

ANATOMICAL STUDIES ON STOMATA OF SOLANACEAE FROM MUZAFFARABAD DIVISION AZAD JAMMU ANDKASHMIR, PAKISTAN

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ABSTRACT: The epidermal features of leaf of family Solanaceae were studied to obtain data on adaxial and abaxial surfaces to appraise their anatomical significance. The study is about the variations in the configuration and allocation of stomata in twenty two species of Solanaceae collected from Muzaffarabad Division. Six types of stomata are examined in which anamocytic is dominant and is present in nine species, while anomotetracytic (3 spp) diacytic (4spp), paracytic (2 spp), anisocytic (2spp) and diacytic type of stomata were found in 2 species. In addition, average density, frequency, length and width of stomata and index are determined.

Key words: Solanaceae, Stomata, Dicot, Abaxial, Muzaffarabad

INTRODUCTION

Solanaceae is a cosmopolitan family in which most of the plants are dioecious. It rarely has trees or shrubs. There are 90 genera and over 3000 species are mostly found in tropical and temperate regions with centers of diversity in the Southern hemisphere, particularly in South America. Center of speciation occurs in Australia, Africa, Europe and Asia [1]. Among angiospermic family, the Solanaceae rank as one of the most important to human being. Species of the family are used for food (e.g. *Solanum tuberosum*, *S. melongena* and *Lycopersicon esculentum*). The family is an important source of more than 300 kinds of alkaloids (e.g. *Atropa acuminata*, *Datura stromanium*, *Hyocyanus niger* and *Solanum nigrum*) [2].

In Pakistan Solanaceae have 14 genera and 52 species, of which 27 species are native, 6 exotic and the rest are cultivated. *Solanum* is one of the largest and diverse genus of this family. Its hyper diversity not only makes it interesting for its taxonomy but benefits to mankind.

Efforts have been made to trace the anatomical characteristics of stomata of leaf and stem of present day Solanaceae. There are different opinions about the phylogeny of this family. In the previous taxonomical studies several other angiospermic families were thought to be a part of the Solanaceae but now considered as separate family [1]. Hutchinson in 1973 placed it in the order Solanales due to herbs, alternate leaves and actinomorphic flowers. Most of the recent taxonomist follow him in this respect [1, 3].

Leaves have a wide range of anatomical and morphological structures that partially isolate stomata from the turbulent atmosphere. The leaf epidermis is considered important in phylogeny and taxonomy [4, 5]. The structure and ontogeny of the stomata has been studied in 26 species of Rubiaceae [6, 7]. Pracytic and anomocytic type of stomata has been studied in the species of *Mallilotus indicus*, *Datura alba*, *Tribulis terrestris*, *Calatropis procera* and *Calatropis brachycarpa* [8]. The leaf epidermis has a number of important diagnostic characters that offer valuable clues for the plant identification, like shape and orientation of stomata. These important tissues play an important roles in taxonomy and determination of number of plant genera [9, 10].

Stomata is usually termed for the opening in the epidermis through which gases exchange [11]. Thirty one different types of stomata were studied among dicot [12]. Different taxonomic groups showed various types of stomata. The

distribution of stomata and their frequency gives the taxonomic as well as phylogenetic significance. The size and shape of stomata are also important characters taxonomically [13].

The present study focused on the stomata leaves and stem of 22 spp. of family Solanaceae, collected from Division Muzaffarabad. Six different types of stomata were observed in 22 species of Solanaceae viz: Amphianisocytic, anisocytic, diacytic, paracytic Tetracytic, anomocytic and anomotetracytic. Muzaffarabad, Azad Kashmir, Pakistan is situated between longitude 73.26-73.30 and latitude 34.12-34.24. The topography of the Division falls into the hilly zone [14].

MATERIALS AND METHODS

Samples of the plant species were collected during March 2014 to July, 2015 from Muzaffarabad Division Azad Jammu and Kashmir. The plants were identified with the help of Flora of Pakistan [15].

