

Comparative Micromorphology of the Seed Surface of *Solanum melongena* L. (eggplant) and Allied Species

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ABSTRAK

Mikrostruktur permukaan biji *Solanum melongena* L., *S. incanum* L. dan beberapa spesies yang lain diteliti dengan menggunakan SEM. Biji-biji telah diberi rawatan pelelasan enzim (*Driselase*) sebelum diteliti untuk mengikis lapisan luar biji yang mengaburkan penglihatan. Kebanyakan biji mempunyai struktur yang kelihatan seperti rerambut atau fibril mengelilingi setiap sel, yang mana merupakan tunjulan daripada penebalan dinding-dinding sisi epidermis luar sel testa. Struktur kulit biji *S. melongena* dan *S. incanum* telah ditemui sebagai seragam dalam keseluruhan masukan yang digunakan, menunjukkan hubungan yang rapat di antara dua takson ini. Bentuk kulit biji *S. torvum* Sw., *S. tomentosum* L., *S. aethiopicum* L., *S. kwebense* Br. and Wr. dan *S. cinereum* R. Br. adalah sangat berbeza bukan sahaja dengan *S. melongena* dan *S. incanum* tetapi juga di antara mereka, menunjukkan perbezaan di antara takson-takson ini.

ABSTRACT

The seed surface microstructure of *Solanum melongena* L., *S. incanum* L. and some other species were examined using SEM. Seeds were given enzyme (*Driselase*) etching treatment prior to examination to sweep off the outer layer of cells which obscured observation. Most seeds were characterised by hair-like structures or fibrils which surrounded each cell and which are strands of thickening in the lateral walls of the outer epidermal cells of the testa. Seed coat structure of *S. melongena* and *S. incanum* was found to be highly uniform in the samples examined indicating a close relationship between them. The seed coat patterns of *S. torvum* Sw., *S. tomentosum* L., *S. aethiopicum* L., *S. kwebense* Br. and Wr. and *S. cinereum* R. Br. were considerably different not only from *S. melongena* and *S. incanum* but also between themselves which supports the distinctness of these taxa.

INTRODUCTION

Solanum melongena L. (eggplant or brinjal) is a variable species and its taxonomic relationship with other taxa is still under discussion. Some experimental taxonomic techniques including morphology, seed protein and isozyme electrophoresis, crossability studies, etc. indicate that the most closely related taxon to *S. melongena* is *S. incanum* L. (Bhaduri 1951; Pearce 1975;

Narasimha Rao 1979; Reayat Khan 1979; Pearce and Lester 1979; Zohary 1983; Choudhury, 1984; Hasan and Lester 1988). *S. incanum* shows great morphological diversity which is distributed over a wide area in southern Asia and eastern Africa (Bitter 1923; Jaeger 1986; Jaeger and Hepper 1986). This variability causes taxonomic confusion in identification and classification of these two taxa as well as of closely related species.

In this study, surface characters of the seed coat were examined by scanning electron microscopy (SEM) to distinguish between several ecotypes *S. melongena*, *S. incanum*, and some other species. The morphology of seed coat is usually stable and is little influenced by external environmental conditions whilst the seeds develop and ripen within the fruit (Heywood 1971; Cole and Behnke 1975; Barthlott 1981). Therefore seed characters can provide valuable information in the delimitation and identification of species.

Various workers have utilised seed coat structures in comparative surveys of members of the genus *Solanum*. Some of them have also carried out studies on embryo and seed development (Edmonds 1983; Morris 1986; Gunn and Gaffrey 1974; Whallen 1979 and 1984). In most species of *Solanum*, the epidermal surface of the seed coat is relatively smooth and featureless due to the presence of the flat tangential wall of the epidermis, or even if these have come off, the inner details may be obscured by a covering of hair-like processes derived from secondary thickening of the radial walls, which have been variously called hairs, spinous hair, pseudohairs, fibrils, or rod-like fibrous thickenings (Edmonds 1983; Lester and Durands 1984). Therefore, any attempt to describe seed coat characters for taxonomic purposes is confounded. However, by breaking down or sweeping off the outer layer, the hidden intricate structures beneath are revealed.

