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ELECTRON MICROSCOPIC AND IR SPECTRAL STUDIES OF THE MEDICINALLY IMPORTANT SPINY SOLANUM- *SOLANUM MELONGENA* VAR *INSANUM* L.

Anil Kumar, V.S[1]. and Murugan, K[2].
[1]*Department of Botany, Govt. College For Women, Thiruvananthapuram 695 014*
[2]*Plant Biochemistry and Molecular Biology Laboratory,*
University College, Thiruvananthapuram 695 034
**Email: harimurukan@gmail.com*

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Abstract: *Solanum* (Solanaceae) is one of the 10 largest genera of flowering plants, with approximately 1400 species including *Solanum melongena* var *insanum* (cheruvazhuthana). It is traditionally used in ayurvedic preparations and also consumed as vegetable. The purpose of the present investigation is to analyze the micromorphological characters using scanning electron microscopic analysis of pollen, spermoderm sculpturing patterns as well as foliar trichomes coupled with IR spectra of the dried leaf tissue samples with a view to unveil the salient features the species and to characterize the chemical profi le related to functional groups using FTIR spectroscopy. Such an approach is highly warranted as this medicinal species is being highly adulterated using other *Solanum* substitutes. Pollen dimorphism was prominent with tri and tetrazono colporate grains with compact projecting spiny mammilate ornamentations.The spermoderm displays a cerebelloid pattern and the lateral testal cell walls were smooth without ornamentation.the distal appendages on the anticlinal cell walls were essentially absent. The upper leaf surface was studded with two different types of hairs such as muliradiate stellate and short stalked glandular trichomes. Similarly, multiradiate stellete non glandular hairs were seen on the lower surface of the leaves. Glandular trichomes secrete secondary metabolites that act as defense cascade against biotic attack. The infra red finger printing analysis provides peaks between 3200-3300 and also another peak at 1635 suggests the characteristic *Solanum* alkaloids. In addition, there are many other minor peaks with different height and shape that can be employed to discriminate this species of *Solanum* from others.

Key words: *Solanum*, Infrared spectra, Spermoderm, Pollen, Trichomes, Scanning electron microscopy

INTRODUCTION

Solanaceaous crops have been subjected to intensive human selection, allowing their use as models to study the evolutionary interface between plants and people. The ancient mode of Solanaceae evolution, coupled with an exceptionally high level of conservation of genome organization at the macro and micro levels make the family a model to explore the basis of phenotypic diversity and adaptation to natural and agricultural environments. Some Solanaceae plants are important model systems for biology; these include tomato for fruit ripening and plant defense, tobacco for plant defense, and *petunia* for the biology of anthocyanin pigments.

In Kerala, the genus is represented by about 30 species. There have been relatively few pollen studies on *Solanum* considering the large size and economic importanceof the genus. *Solanum melongena* var *insanum* is a perennial shrubby plant with violet flowers and globose yellow fruits, often produced singly. The micromorphogical investigations of this medicinally important spiny *Solanum* have not been undertaken so far. Karihaloo and Gottileb (1995) established a close phylogenetic relationship between cultivated *Solanum melongena* with weedy *Solanum insanum* and wild *Solanum incanum* forms existing in South Asia. The plant is widely used as an ingredient of various ayurvedic preparations such as dasamoolarishta and also reported to be used against an array of respiratory disorders.

Despite the numerous economic and agronomic importance of the *Solanum* species, there is still taxonomic lacuna especially in identifying or delineate *Solanum* species. Hence, the objective of this study is to provide

detailed micromorphological information on *Solanum melongena* var *insanum* coupled with IR spectral analysis for accurate description and proper identification of the species.

MATERIALS AND METHODS

Leaf materials used in this study were obtained from freshly collected plants during the expeditions to various parts of Kerala.The plants were identified at the Department of Botany, University of Calicut and a voucher specimen was prepared and deposited in the Herbarium of the Department of Botany, University College, Thiruvananthapuram.

Scanning electron microscopy: Fresh leaf pieces (10 x 10 mm^2) from, *S. melongena* var. *insanum was* immersed in a fixative solution of 3 % glutaraldehyde in 0.1 M phosphate buffer for 24 h. Samples were washed for 15-30 min with the buffer and dehydrated in graded ethanol series. Samples were then critical-point dried using Co2, sputter coated with gold under vacuum and viewed with Hitachi (S-450) scanning electron microscope operating at 15 kV. Images were captured digitally with an Image Slave computer programme for Windows.