Paradermal section of the upper and lower epidermis of many fresh leaves were made with a sharp razor blade and transferred into petridish of water. For preliminary observation, these untreated sections were transferred into glycerin, protected with the cove slip wash in bottle several times, to remove the excess stain and mounted in glycerin. Permanent slides were prepared and viewed under the low power objective of the microscope. For further observations, some of the sections were stained in fast green for about ten minutes, washed in water several times to remove the excess stain and glycerin. Permanent slides were prepared by dehydrating the epidermal sections that have been washed free of excess stain for two minutes in 30, 50, 70 and 90 % alcohol, clearing them in few drops of pure xylene for two minutes and mounted in Canada balsam for microscopic examination [16].

The parameters studied were average length and width size of stomata, type of stomata, density, frequency and index on the abaxial and adaxial surface of amphistomatic leaf surfaces of the species of Solanaceae. Length and width of stomata was measured. The stomatal indexes were estimated using the following formula [17].

The Stomatal Index $i = [S / (E + S)] \times 100$

Where S is the number of stomata per unite area and E is the number of epidermal cells in the same unite area. The percentage of the stomatal complex was computed from the abaxial and adaxial surfaces by randomly observing 45

stomata from five prepared slides. The percentage of occurrence of each type was than obtained as a simple ratio. Density of the stomata was determined by the presence of stomata on the abaxial surface/ total number of stomata on the slide. A total of 45 leaves epidermal characters consisting of qualitative and quantitative features were elucidated. The statistical evaluation of the stomata of epidermal layer included mean and standard error were concluded [17].

RESULTS AND DISCUSSION

Micro characteristics of leaf epidermis and distribution of stomata was examined in the present study. A total of 22 dicot plant species were carried out which were scattered in 11 genera of family Solanaceae for stomatal detail (Table 1). The genus *Solanum* has nine species which are most prevailing while *Capsicum*, *Datura* and *Petunia* have two species and remaining seven genera were represented by single species (Table 1).

Anatomical characteristics have an important role in taxonomy and determining the number of plant genera and species [18, 19]. The foliar epidermis is one of the important taxonomic character from the systematic point of view. The taxonomic studies of a number of families and genera have been made on the basis of leaf epidermis [20-24]. Davis and Heywood [25] emphasized the use of anatomical characters, as these are reliable and fairly constant within a taxon. The micro-anatomical study of epidermis contributed in the classification of Asteraceae, Apocyanaceae, Boraginaceae, Euphorbiaceae, Fagaceae, Polygonaceae, Rosaceae and Solanaceae [20, 26-29].

The maximum average length and width size of stomata was observed in *Physalis minima* ($43.03\mu \pm 1.38\mu$), followed by *Datura stramonium* ($42.23\mu \pm 0.26\mu$), *Datura innoxia* ($40.93\mu \pm 1.08\mu$), *Withania somnifera* ($38.48 \pm 1.09\mu$) and *Hyocymus niger* ($37.96 \pm 1.40\mu$) on abaxial side, (Table 1) and the width was examined in *Solanum nigrum*, *Solanum villosum* and *Solanum surratense* as $27.90\mu \pm 3.20\mu$, $22.30\mu \pm 1.44\mu$ and $22.21\mu \pm 0.98\mu$ respectively. Similarly, on the adaxial surface maximum average length and width size was noted in *Nicotiana glauca* ($37.34\mu \pm 1.17\mu$) *Hyoscymus niger* ($36.85 \pm 2.91\mu$) and *Solanum erianthum* ($33.67\mu \pm 2.82\mu$). Whereas, minimum average length and width size was observed in the species in *Solanum tuberosum* ($14.44 \pm 0.74\mu$, $12.37 \pm 0.81\mu$) *Solanum cordatum* ($19.4 \pm 0.67\mu$, $11.40 \pm 0.52\mu$). Remaining plant species showed intermediate values of stomatal sizes on both amphistomatic leaf surfaces (Table 1).