Lester and Durands (1984) developed a technique involving the enzyme Driselase to remove the outer tangential epidermal cell wall and/or excessive hair-like processes in the radial walls prior to observation. Using this technique, they were able to show that the wild species, *S. anguivi*, was distinct from *S. violaceum*, the taxa of which had been treated previously as a single species under the name *S. indicum*.

This technique was used to evaluate the possible use of seed surface microstructure in the identification and classification of *S. melongena*, *S. incanum* and some other species.

MATERIALS AND METHODS

Seeds from several accessions of *S. melongena* and *S. incanum* in section *Andromonoecum* and some allied species in sections *Oliganthes* and *Torvaria* were used (Table 1).

About 10 clean, dry seeds of each accession were soaked and washed thoroughly in distilled water in 10 cm³ vials for about 1.5 hr. They were then subjected to surface sterilisation by soaking the seeds in 5% (v/v) Domestos solution (sodium hypochlorite) for about 10 min. The seeds were thoroughly sterilised to prevent contamination by bacteria which would digest the enzyme and thus reduce enzyme activity during the digestion period. The seeds were then thoroughly washed with distilled water before being soaked with 1.0 ml of 2% Driselase enzyme (Fluka AG) in Sorensens phosphate buffer pH 5.5 (5 parts 1/15 M Na₂HPO₄ + 95 parts 1/15 M KH₂PO₄) in the vials.

The vials were kept in a waterbath at 30°C for about 24 hr, with mixer blades providing gentle agitation of the vials and their contents. Where appropriate, the seeds were washed and sterilised again, and treated with fresh enzyme for a further 24 hr. The seeds were subsequently thoroughly rinsed in distilled water and were left to dry, by allowing them to stick inside the wall of open vials, at room temperature.

The dry seeds were carefully mounted on SEM stubs dagged with silver conductive paint and were sputter-coated with gold. The seeds were observed under a Cambridge Stereoscan 600 Scanning Electron Microscope. For each accession a standard photograph (X40 μ) of the flank away from the hilum of the seed was taken. Subjective comparisons of the seed surface between accessions were made both directly from the SEM screen and from the photographs produced later.

RESULTS AND DISCUSSION

SEM micrograph of seeds of *S. melongena*, *S. incanum* and related species are shown in Figures 1-3.

In general, seed coat pattern within *S. melongena* and *S. incanum* is fairly uniform between accession. However, there were some differences in size, shape, convolution, fibril shape, and depth of the lumen or pore of the cell. In general, the shape of the cell was more elongated near and at the edges of the seeds. In some accessions such as S.1501, these cells were relatively larger than the others (Figure 2). In a few accessions, i. e. S.0859, S.2052 and S.2024 (Figure 2), convolutions

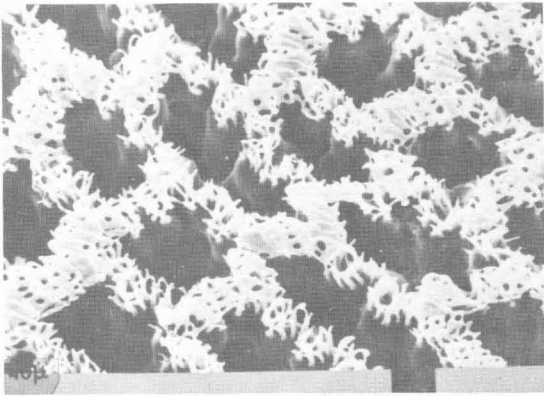
TABLE 1
Species used for the micromorphology of seed
surface examination under SEM