Stomatal Index

The number of stomata in 30 randomly selected microscopic field areas from six leaves was counted per plant to obtain stomatal and epidermal cell frequency. Leaf area served per stoma was calculated based upon the stomatal frequency per unit area. Stomatal index (SI) was calculated according to the formula of Salisbury (1927): SI = S/E + S X 100 where S is the number of stomata per unit leaf area and E is the number of epidermal cells per unit leaf area . The mean values of stomatal indices were statistically analysed using one way ANOVA and t test.

Vein Islet Study

The fully expanded third pair of leaves from the terminal part of the branch was collected from ten representative plants. Leaves were immersed in 80% ethanol for 48-72 h with several changes of solvent to remove chlorophyll pigments. Leaf samples were then washed and treated with 3-5% NaOH at 60U for 24-36 h. The digested leaf tissue was carefully brushed apart to obtain the leaf skeleton. These were further hardened by treating with saturated chloral hydrate solution for several days, washed, dehydrated and preserved. To study minor venation patterns, small bits were cut from the central part of the leaf skeletons (excluding mid rib and marginal parts), stained with safranin and mounted in euparol. Absolute vein islet numbers were calculated by Gupta, (1961); the terminology of Hickey, (1973) is followed for the description of leaf architecture.

Pollen SEM Studies

Anthers from 70% alcohol fixed flower buds are used for pollen acetolysis. Acetolysis was carried out following the standard methodology of Erdtman (1952). Acetolysed pollen is attached to stubs with double-faced carbon tape. The stubs are gold- coated using sputter coater for one minute and examined. The terminologies used are in accordance with Erdtman (1952) and Walker and Doyle (1976).

Seed Surface Studies

Mature dry seeds (without fixation) were glued to aluminum stubs and coated with gold palladium to a thickness of 40 to 50 nm using a JEOL Fine coat Ion Sputter JFL 1100. The specimens were viewed in a scanning electron microscope and photographed at different magnifications. 15 seeds were randomly selected and studied.

IR Spectral Studies

The fresh leaves (approximately 3-4) taken from different plants were pooled as one sample. The samples were immediately dried in an oven for 2d at 60°C. Tablets for FTIR spectroscopy were prepared in an agate mortars, by mixing leaves powder (2 mg) with KBr (1:100 p/p). The absorbance spectra were measured between 300 and 4500 cm^{-1}. At least three spectra were obtained for each sample and was used for IR analysis.

RESULTS AND DISCUSSION

Pollen morphology of Solanaceae is quite heterogeneous (Erdtman, 1952). However, tricolporate grains are common among *Solanum* species. Pollen grains are radially symmetrical, isopolar, prolate-spheroidal to sub-prolate or prolate rarely oblate spheroidal.

Mostly the pollen are tricolporate rarely 4-colporate, colpal membrane finely-coarsely granulated or sub-psilate. Tectum is mostly scabrate (coarse-fine). In addition to this, various grades of rugulate-reticulate, reticulate- rugulate often verrucate or striate tectum are also found. However, tricolporate pollen with scabrate tectum is more commonly found within the family. Most striking variation is found in the shape, apertural types and tectal surface. The pollengrains of *Solanum melongena* var. *insanum* exhibits prominent dimorphism having tri and tetrazonocolporate both with compact granulose projecting elements (Figs. 1a, 1b & 1c).

The taxonomic significance of pollen morphology in Solanaceae is more or less obscure. Sometimes different tribes or subtribes have similar type of pollen or vice versa Genera referred to same tribe or sub tribe may have different type of pollen (Erdtman, 1952). Gbile and Sowunmi (1979) conducted a palynological study in *Solanum* species and found highly significant differences in pollen size and shape within the studied groups. However, tricolporate pollen with scabrate tectum is more commonly found within the family. The morphology and palynology of the species of *Solanum* have proved to be of immense assistance in interpreting

