

Foliar Micromorphologic Features for Climatic Stress Impact Assessment

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Abstract: Foliar epidermal features of seven species of Acanthaceae grown in natural habitat (control) and in areas of environmental stress were analysed using micromorphological and histochemical characters. The parameters include size of stomata, size of epidermal cells, and size of cystolith, palisade ratio, stomatal frequency and stomatal index. Histochemical localisation of starch indicated abnormalities in stomatal complex under environmental stress. However, mesogenous stomatal complex though a vegetative entity is least deviated by the environmental stress; can be considered as reliable as any other conservative taxonomic character for delimiting Acanthaceae. Length and breadth of stomata, epidermal cell and cystolith were analysed on the basis of student's t test. In majority of cases, significant variations were obtained. In the plants grown under environmental stress when tested with chi square, no significant change was observed for palisade ratio and stomatal index. Stomatal frequency showed significant variation under environmental stress, except in *Strobilanthes ciliatus*. *Justicia gendarussa* appears to be the most tolerant species while *Justicia betonica* and *Ruellia tuberosa* are highly susceptible to environmental stress.

Keywords: Micromorphology, Histochemistry, climatic stress

1. Introduction

The effect of climatic stress on plants has been known for long and several incidents of environmental ill effects have started gaining the attention of botanists. Jacobson and Hill (1970) have provided a pictorial atlas based on external symptoms to recognise different damage patterns due to air pollution. Heck, Taylor & Heggestad (1973) and Heath (1980) reviewed the plant damage due to air pollution. Cutter (1978) emphasised the importance of epidermis as it is the tissue that is in direct contact with the environment which is subjected to structural modifications by various atmospheric factors. Investigations of Walker & Dunn (1967); Sharma & Dunn (1968, 1969); Sharma (1972, 1977); Sharma & Tyree (1973); Sahu & Santra (1989) Panday (1990) and Prasad and Inamdar (1990) Cárcer et al., (2017), Drack & Vázquez (2018), Oliveira (2013) Shahid et al., (2017), and Wuyts et al., (2015), have increased the awareness regarding this problem.

Patel & Devi (1985) are of opinion that a comparison of the histochemical localization of metabolites such as starch, insoluble polysaccharides and lipids in the normal and polluted leaves give a clear vision of the physiological status of the plant. The present study aims at a better understanding of the structural as well as histochemical variations due to environmental stress. An effort is also made to find out the parameters which are least deviated by the environmental stress.

2. Methodology

The effect of environmental stress is studied in the following seven species of plants under study.

- (1) *Ecbolium linneanum* Kurz var. *laetevirens*
- (2) *Eranthemurncapense* L.
- (3) *Justicia betonica* L.
- (4) *J. gendarussa* Burm.f.
- (5) *Rhinacanthus communis* Nees
- (6) *Ruellia tuberosa* L.
- (7) *Strobilanthes ciliatus* Nees

The parameters viz., stomatal size, epidermal cell size, size of the cystolith, Stomatal Index, stomatal frequency and palisade ratio are recorded for both control plants from natural habitat and samples from areas of environmental stress. Histochemical characterisation of stomatal complex was done through localisation of starch with I₂ KI solution (Jensen, 1962). P₁ represents plants of natural habitat (control) and P₂ represents plants grown with environmental stress. The significance of variation is statistically analysed with the help of chi square test, student's t test and percentage of variation.

3. Results

3.1. Structural variations

In normal plants from natural habitat, the anticlinal walls of the epidermis show undulations in all the seven species. In plants grown with environmental stress either the degree of undulation is reduced as in the case of *Eranthemurncapense* L. (Pl. 1, Fig. K) or the sinuous walls become straight as in *Strobilanthes ciliatus* Nees., II B (Pl. 1, Fig. B). The typical diallelomesogenous stomata of Acanthaceae is observed in all the species from natural habitat and polluted environment. In samples from plants grown with environmental stress no major change occurs in the morphology of mature stomatal complex. However a reduction in the size of the stomata and subsidiary cells is usually met with in the leaves from stress induced habitat

(Pl. 1, Figs. B,H). In addition to this, stomatal abnormalities like arrested development (Pl. 1, Figs. C.F.K), stomata with single guard cell (Pl. 1, Figs.E) and stomata with aborted or degenerated guard cells (Pl. 1, Fig. H) are frequently recorded in leaves from stress induced habitat. Such abnormal stomatal types are of rare occurrence in the samples from non-polluted environment and their frequency is also insignificant. The difference in the size of epidermal cells, stomata and cystolith of the control and stress induced samples is also charted in Table 1. The comparative observations regarding the Stomatal Index, stomatal frequency and palisade ratio are given in Table 2. Among the plants of the natural habitat, the length of the stomata ranges from 14.52 μm (*S.ciliatus* Nees) to 45.65 μm (*E.linneanum* Kurz var. *laetevirens*), whereas the Stomatal length of the taxa from polluted area varies from 12.50 μm (*S. ciliatus* Nees) to 38.97 μm . (*J.gendarussa* Burm.f.).

