obovate	2.31 ± 0.19 x 1.87 ± 0.18	pale brown	rounded	0.5 x 0.2	striate, disconnected longitudinal furrowed	regular, 4 gonal; elongate in one direction; extremely large	sinous; papillate	absent	slightly concave, reticulate-foveate, papillate
8. *S. lycopersicum*	obovate	3.05 ± 0.91 x 2.32 ± 0.73	yellowish-brown	elliptical	2 x 0.6	without conspicuous cell lumen	4-7 gonal; small nearly isodiametric;	sinous	fibrils radiate type with saw-like margin	slightly concave, papillate
9. *S. macrocarpon*	obovate-± reniform	2.75 ± 0.25 x 2.75 ± 0.25	Pale brown	elliptical	0.4x 0.2	cerebelloid	7-9 gonal; nearly isodiametric; large	sinous	thick ribbon-like forming irregular loops	slightly concave, smooth-obscurely papillate

10. *S. melongena*	reniform	2.92 ± 0.34 x 2.94 ± 0.44	yellowish –pale brown	elliptical	0.8 x 0.3	cerebelloid	regular, 7-9 gonal; nearly isodiametric; small	straight-slightly sinous	very thin ribbon-like deeply divided	slightly concave, smooth
11. *S. nigrum*	obovate	1.84 ± 0.38 x 1.58 ± 0.23	pale yellowish-pale brown	slit-like	0.6 x 0.1	without conspicuous cell lumen	irregular, 5-7 gonal; elongate in one direction; small	slightly sinous	short fibrils palaceous type, with pyramid-shaped base	concave, papillate
12. *S. schimperianum*	reniform	2.42 ± 0.43 x 2.52 ± 0.41	pale yellow-brown	rounded	0.3 x 0.2	reticulate	polygonal 5-7 gonal; large nearly isodiametric;	straight-slightly sinous; papillate	absent	slightly concave, papillate
13. *S. seaforthianum*	broadly ovate- ± reniform	2.75 ± 0.25 x 2.75 ± 0.25	pale yellowish-reddish brown	elliptical	0.3 x 0.1	without conspicuous cell lumen due to profuse surface hairs	irregular, 7-9 gonal; conjugate; large	sinous	fibrils palaceous type, verrucate with thickened base	slightly concave, perforate-microreticulate
14. *S. sinaicum*	Obovate	1.88 ± 0.17 x 1.61 ± 0.24	yellow-brown	elliptical	0.3 x 0.1	reticulate	irregular, 5-7 gonal; small	straight-slightly sinous; densely pusticulate	absent	slightly concave, papillate
15. *S. umbellatum*	broadly ovate- ± reniform	3 ± 0.24 x 2.92 ± 0.45	yellow	rounded	0.6 x 0.3	cerebelloid	irregular, 5-7 gonal; nearly isodiametric; small	slightly sinous; striated	thin minute ribbon in a depression	concave, not prominent due to inconspicuous lumen
16. *S. villosum*	obovate- ± reniform rounded	1.58 ± 0.36 x 1.25 ± 0.16	pale yellow-brown	slit-like	0.5 x 0.1	reticulate	irregular, 5-7 gonal; elongate in one direction; large	sinous	short fibrils palaceous type, with pyramid-shaped base & articulated surface	concave, papillate
17. S. virginianum	Obovate	1.9 ± 0.1 x 2.4 ± 0.1	brown	elliptical	0.4 x 0.2	cerebelloid	irregular, 7-9 gonal; nearly isodiametric; large	sinous	ribbon- like thin forming loops on top of muri	concave