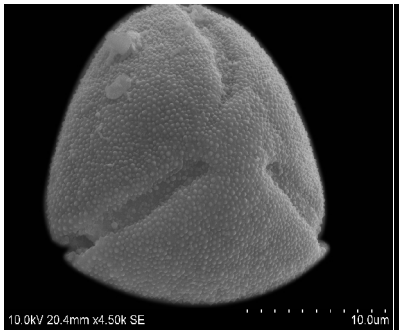

Fig. 1c

Fig. 1b

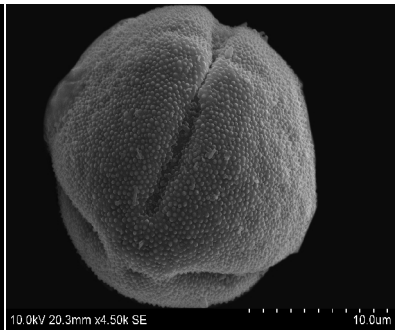

Fig. 1b

The trizonocolporate grains were without a prominent ridge and the tetrazonocolporate grains were not having an operculam. The exine sculpturing at the mesocolpium was different from that of apocolpium. At the mesocolpium region, these elements were having a spiny appearance. Some of the tetrazonocolporate grains were seemed to be in a transition stage between tri and tetra zonocolporate stages. Symon (1981) relates the small size of the pollen grains and the occurrence of the little complex ornamentation or lack of ornamentation in species of *Solanum* with the expulsion process of these grains inside of the poricidal anthers. problems related to plant taxonomy. The results could therefore be utilized with information from other discipline in clarifying taxonomic relationships of the taxa with other genera and species or subspecies.

In *Solanum melongena* var *insanum* both leaf surfaces were studded with stellate non glandular trichomes mainly with 9 radiating arms (Fig.1d & e). Trichomes with 7 and 8 radiating arms could also be observed. Interestingly, the upper leaf surface showed the presence of short stalked multicellular glandular hairs along the blade (Fig. 1d & f).

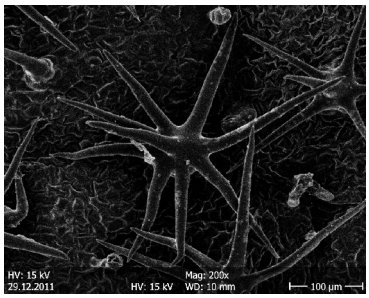

Fig. 1d. leaf upper surface SEM

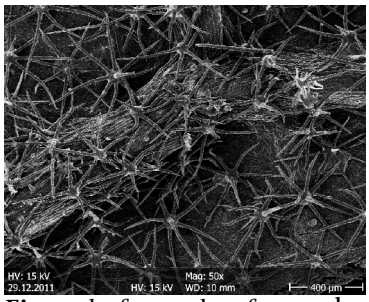

Fig. 1e. leaf ventral surface under SEM

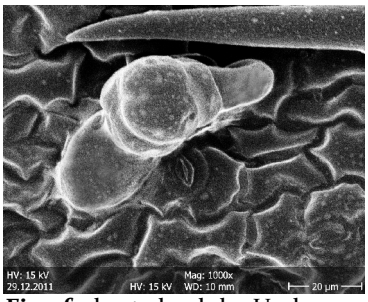

Fig. 1f. short glandular Under trichome

Glandular trichomes are characterized by having glandular head that release, on contact, sticky and/or toxic exudates that may entrap irritate or potentially kill some pests (Simmons *et al.,* 2003). These glands contain important secondary metabolites including terrenes, essential oils, flavonoids and lipophilic components (Afolayan *et al.,* 1995; Ascensao *et al.,* 1999). In most species, the source of these secondary metabolites has been attributed to the trichomes (Buta *et al.,* 1993) The possession of glandular trichomes is characteristic of the genus Solanum and of many other members of Solanaceae, with the exception of *Nicotiana glauca* and *Solandra nitida* (Maiti *et al.,* 2002) The two types of glandular trichomes identified on the leaves of *Solanum* might be responsible for the production, accumulation and release of volatile and secondary metabolites such as the saponins and steroid alkaloids reported by Drewes and Van Staden (Drewes *et al.,* 1995). Although, micro-morphological studies alone do not provide the information required to establish sites of synthesis in cells (Afolayan *et al.,* 1995), it is plausible to assume that the therapeutic compounds in *Solanum* are produced by the glandular trichomes.

