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Research article

TAXONOMIC IMPLICATIONS WITH SPECIAL REFERENCE TO STOMATAL VARIATIONS IN *SOLANUM* SPECIES USING LIGHT AND SCANNING ELECTRON MICROSCOPE

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ABSTRACT: The systematic analysis between 15 different species and one species with two accessions in *Solanum* has been carried out and need all over revisions and investigations. In continuation to our previous morphometric analysis, currently stomatal variation is analyzed using light microscopy and scanning electron microscopy. In addition to classical anomocytic and anisocytic stomatal types, new types of stomata (anisotricytic, paratetracytic, diacytic and paracytic) are noticed for the first time in the genus *Solanum*. The scanning electron microscopic study reveals the nature of stomatal pore, nature of peristomatal rims as well as the presence of epicuticular wax deposits and wax flakes which are of taxonomic significance. This study also indicates that foliar stomatal characteristics are valuable taxonomic traits, which can be utilized to address the taxonomic issues within the genus.

Key Words: *Solanum*; Solanaceae; Anatomy; Leaf epidermis; Taxonomy; Stomata; SEM

INTRODUCTION

Solanaceae show significant diversity in terms of habitats, morphology and ecological features. The family Solanaceae consists of about 98 genera and 2700 species (Olmstead and Bohs, 2007), with cosmopolitan distribution. The genus is known for its economic importance having species used as food, like potato (*Solanum tuberosum* L.), tomato (*S. lycopersicum* L.), egg-plant (*S. melongena* (Mill.) Dunal) etc., as well as species with pharmacological interest like *S. dulcamara* (Moench) Dumort and *S. sodomeum* L., related to anti-neoplasic activity (Cham and Meares, 1987). Foliar fetures, both macroscopic and microscopic, are having great significance in the taxonomic and pharmacognostical realm. The morphology of stomata has long been regarded as a useful taxonomic criterion ie, documented observations suggest that their distribution and arrangement are taxonomically important even above the level of species. The stomatal distribution pattern and features like the most taxonomic characters, is an infallible criterion. The importance of micromorphological features for the taxonomic consideration of Angiosperms is recently mounting up (Parveen et. al., 2000). Micromorphological parameters of different plant parts have been used as aids in the taxonomical recognition of species (Kathiresan et. al., 2011). The foliar epidermis is one of the most noteworthy taxonomic characters from biosystematic point of view. For example, studies are conducted in many families on the basis of the leaf epidermis to delineate taxa (Albert and Sharma, 2013; Aworinde et. al., 2014). In *Solanum*, stomatal studies have been undertaken worldwide in several species. However, there is great controversy regarding the nature of stomata, as observed by various workers (Picoli et. al, 2013, Santhan, 2014). The taxonomic relevance of the foliar epidermal characters of angiosperms has been well documented (Zou et. al., 2008; Yasmin et. al., 2009). Size, distribution, and frequency of stomata have been found to be specific to taxa and are used as significant parameters in taxonomy as well as in elucidating phylogeny (Ahmed, 1979; Rajagopal, 1979; Idu et. al., 2000; Barkatullah et. al., 2014). Despite the scattered reports on few species, a comprehensive report regarding the stomatal patterns of *Solanum* species from Southern Western Ghats of Kerala is lacking and hence the study was undertaken.

MATERIALS AND METHODS

Scanning electron microscope (SEM)

For SEM examination, leaves were fixed in 3% gluteraldehyde overnight followed by washing with 0.1 M phosphate buffer. Then they were passed through gradient ethanol series, subjected to critical point drying and finally sputter coated. The coated specimens were then fixed on aluminum stubs with the help double adhesive tape and observed with SU6600, Hitachi and Zeiss EVO 18 scanning electron microscope. Basic terminology, used for stomatal and epidermal cells identification and explanation, was adopted from Prabhakar (2004).

Light microscopy

The selected leaves were peeled off to get the epidermal peel, stained and observed under the binocular light microscope (Leica ATC 2000) and images were captured.

RESULTS AND DISCUSSION

Stoma, the turgor operated valve is significant in discriminating the taxa at any taxonomic levels. Hayat et. al., (2010) constantly reaffirmed that micromorphological features of plants could be exploited in the biosystematics in the scenario of modern technological revolution. Further, diversity in terms of shapes of epidermal cells, stomatal size, its orientation and trichome nature and vascular bundles distribution are all pivotal in systematics. These characteristics have been employed in many genera to solve some intrinsic taxonomic issues or to contribute to increasing taxonomic database at species and even at family levels. Stomata were initially evaluated by Stresburger (1866) followed by Vesque (1889) to categorize them based on subidiary cells as well as their ontogeny in to four classes. Twenty five stomatal types are recognized based on leaf epidermal arrangement near the guard cells in dicots (Vishal et. al., 2012). Meanwhile, Stace (1984) recognized thirty one diverse types of stomata among seed plants. Stomatal index on leaf surfaces varies greatly among various species of plants. Usually, the lower epidermis of the leaf show increased number of stomata than the upper side. Reports suggests that the stomatal number may vary from zero on the apple leaf upper epidermis to 58,140 / square cm of black oak leaf lower epidermis.