Table 1. Effect Of Environmental Stress On Foliar Epidermis

Data On The Size Of Stomata Epidermis And Cystolith

SPECIES		Stomata *		Epidermis *		Cystolith *	
		L	B	L	B	L	B
<i>Ecbolium linneanum</i> Kurz. var. <i>laetevirens</i>	P ₁	45.65 ± 1.5740	22.72 ± 2.0990	66.8 ± 5.9012	36.520 ± 1.3640	126.92 ± 1.3640	45.64 ± 2.8381
	P ₂	36.250 ± 0.7880	17.26 ± 0.7886	50.25 ± 2.2000	25.0 ± 1.6801	74.60 ± 3.4315	31.73 ± 0.3274
<i>Eranthemum capense</i> L.	P ₁	42.31 ± 0.7898	25.60 ± 1.5750	104.37 ± 4.2982	50.10 ± 8.3520	313.38 ± 2.759	24.39 ± 1.4400
	P ₂	31.17 ± 2.0820	21.15 ± 2.0840	68.75 ± 6.2560	36.25 ± 3.7517	107.99 ± 0.8037	23.38 ± 0.8900
<i>Justicia betonica</i> L.	P ₁	42.31 ± 1.4808	33.96 ± 0.7846	43.83 ± 3.8921	30.89 ± 2.5600	287.80 ± 3.4340	41.76 ± 0.6588
	P ₂	25.05 ± 1.3645	15.58 ± 0.7898	28.48 ± 2.4607	22.24 ± 2.16	156.42 ± 2.0092	24.49 ± 2.0829
<i>J. gendarussa</i> Burm.f.	P ₁	41.19 ± 0.7876	24.49 ± 0.7894	140.46 ± 3.2901	70.97 ± 4.2612	60.53 ± 1.0880	17.69 ± 1.3600
	P ₂	38.97 ± 0.7879	21.71 ± 1.3650	132.15 ± 1.3901	58.86 ± 3.1618	59.28 ± 0.9275	15.03 ± 1.3638
<i>Rhinacanthus communis</i> Nees	P ₁	35.63 ± 1.5740	26.16 ± 1.5745	139.76 ± 5.7124	60.1 ± 2.6742	192.04 ± 0.6678	25.88 ± 1.6760
	P ₂	24.49 ± 2.0837	20.60 ± 0.7890	90.84 ± 2.3450	43.27 ± 3.4210	116.90 ± 0.6818	23.38 ± 1.3651
<i>Ruellia tuberosa</i> L.	P ₁	42.86 ± 2.3710	21.15 ± 0.8736	120.63 ± 6.0864	69.71 ± 4.1771	168.67 ± 2.7258	27.83 ± 2.1290
	P ₂	26.16 ± 0.8887	15.58 ± 2.0840	70.64 ± 7.6902	40.16 ± 1.6770	119.68 ± 2.9510	20.04 ± 2.6047
<i>Strobilanthes ciliatus</i> Nees	P ₁	14.52 ± 0.7758	8.71 ± 0.5507	104.0 ± 4.8208	51.35 ± 4.3440	854.87 ± 1.8391	82.26 ± 3.3352
	P ₂	12.50 ± 0.3106	7.37 ± 0.5542	67.6 ± 4.3406	33.15 ± 2.7612	426.88 ± 2.5446	57.36 ± 2.0174

* All measurements are in microns; L – Length; B – Breadth; P₁- From plants of natural habitat (Control) P₂- From plants grown with environmental stress

The breadth of the stomata from non-polluted samples varies from 8.71 μm (*J. betonica* L.). In the polluted environment, the stomatal breadth ranges from 7.37 μm (*S. ciliatus*) to 21.71 μm . (*J. gendarussa* Burm.f.). The maximum stomatal frequency among the taxa under study is reported in *Rhinacanthus communis* Nees both from control and (160.00/mm²) and stress induced (196.00/mm²) environment. The minimum value of stomatal frequency from stress induced and natural habitat is recorded in *Eranthemum capense* L. (84.00/mm²). From the stress induced samples under study, the lowest stomatal frequency is observed in *Strobilanthes ciliatus* Nees., (93.61 /mm²). From the control taxa under study, the maximum value of Stomatal Index is reported for *Ruellia tuberosa* L., (23.95) and the lowest being 17.13 in *J. betonica* L. Among the plants from stress induced environment, the Stomatal Index ranges from 17.74 (*Eranthemum capense* L.) to 26.31 (*Ruellia tuberosa* L.).