The stomatal density was recorded highest in both upper and lower epidermises of *Physalis minima* ($56.05\mu \pm 1.93\mu$, $19.08\mu \pm 0.59\mu$), *Petunia hybrida* ($40.39\mu \pm 1.75\mu$, $33.10\mu \pm 1.68\mu$) and *Capsicum annum* ($39.79\mu \pm 1.32\mu$, $12.73\mu \pm 0.65\mu$) on the abaxial and adaxial surface respectively (Table 2). The rest of nineteen species of Solanaceae showed least values. Similarly, the highest percentage of stomatal frequency was calculated in *Physalis minima* $68.94\% \pm 2.37$ and $13.20\% \pm 0.40$ on the abaxial and adaxial surface respectively followed by *Datura stromanium* was $62.65\% \pm 1.93$, $25.09\% \pm 0.99$ *Capsicum annum* $60.13\% \pm 1.09$, $21.19\% \pm 1.09\%$ while the smaller value of frequency percentage was recorded in *Petunia hybrida* $20.74\% \pm 0.90$ and

$23.45\% \pm 1.19\%$ on abaxial and adaxial leaf surface respectively (Table 2). Remaining species have lowest frequency value.

The stomatal indices have a wide range of distinction. Although stomatal indices have been given key importance for making comparison in different taxa. Mishra et al recorded stomatal index (upper epidermis) is 30.12 and stomatal index for the (lower epidermis) is 36.31 in the leaves of *Tridax procumbens* [30]. The maximum stomatal index was calculated in *Datura stromanium* (14.40 ± 0.44 , 11.04 ± 0.43), followed by *Petunia hybrida* (13.16 ± 0.57 , 11.64 ± 5.92), *Datura innoxia* 11.62 ± 0.14 , 10.82 ± 0.53 , *petunia alba* 10.98 ± 0.33 , 9.21 ± 0.11 , *Solanum melongana* 9.88 ± 0.39 and 7.00 ± 0.2 on the abaxial side and adaxial side of leaf respectively (Table 3). The rest of the species showed least values of stomatal index on both surfaces of leaves.

During this study very valuable variation in the configuration of foliar epidermal anatomy was explored that can be used as an important taxonomic tool for the identification and differentiation of species. Anatomical studies revealed clear cut differences in size, shapes of epidermal cells, stomatal density, frequency, pavement cells and trichomes.

Stomata were studied on the abaxial and adaxial surfaces of leaves of Solanaceae. Six types of stomata were examined on abaxial and adaxial side of leaves viz; anamocytic anisocytic, anamotetracytic, tetracytic, isocytic and paracytic. Anomocytic type of stomata was represented in *Solanum villosum*, *S. lycopersicum*, *S. pseudo-capsicum*, *S. melangena*, *S. tuberosum*, *Capsicum annum*, *C. frutiscense*, *Hyocymus niger*, *Cestrum nocturnum* and *D. stramonium*. Anisocytic type of stomata was examined in *Solanum surratense*, *Solanum nigrum*, *Datura innoxia*, *Petunia hybrida* and *Atropa accuminata*. Rest of the species showed anamotetracytic, paracytic, tetracytic and isocytic types of stomata. Stomatal characteristics such as the type and index are among the anatomical parameter used in plant taxonomy. Aliero, Asekun, Grierson and Afolayan [31] and Hameed and Hussain [32] studied the stomatal type in selected genera of Solanaceae which was found very important in relation to the taxonomic separation of the different taxa. Different workers studied the stomatal types of various families as some plants of Polygonaceae [33], monocots within flora of Karachi, Pakistan [34], dicot flora of a district Tank in Pakistan [35] and stomatal index differentiation between the leaves of medicinal species of *Ocimum* from non-medicinal ones [36].

Anomocytic type of stomata was common in various members of Solanaceae which is followed by anisocytic type of stomata found in *Solanum cordatum*, *Solanum erianthum*, *Cestrum nocturnum*, *Petunia hybrid*, *Capsicum frutiscense* on the abaxial side which is a typical feature of Solanaceae [31]. The largest size of stomata was examined in *Physalis minima* (Table 1) followed by *Datura innoxia* and *Withania somnifera*. Similarly, *Capsicum frutiscense*, *Nicotiana glauca*, *Hyocymus niger*, *Solanum erianthum* and *Solanum pseudo-capsicum* showed large sized stomatal cells on the adaxial side. Smallest size of stomata was observed in *Solanum nigrum* and *Solanum tuberosum*. Maximum width of stomata was recorded in *Solanum nigrum* and *S.*

Table 1: The size of stomatal cells on the abaxial and adaxial surfaces of leaves of Solanaceae species collected from Division Muzaffarabad.