There are differential reports related to the stomatal nature among *Solanum* species. *S. americanum* showed anisotricytic and tetracytic stomata (Fig. 1 A & B). Maiti et. al., (2002) reported only anisocytic stoma. Further, sharing of the subsidiary cell around the guard cells of adjacent stoma is similar (Fig. 1C) which is not reported earlier for *S. americanum*. This feature of sharing of subsidiary cells has been reported earlier in Asteraceae (Essiett and Archibong, 2014). The stomata of *S. macrocarpon* was reported to be anomocytic (Mbagwu et. al., 2008) while Adedeji et. al., (2007) and Komlaga et. al., (2014) reported it as anisocytic and also as anomocytic. Present investigation reveals the presence of anisocytic and anisotricytic stomata in *S. macrocarpon* (Fig. 2 A & B). Similarly, the stomata of *S. trilobatum* are reported as anamocytic type (Santhan, 2014). However, in the present analysis, it is observed as tetracytic (Fig.3). In addition to the anamocytic nature of stoma in *S. erianthum*, brachyparacytic stomata are also observed (Maiti et. al., 2002) (Fig.4 A & B). The controversy in the stomatal nature of *Solanum* species already exists in the literature. For example, in *S. granulosoleprosum*, Picoli et. al., (2013) reported paracytic type which was earlier reported as anisocytic by Petenetti et. al., (1998). Existence of different types of stomata in the same taxa was also described (Hameed and Hussain, 2011; Essiett and Okono, 2014). Nurit silva et.al., (2011) described anomocytic and anisocytic stomatal types in *S. torvum*. Similarly, Ferreira et. al., (2013) observed anomocytic and anisocytic stomata in *S. capsicoides*. Current observations revealed that the two accessions of *S. capsicoides* (spiny and lax spiny accessions) are having only anisocytic stomata (Fig.5 and Fig.6) while, *S. torvum* showed two types such as anisocytic and tetracytic (Fig. 7 A& B).

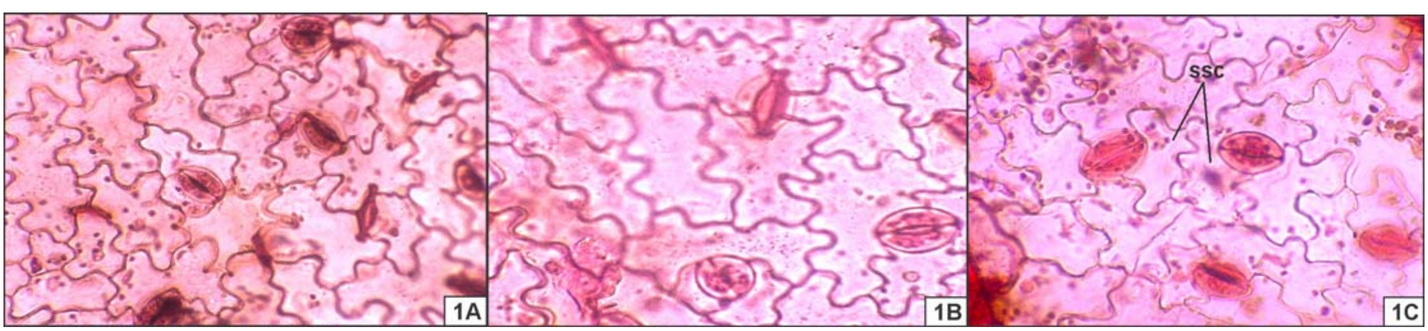


Figure 1 .*Solanum americanum*. A-anisotricytic stoma, B-tetracytic stoma, C- sharing of subsidiary cells by adjacent stoma (ssc- sharing subsidiary cells).

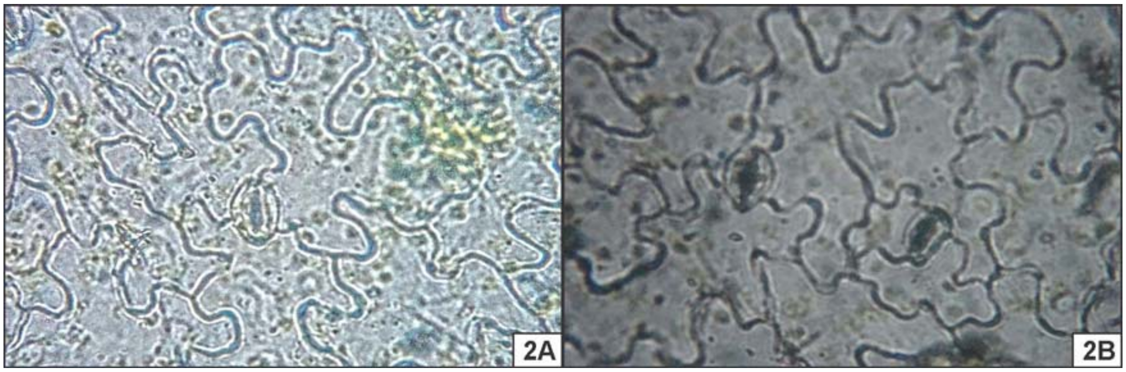


Figure 2. *Solanum macrocarpon*. A- anisocytic stoma, B- anisotricytic stoma.

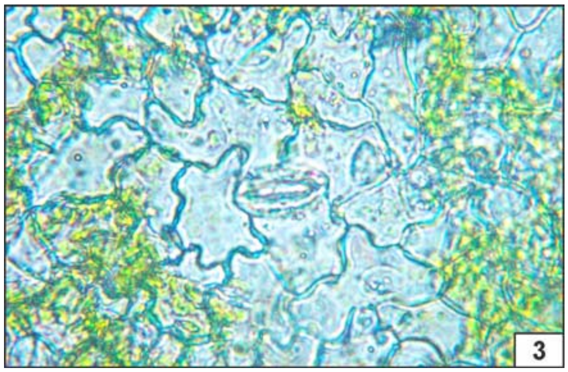


Figure 3.*Solanum trilobatum*- tetracytic stoma.

