



## Variability of Stomatal Index and Chlorophyll Content in four species of Solanaceae Members

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### Abstract

The present investigation focuses on stomatal index and chlorophyll contents of species like *Datura innoxia* L., *Capsicum annum* L., *Lycopersicon esculentum* M., *Solanum nigrum* L. and their comparative account on taxonomic similarity. There were no relation between stomatal size and growth habitat. Our study revealed that *Lycopersicon* significantly showing reduced in stomatal size and more in number at the lower epidermis, in addition to size of the stomata and subsidiary cells of these species were closely allied. All the investigated species has shown anomocytic stomata. The anatomical structure of subsidiary cells shows irregular in shape, size, and numbers. Irrespective of leaf surface they have peculiar types of anomocytic stomata, stomatal type, and stomatal index. Quantification study of chlorophyll pigment revealed that there is no significant difference among the four species in terms of quantity and taxonomical variations in these species.

**Keyword:** Stomata, chlorophyll, Solanaceae.

### Introduction

The present work was carried in four members of solanaceae family, in order to know the taxonomic similarities in terms of stomatal index, types and quantity of chlorophyll in the selected candidate species. The solanaceae family belongs to herbs. Interestingly most of the members of solanaceae are herbs while some species are climbing habit. The family solanaceae consists of about 90 genera and nearly 3000 species<sup>1,2</sup>. The morphological and leaf epidermal features has been found to be key element in the taxonomical studies. One of the excellent review studies highlights the application of morphological features in plant systematic studies<sup>2-4</sup>. The introduced the term stomatal index to express stomatal frequency independent to the size of the epidermal cells<sup>4</sup>. He points out that the stomatal index is a useful taxonomic character in leaf area studies<sup>5</sup>. The taxa collected from different localities showed more or less constant stomatal index values and can be used in distinguishing different taxa. Systematic studies on the development and morphology of stomatal types may be expected to give evidence for various evolutionary trends among the angiosperm families and may aid in assigning taxa of uncertain to proper positions<sup>6</sup>. Different taxonomic groups differ in the type of stomata, their distribution and frequency, thus analysis of stomatal characters gives the taxonomic as well as phylogenetic significance.

The plant morphologists, physiologists and taxonomist have long been studied the stomatogenesis. The morphology and ontogenies of taxa are important in intrageneric systematics. Diversity in stomata types, even on the same surface of an organ, indicates the limitation for using stomata as a taxonomic

character<sup>7</sup>. In addition to diversity, the most repetitively stomata type can also be used as a taxonomic character for the morphological and phylogenetic studies<sup>8</sup>. Therefore the present work has been undertaken, to elucidate the diagnostic significance of stomata. Many groups highlighted the importance of folio epidermal characters are important in systematic botany, the use of modern techniques and chemical composition<sup>4,9</sup>.

### Material and Methods

The four candidate species with four different genera were collected at Karnataka State Women's University Campus Bijapur (Torvi). The selected members were *Solanum nigrum*. L. *Datura innoxia* L, *Lycopersicon esculentum*. M and *Capsicum annum*. L. The fresh leaves are collected and used immediately for the study. The collected plants were healthy and have no any physiological deforms on leaves. All candidate species were collected from open spaces.

**Macroscopic and Surface Study:** The collected leaves are washed thoroughly with water and both upper and lower epidermises were peeled off by freehand and the 1mm square section of peeled leaves taken for the microscopic examination. Stomatal Index and number of stomata of the both surface of leaves were carefully observed and recorded for further analysis as per API standard.

**Determination of Stomatal Density and Stomatal Index:** The stomatal density was determined as the number of stomata per square millimeter of leaf. The index was determined as the

number of stomata per square millimeter divided by the number of stomata plus number of epidermal cells per square millimeter multiplied by 100. The lengths of stomata were measured to determine the stomatal size. The stomatal index (SI) was intended using the equation described by Salisbury, that is:

$$\text{Stomatal Index} = \frac{S}{E + S} \times 100$$

Where, S = donates the number of stomata per unit area and E = the number of epidermal cells in the same unit area.