Taxon	Abaxial (length) μm Mean \pm SEM	Abaxial (width) Mean \pm SEM	Adaxial (length) Mean \pm SEM	Adaxial (width) Mean \pm SEM
1 <i>Solanum americanu</i>	24.77 \pm 4.35	27.90 \pm 3.20	24.76 \pm 1.309	16.817 \pm 0.86
2 <i>Solanum villosum</i> L.	31.00 \pm 11	22.30 \pm 0.98	27.60 \pm 1.38	21.45 \pm 0.79
3 <i>Solanum surratense</i>	28.10 \pm 2.40	22.21 \pm 1.44	32.18 \pm 0.40	20.53 \pm 1.00
4 <i>Solanum cordatum</i>	28.50 \pm 1.18	15.60 \pm 0.52	19.40 \pm 0.67	11.40 \pm 0.52
5 <i>Solanum pseudo</i>	35.40 \pm 2.06	20.85 \pm 0.36	32.00 \pm 0.66	16.41 \pm 0.93
6 <i>Solanum melangena</i>	36.93 \pm 1.76	17.88 \pm 1.27	20.24 \pm 1.82	21.73 \pm 2.37
7 <i>Solanum tuberosum</i>	32.30 \pm 2.55	21.64 \pm 1.65	14.44 \pm 0.74	12.37 \pm 0.8
8 <i>Solanum erianthum</i>	36.13 \pm 2.55	20.88 \pm 1.04	33.67 \pm 2.82	14.66 \pm 0.82
9 <i>Solanum dalcumara</i>	33.77 \pm 1.40	16.73 \pm 0.41	24.63 \pm 0.54	11.48 \pm 0.46
10 <i>Hyoscyamu s niger</i> L.	37.63 \pm 0.40	17.91 \pm 1.30	36.85 \pm 2.91	15.50 \pm 0.85
11 <i>Capsicum annuum</i> L.	34.70 \pm 1.35	19.40 \pm 2.13	26.86 \pm 0.68	10.00 \pm 0.61
12 <i>Capsicum frutescens</i>	26.07 \pm 1.62	18.15 \pm 1.62	33.18 \pm 1.17	15.83 \pm 0.60
13 <i>Petunia alba</i> Lindl.	37.33 \pm 2.39	19.27 \pm 0.60	27.27 \pm 0.62	9.57 \pm 0.24
14 <i>Petunia hybrida</i> L.	35.40 \pm 0.34	18.38 \pm 0.31	20.88 \pm 2.12	12.40 \pm 0.64
15 <i>Withania somnifera</i>	38.48 \pm 1.09	22.28 \pm 1.253	23.64 \pm 1.65	11.80 \pm 1.27
16 <i>Cestrum noctrunum</i>	32.05 \pm 1.40	14.670 \pm 1.098	27.35 \pm 2.23	1558 \pm 1.32
17 <i>Datura Stramoniu</i>	42.30 \pm 0.26	20.770 \pm 0.589	31.030 \pm 2.71	11.30 \pm 0.26
18 <i>Datura innoxia</i>	40.93 \pm 1.08	18.37 \pm 2.03	31.54 \pm 0.51	11.96 \pm 0.54
19 <i>Atropa acuminata</i>	33.23 \pm 2.43	8.20 \pm 0.72	24.27 \pm 1.04	9.50 \pm 0.72
20 <i>Physalis minima</i>	43.30 \pm 1.38	18.90 \pm 0.99	32.18 \pm 0.50	13.83 \pm 0.93
21 <i>Nicotiana tabaecum</i>	33.51 \pm 3.27	15.74 \pm 1.08	37.34 \pm 1.17	14.73 \pm 0.65
22 <i>Lycopersic on</i>	30.96 \pm 1.50	19.41 \pm 0.77	27.95 \pm 1.44	12.60 \pm 1.12

Table 2: Stomatal density and stomatal frequency on the abaxial and adaxial surface of Solanaceae species collected from Division Muzaffarabad.