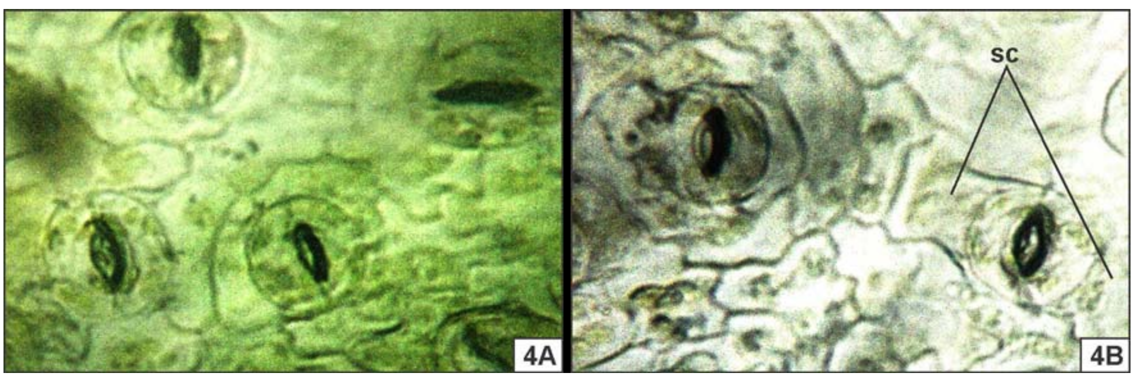


Figure 4. *Solanum erianthum*. A- anamocytic stoma, B-brachyparacytic stoma (sc- subsidiary cells).

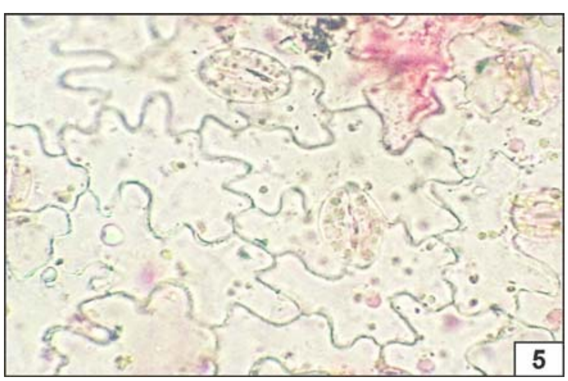


Figure 5. *Solanum capsicoides* (spiny accession)- anisocytic stoma.

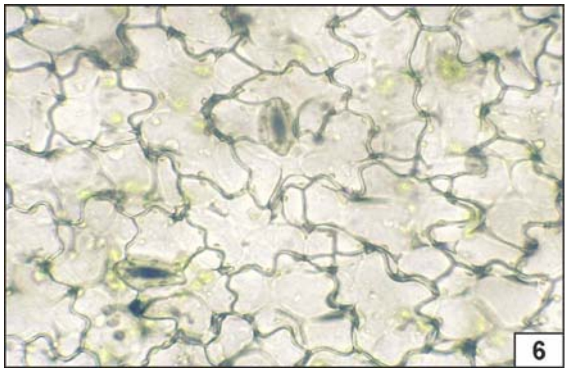


Figure 6. ***Solanum capsicoides*(spiny accession)- anisocytic stoma.**

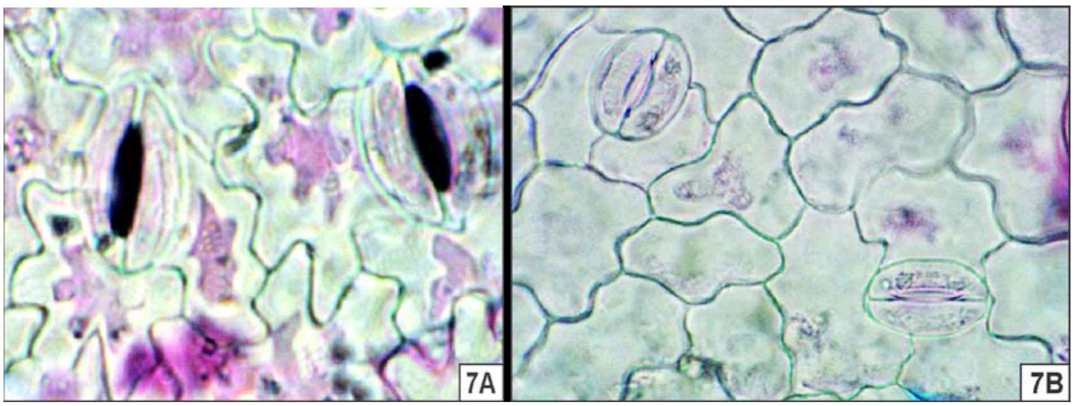
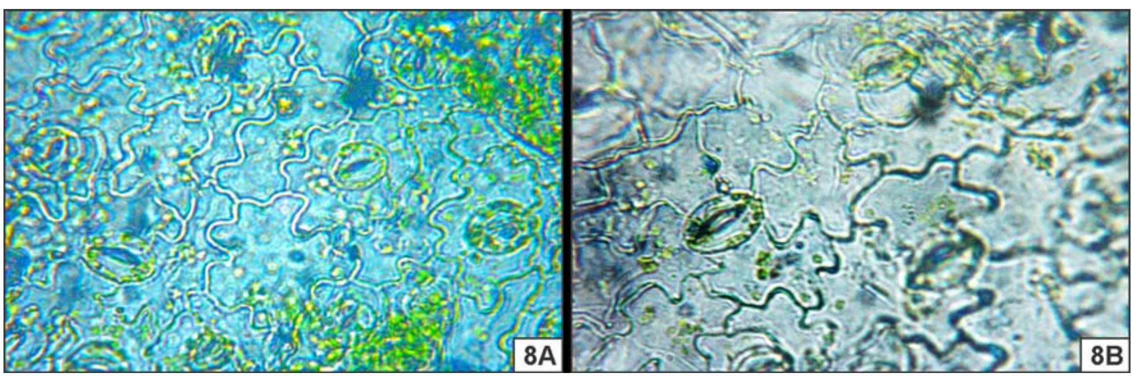