Sections	Species	Accessions no	Status/group	Country of origin
<i>Andromonoecum</i>	<i>S.melongena</i> L.	S.2458	advanced cultivar; large fruit.	United Kingdom
<i>Andromonoecum</i>	<i>S.melongena</i> L.	S.2444	primitive cultivar, small round fruit.	Thiland
<i>Andromonoecum</i>	<i>S.melongena</i> L.	S.2426	wild/weedy; prickly.	Malaysia
<i>Andromonoecum</i>	<i>S.melongena</i> L.	S.2424	primitive cultivar; small round fruit.	Malaysia
<i>Andromonoecum</i>	<i>S.melongena</i> L.	S.2310	wild/weedy;prickly.	Malagasay
<i>Andromonoecum</i>	<i>S.melongena</i> L.	S.1554	weeds; hairy-prickly, low and stranggling habit.	India
<i>Andromonoecum</i>	<i>S.incanum</i> L.	S.0931	truly wild; ovate broad-leaved.	Israel
<i>Andromonoecum</i>	<i>S.incanum</i> L.	S.1518	truly wild; lanceolate broad-leaved.	South Africa
<i>Andromonoecum</i>	<i>S.incanum</i> L.	S.1501	truly wild; elongated, narrow leaved.	Zambia
<i>Andromonoecum</i>	<i>S.incanum</i> L.	RNL 336	truly wild; elongated, broad leaved.	Zimbabwe
<i>Andromonoecum</i>	<i>S.incanum</i> L.	S.2024	truly wild; broad- lanceolate, narrow leaved	Kenya
<i>Andromonoecum</i>	<i>S.incanum</i> L.	S.2052	truly wild; broad- lanceolate, narrow leaved	Kenya
<i>Andromonoecum</i>	<i>S.incanum</i> L.	S.0859	truly wild; broad- lanceolate, narrow leaved.	Uganda
<i>Andromonoecum</i>	<i>S.incanum</i> L.	S.1782	truly wild; broad- lanceolate, narrow leaved.	Tanzania
<i>Oliganthes</i>	<i>S.aethiopicum</i> L.	S.2339	truly wild	Upper Volta
<i>Oliganthes</i>	<i>S.tomentosum</i> L.	S.1040	truly wild	Bostwana
<i>Oliganthes</i>	<i>S.cinereum</i> R.Br.	S.0202	truly wild	South Australia
<i>Oliganthes</i>	<i>S.kwebense</i> Br. & Wr.	S.1789	truly wild	South Africa
<i>Torvaria</i>	<i>S.torvum</i> Sw.	S.2415	wild/weedy	Malaysia

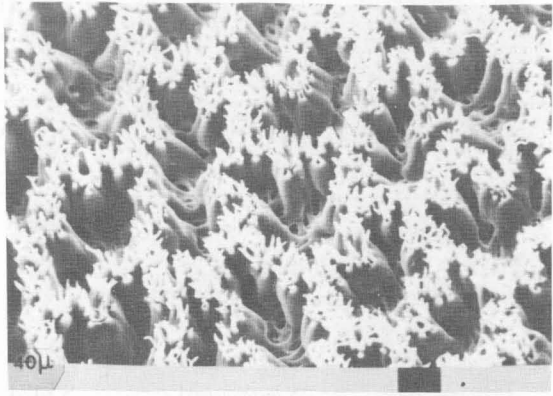
surrounding the lumen were more invaginated than in others. For some accessions, such as S.1782, S.1518 and RNL336 (*Figure 2*), the cell lumen or pore was relatively deep, but in some others such as S.2052 (*Figure 2*) the lumens were

shallow and narrow which might be due to more extensive secondary cell wall thickening.

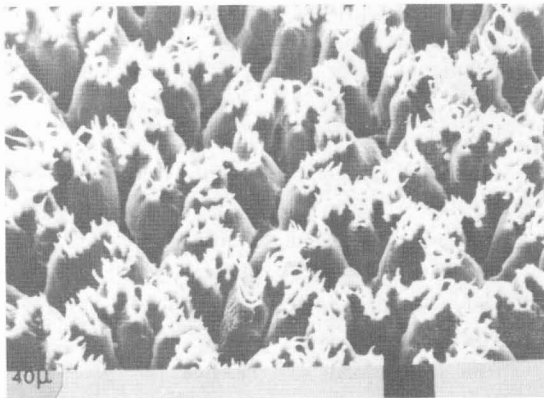
The fibrils surrounding each cell were strands of lignified thickening in the radial walls of the epidermal cell (Edmonds 1983), which arise