Description of seed and pollen micromorphology

Table 3. Pollen characteristics in the studied Solanum taxa

Taxa	PA (µm)	EA (µm)	P/E	Amb	Shape	Aperture type	Pore size LxW (µm)	Colpus	Colpus length (µm)	Colpus apex	Exine ornamentation
1. S. abutiloides	20.63 ± 1.37	12.03 ± 1.97	1.71	Triangular	Prolate	Tricolporate	2.34 x 2.19	Syncolpate	17.19	Acute	Scabrate
2. S. coagulans	31.52 ± 2.48	20 ± 2	1.58	Triangular	Prolate	Tricolporate	4.35 x 3.91	Syncolpate	27.69	Acute	Verrucate
3. S. diphyllum	15.94 ± 0.09	9.06 ± 1.94	1.76	Triangular	Prolate	Tricolporate	2.34 x 1.56	Apocolpate	13.75	Acute	Psilate
4. S. elaeagnifolium	27.83 ± 2.63	21.74 ± 2.68	1.28	Triangular	Sub-prolate	Tricolporate	5.43 x 6.52	Syncolpate	23.26	Obtuse	Psilate
5. S. forskalii	22.39 ± 2.36	19.35 ± 1.65	1.16	Circular	Sub-prolate	Tricolporate	4.35 x 4.35	Syncolpate	20.65	Obtuse	Psilate
6. S. incanum	23.85 ± 1.45	23.85 ± 1.65	1	Triangular	Spherical	Tricolporate	4.0 x 4.8	Syncolpate	22.69	Obtuse	Scabrate
7. S. laciniatum	34 ± 2	22 ± 2	1.55	Elliptical	Prolate	Tricolporate	Not distinct	Apocolpate	30	Acute	Granulate
8. S. lycopersicum	28.69 ± 1.11	13.26 ± 1.74	2.16	Elliptical	Perprolate	Tricolporate	Not distinct	Apocolpate	24.35	Acute	Psilate
9. S. macrocarpon	26.09 ± 2.01	25 ± 2	1.04	Circular	Prolate-spheroidal	Tricolporate	4.35 x 3.48	Syncolpate	19.57	Acute	Granulate
10. S. melongena	19.13 ± 2.07	15.22 ± 2.18	1.26	Circular	Sub-prolate	Tricolporate	3.48 x 1.3	Syncolpate	15.87	Obtuse	Granulate
11. S. nigrum	28.48 ± 0.48	17.61 ± 0.59	1.62	Trilobed	Prolate	Tricolporate	2.39 x 1.3	Apocolpate	24.57	Obtuse	Scabrate
12. S. schimperianum	26.09 ± 1.09	22.39 ± 2.29	1.17	Triangular	Sub-prolate	Tricolporate	3.26 x 4.35	Syncolpate	22.61	Obtuse	Granulate
13. S. seaforthianum	15 ± 0.50	15.16 ± 1.46	0.99	Trilobed	Oblate-spheroidal	Tricolporate	4.69 x 1.56	Syncolpate	14.06	Acute	Psilate
14. S. sinaicum	33.04 ± 2.04	15.65 ± 1.35	2.11	Elliptical	Perprolate	Tricolporate	Not distinct	Apocolpate	28.26	Acute	Psilate
15. S. umbellatum	20.65 ± 1.35	13.69 ± 1.31	1.51	Triangular	Prolate	Tricolporate	2.17 x 3.48	Apocolpate	17.39	Acute	Psilate
16. S. villosum	31.52 ± 1.48	16.52 ± 1.48	1.91	Elliptical	Prolate	Tricolporate	Not distinct	Apocolpate	27.83	Acute	Granulate

PA= Polar axis length, EA= Equatorial axis length, P/E= Polar/Equatorial, LxW= Length x Width

Description of seed and pollen micromorphology

Identification Key based on palynological characters

1a. Pollen grain small, 15–19 x 9–15.22 µmm…….……..…….2
1b. Pollen grain large, 20.6 – 34 x 12–25 µmm…….…….……...……4
2a. Exine granulate, colpus apex obtuse……*S.melongena*
2b. Exine psilate, colpus apex acute……..…….……..…..……..…3
3a. Pollen grain prolate…….……...…….. *S. diphyllum*
3b. Pollen grains oblate-spheroid…….……...…… *S. seaforthianum*
4a. Exine verrucate ……...…..……. *S. coagulans*
4b. Exine psilate, granulate or scabrate…….....……..5
5a. Exine scabrate …..……..……...……..……..6
5b. Exine granulate or psilate……..…….……..…..8
6a. Pollen grains spheroidal……..…….……. *S. incanum*
6b. Pollen grains prolate ……..……..7
7a. Pollen grains 28.48 x 17.6 µmm; colpus apex obtuse……..… …...*S. nigrum*
7b. Pollen grains 20.63 x 12.03 µmm; colpus apex acute…….…….…..*S. abutiloides*
8a. Exine psilate……..……9
8b. Exine granulate…….……13
9a. Pollen grain 33.05 x 15.56 µmm; perprolate……..…….....*S. sinaicum*
9b. Pollen grain 20.65 – 28.69 x 13.69 – 21.74 µmm; prolate- subprolate……10
10a. Colpus apex obtuse ……..……...……...…11
10b. Colpus apex acute……..……..……..12
11a. Grains circular in equatorial view ……..…….…..…... *S. forskalii*
11b. Grains broadly elliptic in equatorial view….…… *S. elaeagnifolium*
12a. Pollen grains perprolate…...…….…….*S. lycopersicum*
12b. Pollen grains prolate …….……...…*S. umbellatum*
13a. Colpus apex obtuse……... *S. schimperianum*
13b. Colpus apex ocute……...……14
14a. Equatorial view circular; colpus short 19.57 µmm…….. *S. macrocarpon*
14b. Equatorial view elliptic; colpus large 27 – 30 µmm……..….15
15a. Pollen 31.52 x 16.5 µmm; colpus 27.8 µmm……...……....*S. villosum*
15b. Pollen 34 x 22 µmm; colpus 30 µmm …….…..……*S. laciniatum*

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