Figure 7. ***Solanum torvum*. A-tetracytic stoma, B-anisocytic stoma**



S. giganteum revealed 3 distinct types of stomatal types- diacytic, anomocytic and anisocytic (Fig.8 A, B & C). Out of the different species selected for the present investigation, no other species revealed three stomatal types. Sharing of subsidiary cells by adjacent guard cells was also exhibited by *S. mammosum* (anisocytic stoma) (Fig.9) and *S. melongena* var. *insanum* with paracytic and anisocytic stomata (Fig.10 A& B). Variations in the structure and distribution of stomatal types have been reported in members of other families also (Gill et. al., 1982). One of the probable reasons for the differences in stomatal types as observed by different workers can be correlated to the observations done by Patel and Inamdar (1971) i.e., stomatal development varies during the life history of a species. It may be the stage of development or age of the leaf that largely accounts for the observed variations between previous and present reports among certain solanaceous taxa. *S. pseudocapsicum* was having anisotricytic stomata which apparently look like paracytic forms (Fig.11). Aliero et. al., (2006) has reported the anisocytic stomatal nature in *S. pseudocapsicum.* Anisotricytic stomata are also noticed in *S. seaforthianum, S.wendlandii, S. mauritianum* and *S.violaceum* ssp. *multiflorum* (Figs. 12, 13A, 14 and 15 A). However, *S. wendlandii* showed anisocytic types (Fig.13 B) and *S.violaceum* ssp. *multiflorum* displays tetracytic types also (Fig.15 B).

S. violaceum ssp. *violaceum* is characterized by anomocytic stoma (Fig.16) while, *S. aculeatissimum* with anisocytic type (Fig.17). Nurit silva et. al., (2012) employed the type and distribution of stomata along with other foliar features for differentiating 10 species of *Solanum* belonging to the section Torva which supports the feasibility of using the stomatal features in taxonomic species discrimination.

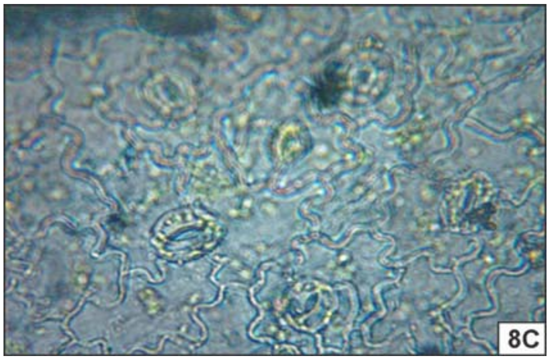


Figure 8. *Solanum giganteum*. A- diacytic stoma, B-anamocytic stoma, C-anisocytic stoma.

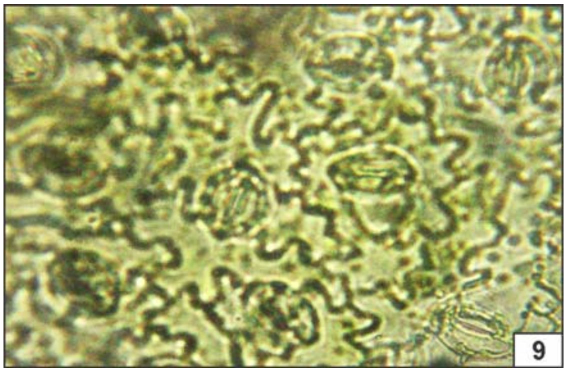


Figure 9. *Solanum mammosum*- anisocytic stoma.

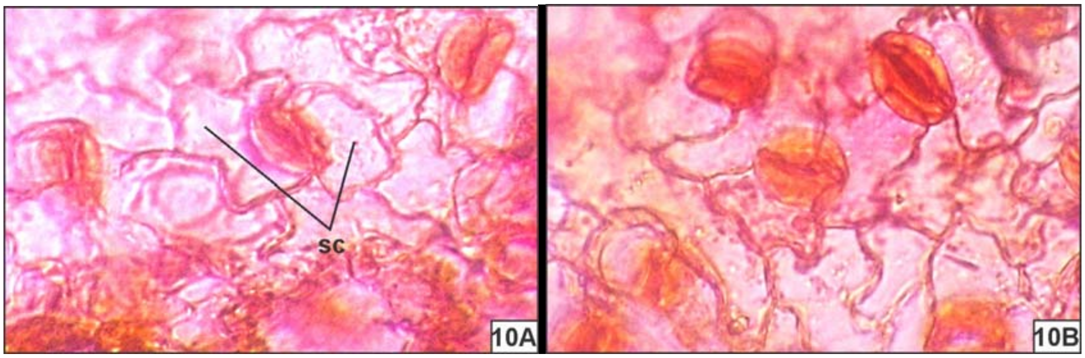


Figure 10. *Solanum melongena* var. *insanum*. A- paracytic stoma (sc- subsidiary cells), B-anisocytic stoma.

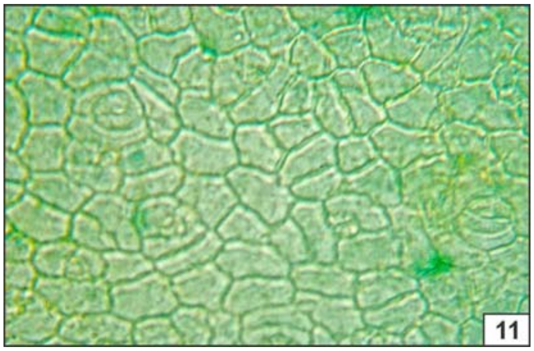


Figure 11. *Solanum pseudocapsicum*- anisotricytic stoma.