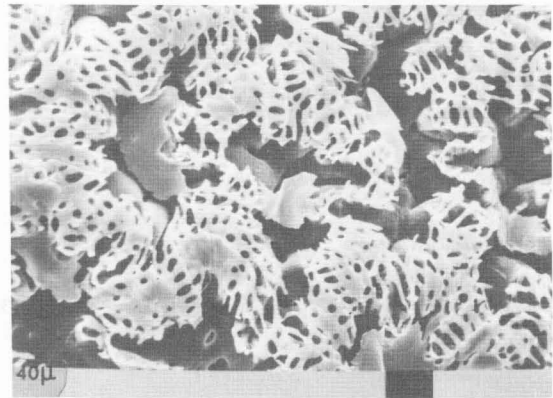
S.2426



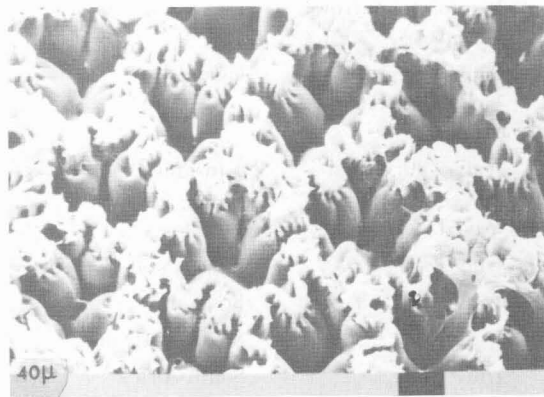
S. 2444



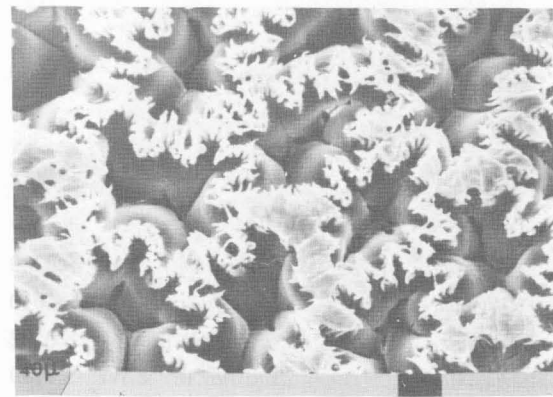
S.2424



S. 2458

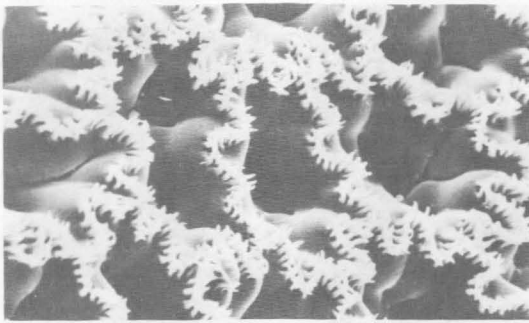


S. 2310

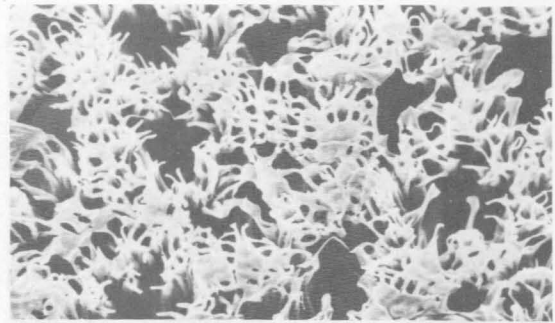


S. 1554

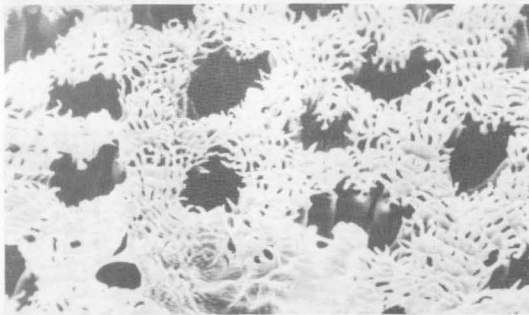
Figure 1: Seed surface micrographs for several accessions of *S.melongena*