The stomatal indices of both adaxial and abaxial leaf epidermises of *Solanum melongena* var. *insanum* were examined. The values of stomatal index varied from 6.00 for upper epidermis and 13.37 for the lower. Stomata are turgor operated valves regulating transpiration and therefore stomatal frequency has a direct relationship with the habitat of the plants. Stomatal index is constant for a particular species and hence taxonomically significant. Stomatal density is consistently higher in heliophytes than others (Wagner, 1998). Gupta (1961) studied the absolute vein islet termination numbers and absolute vein islet numbers in leaves of some Solanaceous plants. *Solanum melongena* var. *insanum* showed a value of 8.03 for the vein islet as in the present study. The ultimate veins of the leaf are either simple or branched. Simple vein endings may be linear or curved. The branched ones may divide dichotomously once or twice and branches may be symmetrical or asymmetrical. Usually a large number of vein endings are present in a big areole, which are the smallest areas of the leaf tissue surrounded by major veins and form a contiguous field over most of the leaf area. The ultimate vein endings were merged in *Solanum melongena v*ar. *insanum*.

The morphology of seed coat is usually stable and little influenced by external environmental conditions whilst the seeds develop and ripen within the fruit (Barthlott, 1981). Seed morphology has been shown to provide useful characteristics for the analysis of taxonomic relationships in a wide variety of plant families (Gontchaova *et al.,* 2009). In addition to gross morphology of seeds, sculpturing details of outer seed coat are quite variable between different species and can be of systematic importance. Edmonds (1983) used SEM for described in detail of spermoderm features of *Solanum* section *Solanum*, found all species had very similar features of their bands at the lateral walls of the outer epidermal cells of the testa. Lester (1991) used micromorphology of the spermoderm cells and presented evolutionary relationships of tomato, potato and other *Solanum*. Seed coat micromorphology of *Solanum melongena* and allied species was reported by Sayed Mohammed Zain Hasan *et al*. (1990). Irregular shape and size of the spermoderm cells , sinuate undulate wall and lack of fibrils and other ornamental are the characters. The lumen is shallow and thick ribbon of radial cell wall is found in this species. SEM of seed coat, as in the present study, showed slight difference in pattern of the two seed surfaces- with respect to the thickness and compactness of the ornamentation units as well as the lumen size. The seed is somewhat circular in shape with about 2.09 mm in width and 2.30 mm in length (Fig. 2a). The surface sculpturing units on the lower seed surface appeared to be sinuous, continuous and broad which are irregular. The lumen was somewhat prominent with 114 um x 86.6 um being the highest dimension (Fig. 2b & c). The muri showed an upper maximum dimension of 49.5 um. The sculpturing units were found to be faint towards the hilum region (Fig. 2d). Interestingly, the other surface i.e. the upper surface showed units which are comparatively widely placed having 127 um x 63.2 um being the highest lumen dimension (Fig. 2e & f). The muri had a thickness of 46.8 um being the upper value.