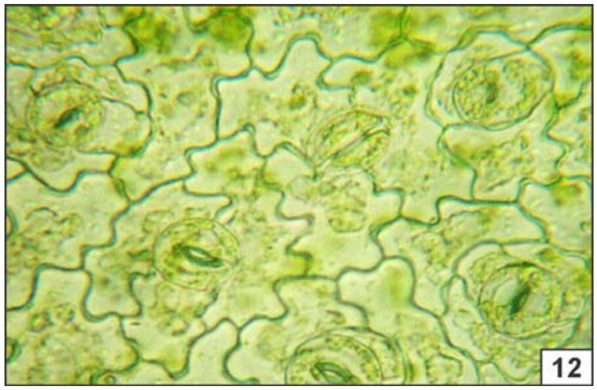


Figure 12. ***Solanum seaforthianum*- anisotricytic stoma.**

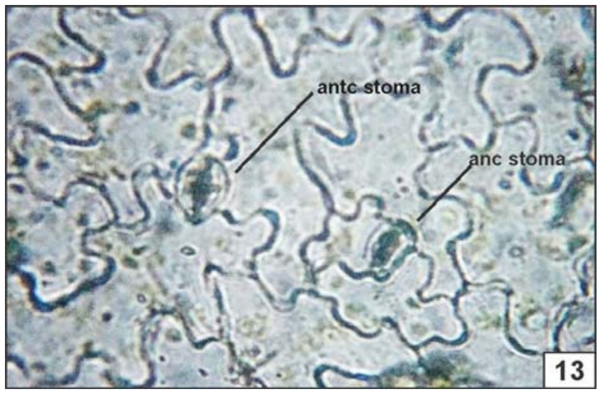


Figure 13. ***Solanum wendlandii*- anisotricytic (antc stoma) and anisocytic stoma (anc stoma).**

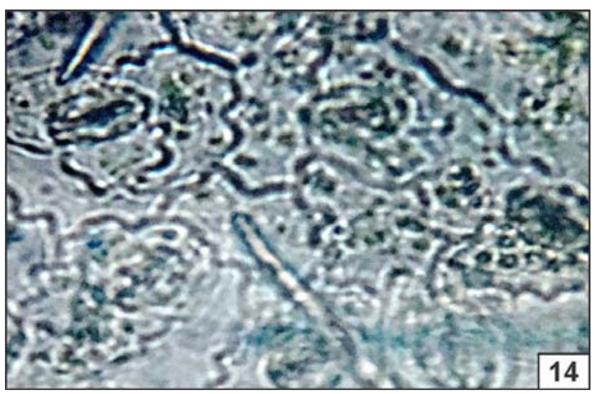


Figure 14. ***Solanum mauritianum*- anisotricytic stoma.**

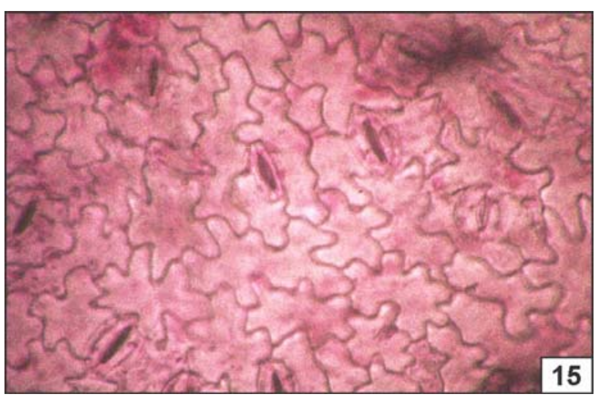


Figure 15. ***Solanum violaceum* ssp. *multiflorum*- anisotricytic and tetracytic stomata.**

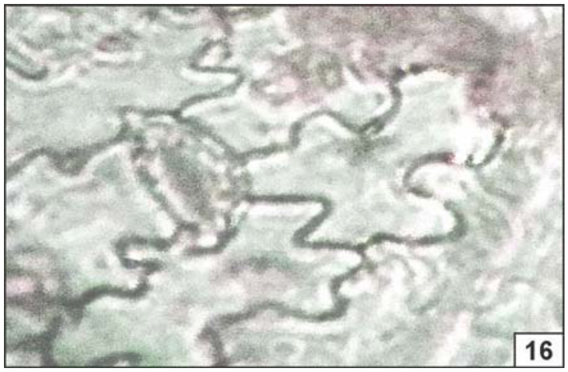


Figure 16. *Solanum violaceum* ssp. *violaceum*- anomocytic stoma.

Figure 17. *Solanum aculeatissimum*- anisocytic stoma.

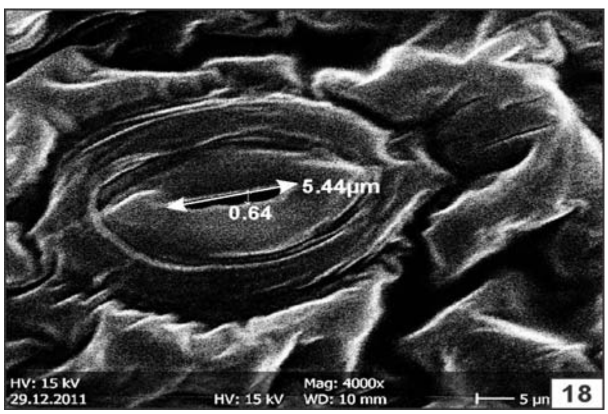


Figure 18. *Solanum aculeatissimum*.

Stomatal studies of *Solanum* species viz. *S. arboretum*, *S. falconense*, *S. gratum*, *S. lucens*, *S. ripense*, *S. tanysepalum*, *S. imberbe* and *S. sieberi* was attempted by Benitez de Rojas and Ferrarotto (2009). The stomata were anisocytic except *S. lucens*, *S. tanysepalum* and *S. imberbe*, which showed staurocytic stomata. Adedeji et. al., (2007) conducted epidermal studies in *S. macrocarpon*, *S. torvum* , *S. americanum* and reported that stomata of *S. torvum* were largely anisocytic, occasionally anomocytic and brachyparacytic while those of *S. macrocarpon* were anisocytic and anomocytic, occasionally brachyparacytic. *S.americanum* displays anisocytic stomata, occasionally ranging from para, dia, hemipara and anomocytic types. The early history of stomatal nomenclature dates back to Prantle (1881). Despite several classificatory systems and review works (Kidwai, 1981, Baranova, 1992), precise definition for subsidiaries and stomatal types is still lacking. Definitions provided by various workers for different stomatal types are ambiguous. New classification and modified definitions was introduced by Prabhakar (2004) based on his observations on more than 500 species of angiosperms. In the present study, stomatal patterns of fifteen species of *Solanumi* and two accessions of *Solanum capsicoides* from Southern Western Ghats of Kerala has been analyzed and show marked variations (Fig.1 – 17).