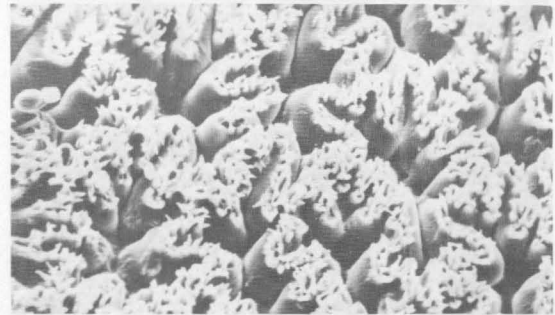
S. 1501



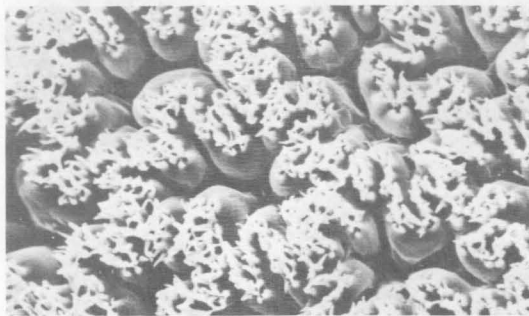
RNL 336



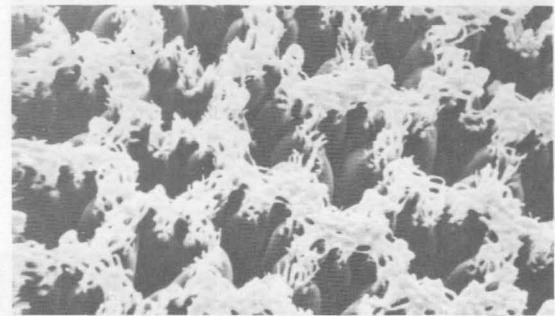
S. 1518



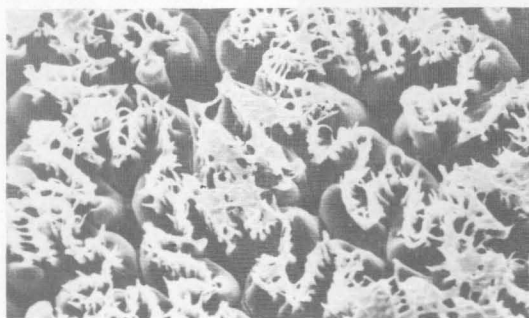
S. 0931



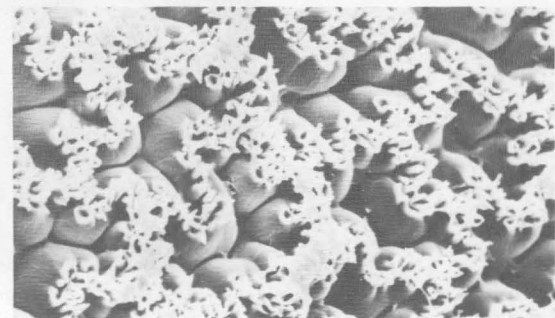
S. 0859



S. 1782



S. 2024

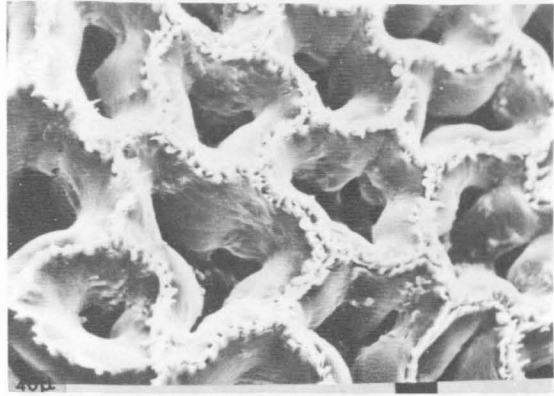


S. 2052

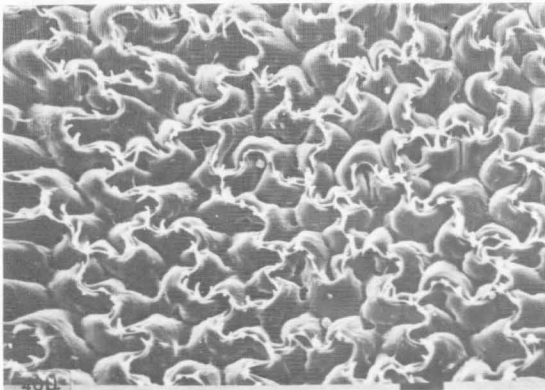
Figure 2: Seed surface micrographs for several accessions of *S. incanum*