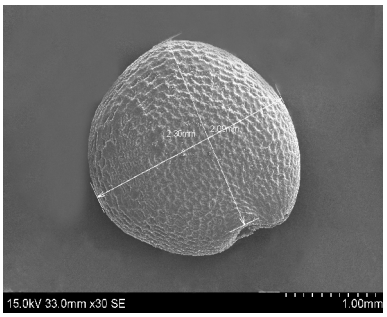

Fig.2a. entire seed under SEM

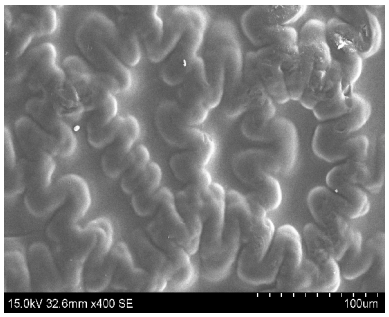

Fig.2b. SEM image of the surface showing thick muri

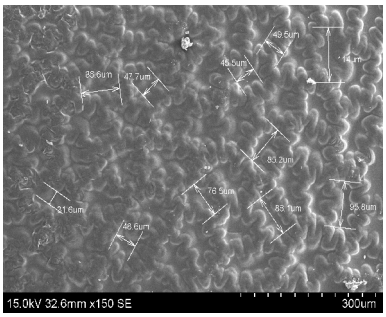

Fig. 2c. SEM image of the seed surface showing comp act muri and lumen

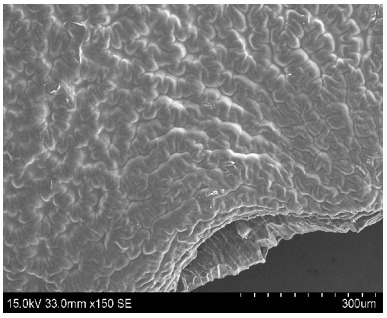

Fig. 2d. seed hilum region

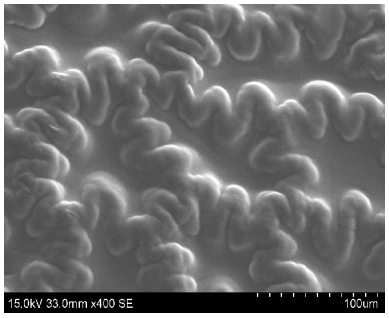

Fig. 2e. seed hilum region Surface with comparatively less thickened muri

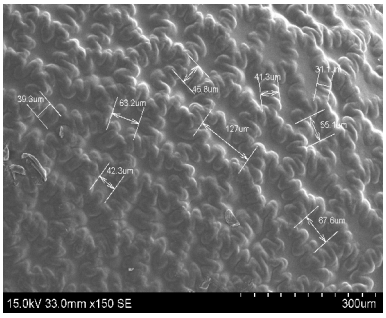

Fig. 2f. SEM image of the seed surface showing wider lumens

IR spectral analysis was conducted for five species of *Solanum* viz. *S. jasminoides*, *S. giganteum, S. nigrum, Solanum melongena* var. *insanum* , *S. capsicoides* and *S. mammosum* that yielded peaks between 3200 - 3500 and also a peak range 1600-1700. This can be used as a marker character to identify the genus. The peak 3200- 3300 may represent NH group of Solanum alkaloids. Similarly the peak at 1635 forms C=N group contain alkaloids. The peaks above 3200 and below 3300 may be alcohol, phenols or flavones like quercetin derivatives. The peak at 1700 represents C=O contains non conjugated compounds, while 1600 forms CH aromatic compounds. The peaks above 1700 and below 1800 represent unconjugated aliphatic ketones. The peaks at 1600, 1200 form the flavonoids and 1400 - 1500 forms C - O ether compounds or flavonoids (Fig. 3a).

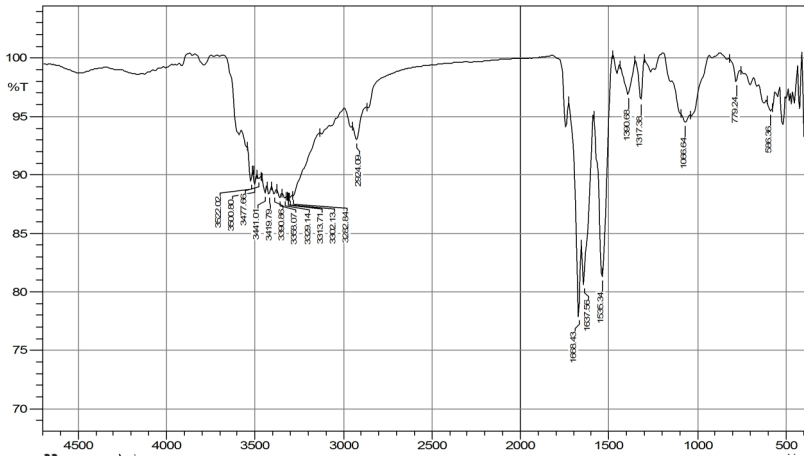

Fig. 3a. IR Spectrum of *Solanum melongena* var. *insanum*

Botanical identification is classically based on anatomical and morphological data. However, when the herbal products to be identified such as extracts, powder or the vegetative plant organs requires other tools for identification. Chromatographic techniques to analyse crude extracts, such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC) or gas chromatography (GC) hyphenated with detection techniques such as ultraviolet (UV), mass spectrometry (MS) and nuclear magnetic resonance (NMR) have been successfully employed (Yang *et al.*, 2006). Also, DNA-based techniques have been widely used for authentication of medicinal plants especially in the case when those plants are substituted or adulterated with other species morphologically or chemically indistinguishable (Joshi *et al.,* 2004)

The unique IR fingerprints for the species are 1317, 1515, 2956 and 3589 in comparison with other species like *S. jasminoides*, *S. giganteum*, *S. nigrum, Solanum melongena* var *insanum* and *S. capsicoides* and can be employed in identifying the species. Similarly, the peaks also explain the possible phytochemical profile of the species that can be explored and utilized in pharmacology.

CONCLUSION

Micromorphological investigation of Solanum melongena var insanum was carried out using electron microscopy and the surface features of leaf, pollen and seed was analysed. The IR spectral peaks also display the presence of potential bioactive components that may be of immense biological values. Further exploration in this regard is warranted.

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