Hayat et. al., (2010) analyzed stomatal diversity in 24 taxa of the genus *Artemisia* by using light microscopy and scanning electron microscopy. In addition to previously studied classical types, new types of stomata (anomotetracytic, paratetracytic, diacytic and paracytic) are reported. The leaf epidermal morphology of *Onosma* species has been attempted by Ergen Akcin et. al., (2013) and reported that the stomatal rims and wax ornamentations are important characters for delimiting the taxa. In the present study, SEM investigation revealed remarkable microsculpturings of the stoma in 12 species and the two variant accessions of *Solanum capsicoides*. In species like *S. torvum*, *S. erianthum* and *S.mauritianum* , stomata are hardly visible due to their thick mat of stellate trichomes . Mostly, the stomata were having nearly smooth inner margin of the outer stomatal rim (Figs.18,19,20,21,23,25,26,28,29,30) with the exception of *S. melongena* var. *insanum*, *S. mammosum* and *S. pseudocapsicum* display a sinuolate inner margin (Figs.22,24,27) and *S. wendlandii* with fine sinuous inner margin(Fig.31). The outer stomatal rim is generally raised in *S. melongena* var. *insanum*, *S.macrocarpon*, *S. mammosum* and *S.trilobatum*. A slightly raised rim is characteristic of spiny accession of *S. capsicoides*, *S. pseudocapsicum* and *S. americanum*. In the lax spiny accession of *Solanum capsicoides*, *S. giganteum*, *S. violaceum* ssp. *multiflorum* , , *S.seaforthianum*, *S.violaceum* ssp. *violaceum* and *S.wendlandii* , the stomatal rims are almost at the same level of the epidermis. Measurements of pore size as given in the table 2 indicate that the longest aperture size is characteristic of *S.americanum* (11.25 µm) and the least for lax spiny accession of *S.capsicoides* (5.12 µm). The width of the stoma ranged from 0.64 µm in *S.aculeatissimum* to 4 µm in *S.americanum*. WU Ding et. al., (2005) used the SEM and light microscopic data of stomata for comparative morphologic analysis of the leaf epidermis in *Parnassia* from China. Wax deposits and wax flakelets were observed in some of the studied taxa (Figs.19,21,24,26,27,28). Similar observations were made by Ergen Akcin et. al., (2013) in species of *Onosma* from Turkey. Epicuticular wax flakelets are noticed in lax spiny accession of *Solanum capsicoides*, *S. giganteum* and. *pseudocapscum* while wax deposits are noticed along the peristomatal rims in *S. mammosum*, *S. americanum* and *S.seaforthianum*. the wax ornamentations are considered useful in delimiting the taxa (Ergen Akcin et. al., 2013).

Figure 18 – 31. SEM images of the stomata of *Solanum* species showing pore size and nature of inner peristomatal margins.

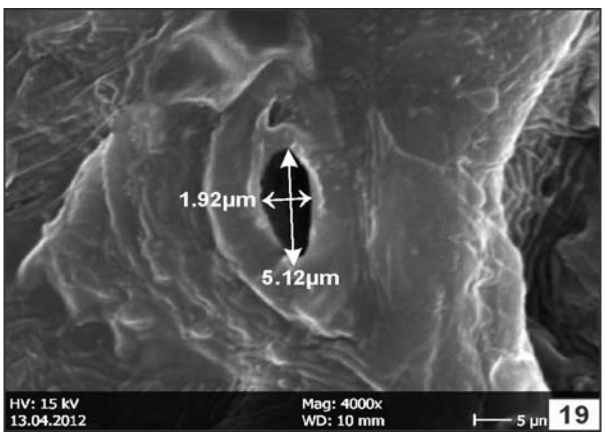


Figure 19. *Solanum capsicoides* (lax spiny accession).

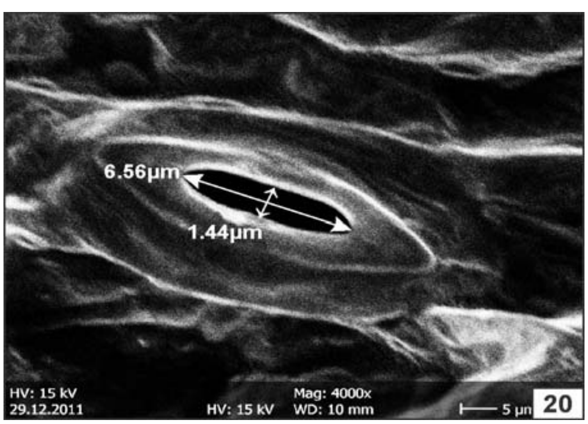


Figure 20. *Solanum capsicoides* (spiny accession).