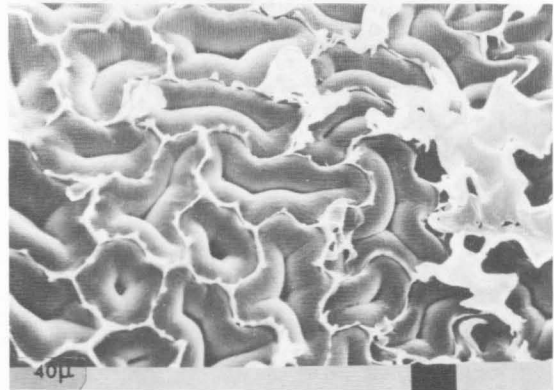
Solanum aethiopicum



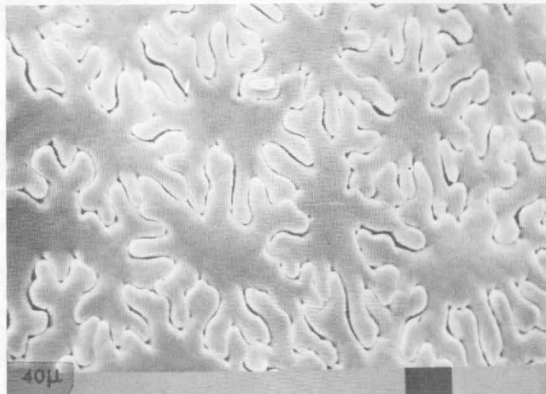
Solanum tomentosum



Solanum cinereum



Solanum kwehense



Solanum torvum

Figure 3: Seed surface micrographs for several others species in genus Solanum.

from pyramid-shaped bases. These structures showed considerable variation both between and within *S. melongena*, *S. incanum* and the other species.

The fibrils of some accessions extend as thickenings of the outer tangential wall, giving a net-like structure, such as those found in S.2426, S.2458 and S.2310 (Figure 1). In some other accessions, including S.2024 and RNL336 (Figure 2), the fibrils were long, curved and irregularly spread, Accessions S.1501, S.0931, S.0859 (Figure 2), S.2424 and S.2444 (Figure 1) have short or medium-length fibrils which were mostly upright. However, variation in fibril structure was also affected by the efficiency of the etching treatment by the enzyme. Careful and detailed observations of the fibril structures of the seed coat failed to distinguish consistently between seed of *S. melongena* and *S. incanum* nor between them and the other species examined, confirming that there was general similarity between them.

This uniformity in seed coat characters not only indicates the coherence of these species, but that these characters are of little taxonomic value in discriminating within this species complex. Edmonds (1983) came to a similar conclusion based on the results of her study on seed surface structure in taxa belonging to *Solanum* section *Solanum*, where again many morphologically distinct species had similar seed surface features.

Nevertheless, seed coat structure may yet provide useful taxonomic characters at the sectional and generic levels. This study indicates that the seed surface structure of *S. torvum* Sw. (Figure 3) in section *Torvaria* is completely different from the other species surveyed, having convoluted cells walls with a sinuous pattern. Cell lumens and fibril are also lacking.

S. kwebense Br. & Wr. and *S. cinereum* R.Br. (Figure 3) in section *Oliganthes* are also distinguishable from the other species examined. But they are somewhat similar to each other in size and shape although the convolutions are more invaginated in *S. cinereum*.

The seed coats of *S. aethiopicum* L. and *S. tomentosum* L. (section *Oliganthes*) (Figure 3), are rather different from those of *S. melongena*-*S. incanum* complex, although they are similar in some aspects such as cell size.

CONCLUSION

The micromorphological variation in seed surfaces failed to distinguish *S. melongena* from *S. incanum* indicating that these highly variable taxa belong to the same closely-knit group. However, from the small sample examined, seed coat microstructure may be useful in the identification and classification of the sectional and generic levels in the *Solanaceae*.

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