**Chlorophyll quantification:** Collected fresh healthy leaves of selected four genera of solanaceae members and washed thoroughly with running tap water then allowed to dry it at room temperature. Taken 5gm of each leaf sample in mortar and homogenised it with 90% acetone by using pestle. The homogenised extracts were subjected for centrifugation at 5000 rpm at 15 minutes at room temperature, then filtered by whatsmann's filter paper and collected the supernatant in clean test tubes and mixed with equal amount of supernatant and 90% acetone then measured the absorbance of solutions by using spectrophotometer (shimadzu) at 663nm and 645nm wave lengths and 90% acetone taken as standard blank solution. The calculation was done as per Arnon's method.

## Results and Discussion

Microscopic studies of the stomatal density of adaxial surface leaf shows that the maximum numbers of stomata were found in *L.esculentum* 172 mm<sup>2</sup> and minimum stomatal density recorded in 19 mm<sup>2</sup>. The size of stomata are very minute, because of the minute size density of stomata occurrence and their distribution more on *Lycopersicon* in other hand the *C. annum* has sparsely distribution and their density less at adaxial surface as compared with abaxial side (table-1). Another interesting taxonomic feature out of four species only *L. esculentum* has more density of stomata on adaxial surface as compared to abaxial surface. Many plant systematics and morphologists were revealed that the distribution and frequency of stomata are useful in solving several problems of plant systematic<sup>11-13</sup>. He was the first to report that the frequency of stomata is high when the size of epidermal cells is low and the frequency is low when the epidermal cells are large<sup>14</sup>.

Abaxial surface of leaf shown an interesting features of stomatal distribution in *C.annum* it has three times more than adaxial surface but in case of other three species comparatively less even though *Lycopersicon* showing less number of stomatal density 86.81 mm<sup>2</sup> table-1. Similar kinds of observation noticed<sup>15</sup>, the quantity of stomata per square millimetre and stomatal index were found to be useful in deciphering the individual species, among the genus *Cinnamomum*.

The type of stomata is also of considerable significance in delimiting the taxa, both at the species and generic levels. In *L.*

*esculentum*, *C. annum* and *S.nigram* have shown typical anomocytic stomata and *D. innoxia* has both anomocytic and anisotypic stomata on their upper and lower surface. The most of species of solanaceae shown anomocytic and anisocytic types of stomata it indicates that variability of stomatal types in family and generic and species level. Similar work was conducted<sup>16,17</sup>. In some cases, the dominant type of stomata is anomocytic or anisocytic in the leaves of *Wedelia urticifolia* DC and *Wedelia trilobata* (L.)AS.Hith. In addition to these two types, polocytic and hemiparacytic stomata are observed in *Wedelia chinensis* (Osbeck) Merr.The different species of *Spilanthes jacq* possess diacytic stomata as the most frequent type.

**Table-1**  
**Stomatal index of selected species of solanaceae members**

S. No	Plant species	Adaxial surface 10X	
		No of stomata	Stomatal index (%)
1	<i>Datura innoxia</i> , L.	67.00	25%
2	<i>Lycopersicon esculentum</i> M.	172.00	19%
3	<i>Capsicum annum</i> L.	19.00	13%
4	<i>Solanum nigrum</i> L.	30.00	21%
Abaxial surface			
1	<i>Datura innoxia</i> , L.	71.00	25%
2	<i>Lycopersicon esculentum</i> M.	87.00	55%
3	<i>Capsicum annum</i> L.	59.00	16%
4	<i>Solanum nigrum</i> L.	46.00	20%

**Stomatal Index:** The comparative microscopic study of stomatal index in four species of solanaceae emphasised that the least stomatal index (13%) of *C.annum* at abaxial surface Interestingly, the highest stomatal index *D. innoxia* shown at adaxial surface has 25% and same index were at abaxial side also it indicates that there is no variability in both surfaces; on the other abaxial surface *L.esculentum* has maximum stomatal index (55%) but has least stomatal index of *C. annum* (16 %) in table-1. The comparative microscopic study of stomatal index in four species of solanaceae emphasised that the least stomatal index (13%) of *C.annum* at abaxial surface Interestingly, the highest stomatal index *D.innoxia* shown at adaxial surface has 25% and same index were at abaxial side also it indicates that there is no variability in both surfaces; on the other abaxial surface *L. esculentum* has maximum stomatal index (55%) but has least stomatal index of *C.annum* (16 %) shown in table-1. The stomatal index of the individual species within a genus is different, except in few cases, which makes it a useful diagnostic feature. These studies revealed that stomatal index of various genus of the same family significantly varied. The presence or absence of stomata on the epidermis of leaves is extremely useful in delineating taxa both at the species and generic levels. Other studies on stomata revealed the application of the character at various taxonomic levels<sup>18,19</sup>. The term stomatal index was first introduced to express stomatal