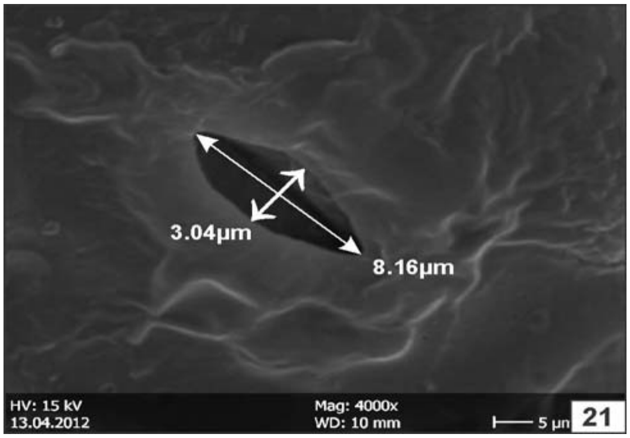


Figure-21. ***Solanum giganteum***

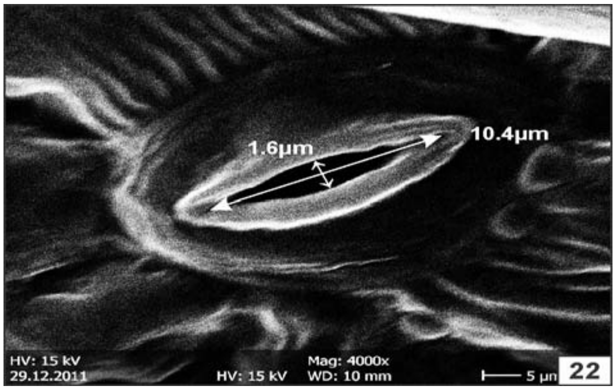


Figure-22. ***Solanum melongena* var. *insanum***

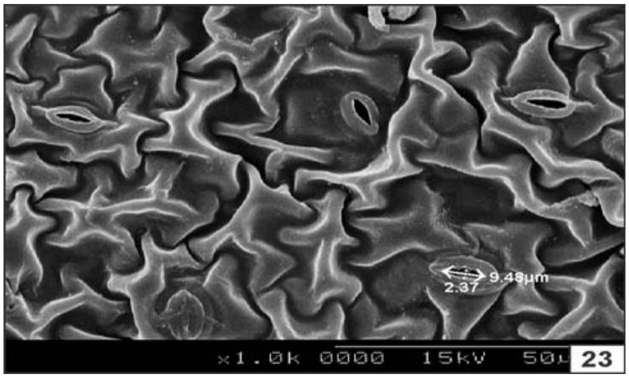


Figure-23. ***Solanum macrocarpon*.**

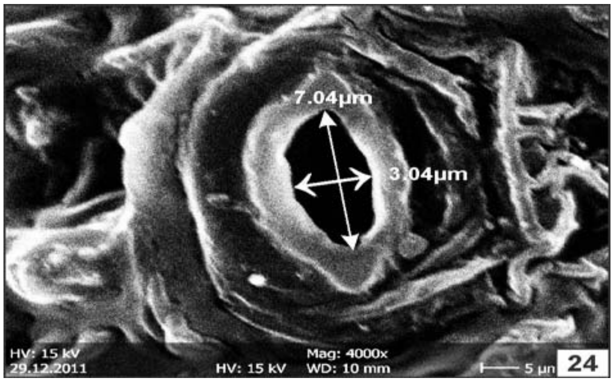


Figure-24. ***Solanum mammosum*.**

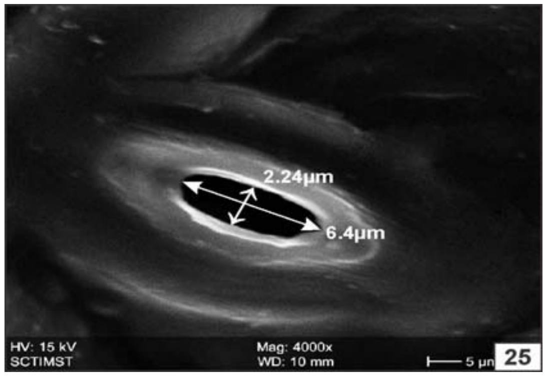


Figure-25. *Solanum violaceum* ssp. *multiflorum.*

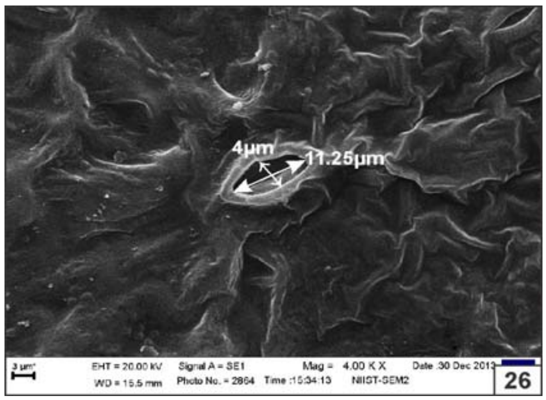


Figure 26. *Solanum americanum*.

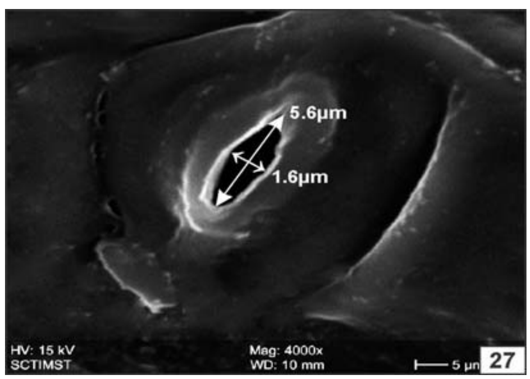


Figure 27. *Solanum pseudocapsicum*.

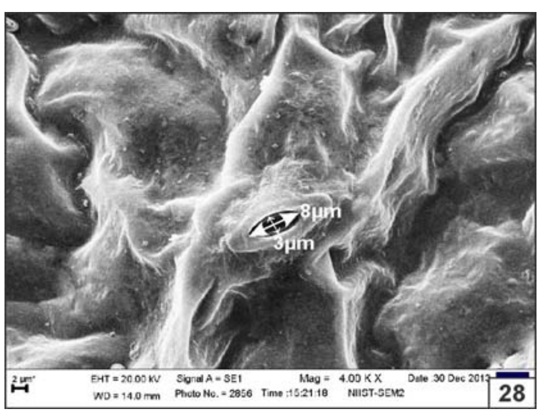


Figure 28. *Solanum seaforthianum*.

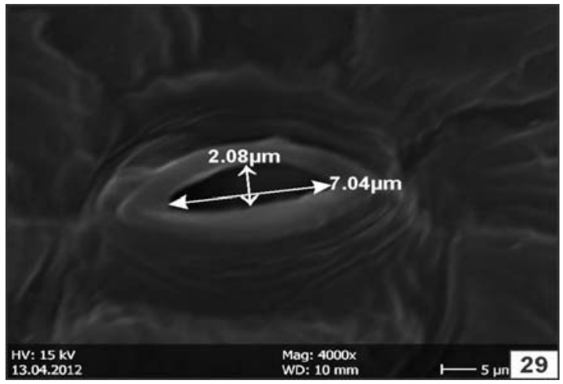


Figure-29.*Solanum trilobatum*.