Taxon	Stomatal Density (ab)	Stomatal Density (ad)	Stomatal	Mean \pm SEM
1 <i>Solanum americum</i> L.	19.33 \pm 1.02	17.03 \pm 0.47	30.95 \pm 1.45	25.22 \pm 0.70
2 <i>Solanum villosum</i> L.	29.75 \pm 1.06	13.92 \pm 0.39	39.22 \pm 1.40	15.65 \pm 0.43
3 <i>Solanum surratense</i> Brunf.s.	38.68 \pm 0.83	15.58 \pm 0.46	56.04 \pm 1.21	19.31 \pm 0.58
4 <i>Solanum cordatum</i> Forssk.	33.31 \pm 0.19	12,34 \pm 0.69	59.49 \pm 0.40	19.67 \pm 0.80
5 <i>Solanum pseudo capsicum</i> L.	2130 \pm 0.76	17.11 \pm 0.21	28.07 \pm 1.08	15.72 \pm 0.57
6 <i>Solanum melangena</i> L.	21.73 \pm 0.08	18.80 \pm 0.5	39.74 \pm 0.15	16.03 \pm 0.61
7 <i>Solanum tuberosum</i> L.	17.04 \pm 0.59	14.20 \pm 0.11	3.38 \pm 1.03	28.31 \pm 0.22
8 <i>Solanum erianthum</i> D.Don	12.37 \pm 0.47	16.82 \pm 0.29	25.81 \pm 0.98	23.36 \pm 0.50
9 <i>Solanum dalcumara</i> L.	37.75 \pm 0.85	29.94 \pm 1.02	47.65 \pm 10.82	49.92 \pm 1.71
10 <i>Hyoscyamus niger</i> L.	16.87 \pm 0.29	15.47 \pm 0.57	23.10 \pm 0.00	15.57 \pm 0.57
11 <i>Capsicum annuum</i> L.	39.92 \pm 1.32	12.73 \pm 0.65	60.34 \pm 1.99	21.19 \pm 1.09
12 <i>Capsicum frutescens</i> L.	34.58 \pm 1.11	9.24 \pm 0.20	43.61 \pm 1.40	13.93 \pm 0.31
13 <i>Petunia alba</i> Lindl.	24.13 \pm 0.72	29.38 \pm 0.33	39.21 \pm 1.18	54.08 \pm 0.65
14 <i>Petunia hybrida</i> L.	40.91 \pm 1.75	33.06 \pm 1.63	20.74 \pm 0.00	23.45 \pm 1.19
15 <i>Withania somnifera</i> (L.)Dunal	2944 \pm 0.77	25.45 \pm 0.56	33.36 \pm 0.82	17.88 \pm 0.39

16	<i>Cestrum nocturnum</i> L.	34.00	±1.26	23.01	±0.33	49.64	±185	64.02	±0.94
17	<i>Datura Stramonium</i> L.	36.13	±1.11	17.89	±0.7	62.65	±1.30	25.09	±0.99
18	<i>Datura innoxia</i> Miller	21.37	±0.27	22.19	±1.09	31.27	±0.35	43.33	±2.14
19	<i>Atropa acuminata</i> Royle	11.35	±0.21	15.93	±0.53	23.21	±0.41	19.41	±0.64
20	<i>Physalis minima</i> Auctt.	56.05	±1.90	19.87	±0.59	68.94	±2.73	13.20	±0.40
21	<i>Nicotiana tabaeum</i> L.	12.61	±0.17	15.33	±1.00	31.77	±0.35	21.19	±1.38
22	<i>Lycopersicon esculentum</i>	23.34	±0.65	30.63	±0.61	43.68	±1.22	49.50	±0.99

Table 3: Stomatal index on the abaxial and adaxial surface of Solanaceae species collected from Division Muzaffarabad.