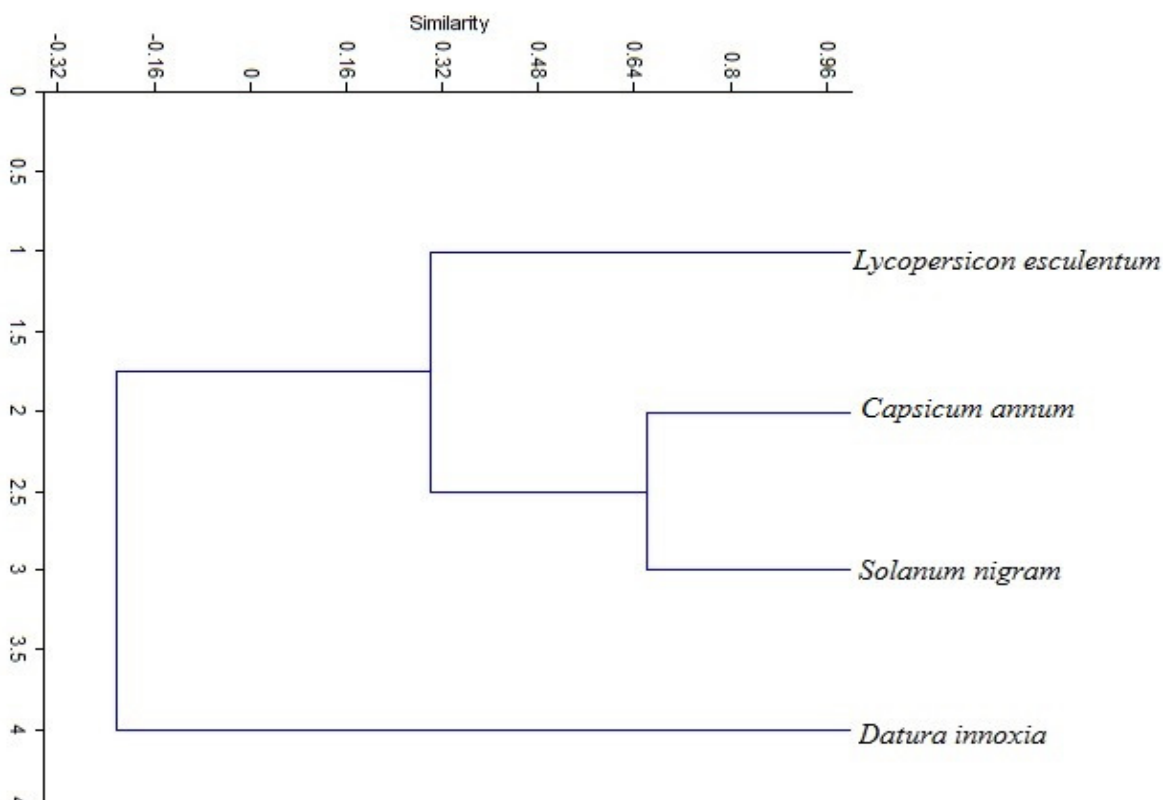
occurrence independent of the size of epidermal cells<sup>10,14</sup>. He points out that the stomatal index is a useful taxonomic character when comparable leaf areas are used<sup>5</sup>. Stomatal index is one of the extensively used features for pharmacognostic studies. Similar kinds of work was done in the of Cassia genus made very careful tests of stomatal index on the genus Cassia, Linn and inferred that stomatal index is the same for different varieties within a species<sup>20,21</sup>. In this studies of stomatal indexes were varied in adaxial and abaxial sides of same leaf. So, the

classification of taxa based on stomatal index is deceiving actual classification of plants.