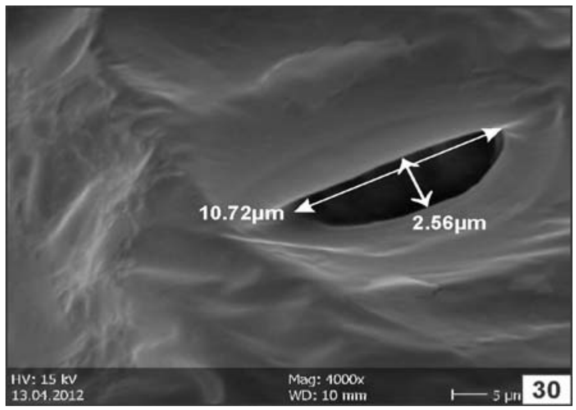


Figure 30. *Solanum violaceum* ssp. *violaceum*.

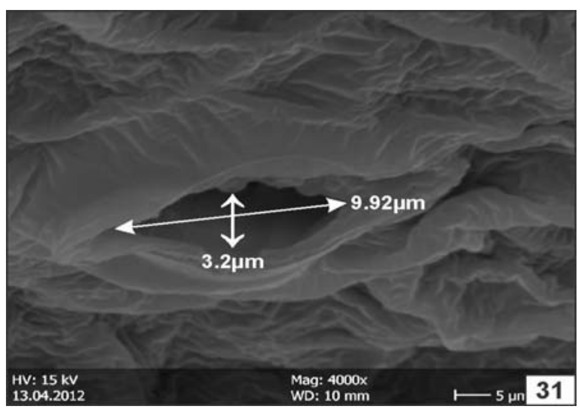


Figure 31. *Solanum wendlandii*.

Malik et. al., (2014) reviewed eight species of *Minuartia* with certain structural characteristics significantly important in separation of these taxa, such as anticlinal walls of epidermal cells along with stomatal features. Six *Phyllanthus* species epidermal morphology showed variations in shapes of the epidermal cells and types of stomata, which varied from wavy, polygonal to sinuous and anisocystic, tetracytic to paracytic respectively in the different species. Differences were also found in the distribution of the stomata as well as the variation in the cell wall contours and thickness. Also, a quantitative trait such as the stomatal index (SI) was described in details by Uka et. al., (2014). Daniel and Atumeyi (2011) analyzed stomata complex type found in four *Dioscorea* species from anomocytic type to other variations in all the analysed species. Al-Edany and Al-Saadi (2012) studied five cultivated species belong to five genera of Myrtaceae such as *Callistemon viminalis*, *Eucalyptus camaldu- lensis*, *Myrtus communis*, *Psidium guajava* and *Syzygium aromaticum*. It was clear that certain stomatal structural characteristics were of significant importance in separation of these taxa, Ahmad et. al., (2009) screened 34 genera and 20 families for stomatal diversity. Seven types of stomata were found in which amphianisocytic was the dominant one found in 12 species while staurocytic and diacytic were found in 7 and 6 species respectively.

Table 1: Dimensions of stomatal pore in different *Solanum* species

S. No.	*Solanum* species	Length of stomatal pore (μm)	Width of stomatal pore in (μm)
1	*S. aculeatissimum* Jacq.	5.44	0.64
2	*S. capsicoides* All.-*lax spiny*	5.12	1.92
3	*S. capsicoides* All.- *spiny*	6.56	1.44
4	*S. giganteum* Jacq.	8.16	3.04
5	*S. melongena* L. var. *insanum*(L)Prain	10.4	1.6
6	*S.macrocarpon* L.	9.48	2.37
7	*S.mammosum* L.	7.04	3.04
8	*S. violaceum* Ortega ssp. *Multiflorum* (Clarke) Matthew	6.4	2.24
9	*S. americanum* Mill.	11.25	4
10	*S. pseudocapsicum* L.	5.6	1.6
11	*S.seaforthianum* Andr.	8	3
12	*S. trilobatum* L.	7.04	2.08
13	*S.violaceum* O rtega ssp. *violaceum*	10.72	2.56
14	*S. wendlandii* Hook	9.92	3.2

In 7 species two and in one species three different types of stomata were found. Tahir and Tahir (2009) analyzed nine species of *Sibbaldia* L. (Rosaceae) by scanning electron microscope. Stomata are mostly anomocytic type, usually present on both surface of the leaves. Differences in shape, size, distribution and the orientation of stomata have been observed. *Cleome* species viz., *Cleome chelidonii*, *C. gynandra*, *C. simplicifolia* and *C. viscosa* herbs growing at same locality but in different soil types as *Cleome chelidonii* grows luxuriously in moist places and also in the rocky regions, while *C. simplicifolia* and *C. viscosa* grow luxuriantly in the black soil in rainy season. *Cleome simplisifolia* has short life span up to 3-4 months only. *C. viscosa* and *C. gynandra* grow throughout the year but more vigorously during rainy season. *C. gynandra* grows predominantly in waste places along waste water. *C. speciosa* is cultivated species growing widely in shadow places in the red soil particularly during rainy season. It is famous for its beautiful showy inflorescence and hence cultivated in gardens. Several researchers have provided evidences that stomatal densities change in response to changing atmospheric levels of carbon dioxide. Stomata may also vary in response to the amount of annual rainfall in different localities (Vishal et. al., 2012).

CONCLUSION

Thus, it can be concluded that different species of *Solanum* display variations in their stomatal nature along with other features that tend to support their taxonomic discrimination. These *Solanum* species also show variations in other foliar features like vein islet, stomatal index, palisade ratio as well as in the nature and distribution of foliar trichomes.

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