Taxon		Stomatal Index (ab)		Stomatal Index (ad)	
1.	<i>Solanum americanum</i> L.	4.44	±0.23	3.88	±0.10
2	<i>Solanum villosum</i> L.	5.62	±0.60	4.58	±0.12
3	<i>Solanum surratense</i> Brunf.s.	5.93	±0.19	5.66	±0.17
4	<i>Solanum cordatum</i> Forssk.	7.92	±0.04	6.88	±0.27
5	<i>Solanum pseudo capsicum</i> L.	6.39	±0.29	5.09	±0.20
6	<i>Solanum melangena</i> L.	9.88	±0.09	7.50	±0.26
7	<i>Solanum tuberosum</i> L.	4.71	±0.16	3.69	±0.03
8	<i>Solanum erianthum</i> D.Don.	8.38	±0.20	8.93	±0.19
9	<i>Solanum dalcumara</i> L.	11.19	±0.24	9.68	±0.33
10	<i>Hyoscyamus niger</i> L.	5.64	±0.09	6.40	±0.24
11	<i>Capsicum annum</i> L.	5.06	±0.19	4.49	±0.22
12	<i>Capsicum frutescens</i> L.	6.49	±0.20	5.08	±0.11
13	<i>Petunia alba</i> Lindl.	10.94	±0.33	9.22	±0.11
14	<i>Petunia hybrida</i> L.	13.67	±0.57	11.64	±0.59
15	<i>Withania somnifera</i> (L.)Dunal	3.46	±0.09	2.85	±0.06
16	<i>Cestrum nocturnum</i> L.	7.30	±0.27	5.60	±0.08
17	<i>Datura Stramonium</i> L.	14.49	±0.44	11.48	±0.43
18	<i>Datura innoxia</i> Miller	11.25	±0.15	10.24	±0.53
19	<i>Atropa acuminata</i> Royle	5.87	±0.10	5.06	±0.16
20	<i>Physalis minima</i> Auctt.	3.56	±0.23	2.92	±0.09
21	<i>Nicotiana tabaeum</i> L.	8.49	±0.16	6.76	±0.44
22	<i>Lycopersicon esculentum</i> Miller	4.76	±0.13	4.09	±0.08

melongena on both sides (Table 1) which were followed by *Solanum villosum*, *Solanum surratense*, *Solanum pseudocapsicum*, *Solanum melongena* and *Solanum erianthum* respectively (Table 1).

The stomatal density, frequency, stomatal index, their mean and standard error were recorded. The highest stomatal density was recorded in *Physalis minima* and *Petunia hybrida* on the abaxial and adaxial surfaces (Table 2, 3) which was followed by *Capsicum annum*, *Solanum surratense*, *Lycopersicon esculentum*, *Solanum dalcumara* and *Petunia alba*. The stomatal frequency was recorded maximum in *Physalis minima* and *Datura stramonium* and minimum in *Petunia hybrida* and *Hyoscyamus niger* (Table 3) in the abaxial surface whereas, it was high in *Cestrum nocturnum* and *Petunia alba* while it was low in *Solanum villosum* and *Solanum pseudo-capsicum* (Table 3). Stomatal indices showed wide range of variations among 22 species of Solanaceae. The stomatal index was highest in *Solanum erianthum* and *Petunia alba* while it was recorded lowest in the stomata of *Atropa acuminata* and *Withania somnifera* on

the abaxial and adaxial surfaces of leaves. Mishra, Sangameswaran, Singh and Dubey [37] and Krishnaveni and Thaakur [38] reported stomatal index of *Solanum nigrum* and *Withania coagulense*.

It has been reported that paracytic and anomocytic stomata in order Centrospermae and Polygonales and in *Salix* species while anisocytic and diacytic stomata was observed in Amaranthaceae [39]. Correlation of features provides a base to assign taxonomic categories. Stomatal variations are useful at all levels of taxonomic hierarchy [8].

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