Micrometric analysis table-2 of stomata complex was presented as paired group correlation (Coph correlation 0.7459) dendrogram (cluster analysis) shown that *C. annum* and *S. nigrum* + 0.64 similarity with paired group ; only + 0.32 similarity with *L. esculentum* and other genera *D. innoxia* has - 0.16 least similarities among themselves figure-1.

**Table-2**  
**Micrometry of stomatal complexes (at 10x field) of selected species**

Sl. No	Species	Adaxial surface 10X			
		Length of subsidiary cells(µm)	Breath of subsidiary cells(µm)	Length of guard cell(µm)	Breadth of guard cells(µm)
1	<i>Datura innoxia</i> , L.	40.04±10.37	30.94±12.21	35.13±28.04	18.2±0.00
2	<i>Lycopersicon esculentum</i> M.	43.68±9.96	43.68±14.95	9.1±0.00	18.2±0.00
3	<i>Capsicum annum</i> L.	41.86±4.98	58.24±16.53	18.2±0.00	25.48±4.07
4	<i>Solanum nigrum</i> L.	45.5±11.14	43.68±9.96	30.94±4.98	20.02±4.07
Abaxial surface 10X					
1	<i>Datura innoxia</i> , L.	34.58±7.61	20.02±4.07	25.48±4.07	36.40±6.43
2	<i>Lycopersicon esculentum</i> M.	63.70±11.15	21.84±8.14	18.20±0.00	10.92±4.07
3	<i>Capsicum annum</i> L.	23.66±18.56	20.57±6.06	45.5±33.44	10.74±1.00
4	<i>Solanum nigrum</i> L.	38.22±7.61	34.58±18.65	36.40±0.00	10.92±4.07



**Figure-1**  
 Paired group correlation dendrogram of subsidiary cells and guard cell the lower epidermis of selected species

**Taxonomy Related To Chlorophyll Pigments :** Chlorophyll are the principal component of pigment protein complexes which take part in a most important role in the photosynthesis, any variation in chlorophyll content is normal to bring change in photosynthetic rate. In this investigation, it found that higher total chlorophyll content in *D.innoxia* (8.01mg/l) and least number of total chlorophyll content in *C. annum.* (7.20mg/l) table-3. The diversity of chlorophylls such as chl-a, chl-b etc and total chlorophyll contents does not give any valid information for classification of higher plant because these pigments are commonly distributed all the vascular and non-vascular plant with slightly varied its concentration with species to species. The chlorophyll-a, chlorophyll-b and total chlorophyll contents of four species had shown comparatively slight differences among themselves. Even though, chlorophyll content angiosperms did not give any valid information classification of taxa. Comparative account of chlorophyll and other accessory pigments are principle role in classification of algae because of their cell contain higher concentration other than the principle pigments such as chlorophyll. If the plants growing in shade, it contain less chlorophyll a and more chlorophyll b. Replacing Mg with Fe ions gives a grey-brown chlorophyll product and the presence of Zn and Cu ions increases the stability of the natural green colour<sup>22</sup>. In this regards the chlorophyll is a principle pigments in all autotrophic green plants hence taxonomic classification of green plants on the bases of chlorophyll along impossible but its concentration will vary species to species. Even though, plant pigments are mainly used for classification lower groups of plants particularly algae.

**Table-3**  
**Estimated chlorophyll contents of selected species,**  
**solanaceae members**

S. No	Species	Chl-a(mg/l)	Chl-b(mg/l)	Total chl (mg/l)
1	<i>Datura innoxia</i> L.	2.70	0.59	8.01
2	<i>Lycopersicon esculentum</i> M.	2.78	0.31	7.27
3	<i>Capsicum annum</i> L.	2.79	0.43	7.70
4	<i>Solanum nigrum</i> L.	2.84	0.23	7.20

## Conclusion

All investigated four species of solanaceae shown a massive variation in the epidermal cells character at different level of taxonomy. Leaf epidermal anatomy with reference to stomata type, density, stomatal indexes and chlorophyll contents very constructive way for taxonomic point. Among four species *D.innoxia* shown higher variability in all aspects except the stomatal type; it show a typical anomocytic stomata. Further molecular level study enhances understanding of stomatal properties. This study may provide a platform for future researcher to make out a distinguishing feature among the four species.

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