Table 2. EFFECT OF ENVIRONMENTAL STRESS ON FOLIAR EPIDERMIS					
Data on Palisade Ratio, Stomatal Frequency and Stomatal Index					
Species		Palisade Mean	Ratio Range	Stomatal Frequency/mm ²	Stomatal Index
Ecbolium linneanum Kurz. var. laetevirens	P ₁	4.10	(3.0 - 5.0)	96.00	15.81
	P ₂	4.00	(3.25-4.75)	105.88	18.76
Eranthemum capense L.	P ₁	11.90	(9.0 -13.75)	84.00	15.96
	P ₂	11.66	(10.0 -14.0)	102.90	17.74
Justicia betonica L.	P ₁	6.33	(5.50 - 7.50)	148.00	17.13
	P ₂	6.00	(5.00 - 7.00)	172.00	19.95
J. gendarussa Burm.f.	P ₁	7.05	(6.25 - 8.25)	129.66	19.50
	P ₂	7.01	(6.0 - 7.75)	135.30	20.10
Rhinacanthus communis Nees	P ₁	5.70	(4.50 - 7.50)	160.00	20.95
	P ₂	5.66	(4.50 - 6.50)	196.80	24.56
Ruellia tuberosa L.	P ₁	16.50	(13.00-17.25)	136.00	23.95
	P ₂	14.43	(12.75-17.00)	192.00	26.31
Strobilanthes ciliatus Nees	P ₁	10.20	(8.00 - 12.00)	88.00	15.74
	P ₂	9.25	(8.00 - 9.75)	93.61	18.95

P₁- From plants of natural habitat (Control); P₂- From plants grown with environmental stress

The size of the epidermal cells shows considerable variations with respect to different taxa. Among the control species under study, the maximum length of epidermal cells noted is 140.46 μm (J. gendarussa Burm.f.) while the minimum value is 43.83 μm (J. betonica L.). In the stress induced area, the length of the epidermal cells varies from 28.48 μm (J. betonica L.) to 132.15 μm (J. gendarussa Burm.f.). In normal plants, the breadth of the epidermal cells ranges from 30.89 μm (J. betonica L.) to 70.97 μm (J. gendarussa Burm.f.), while the stress induced taxa give a variation from 22.24 μm (J. betonica L.) to 58.86 μm (J. gendarussa Burm.f.).

Both in control and stress induced materials under study, the size of the cystolith varies with species. In taxa from natural area the length of the cystolith gives a minimum value of 60.53 μm in J. gendarussa Burm.f. and a maximum value of 854.87 μm in S. ciliatus Nees. The length of cystolith in stress induced samples varies from 59.28 μm (J. gendarussa Burm.f.) to 426.88 μm (S. ciliatus Nees). From the plants of control area the lowest value for the breadth of cystolith is recorded in J. gendarussa Burm.f. (17.69 μm) and the highest being 82.26 μm., as in S. ciliatus Nees. The leaves from stress induced environment show a difference in the breadth of the cystolith ranging from 15.03 μm (J. gendarussa Burm.f.) to 57.36 μm (S. ciliatus Nees). Among the taxa investigated, the highest value of palisade ratio is recorded for Ruellia tuberosa L., both for natural and (16.50 μm) and stress induced (14.43) materials. The lowest value of palisade ratio is represented by Ecbolium linneanum Kurz var. laetevirens and the mean palisade ratio values of the species from the control and stress induced area being 4.10 μm and 4.00 μm respectively.

3.2. Distribution of starch

When tested with I₂ -KI, starch grains appear blue-black in colour, and in all the samples they are confined to the guard cells. The stomata from normal healthy show the presence of large globular starch grains (Pl. 1, Figs- A,D,G,J,L). Stress induced samples are characterised by stomata having ill-formed starch grains which are found to be reduced (Pl. 2, Figs.E,H) when compared with that of the control samples. In abnormal stomata the cytoplasm of the stomatocyte appears to be filled with amorphous blue-black fluid (Pl. 1, Figs. F,I,K) indicating the presence of starch. However the grain formation is found to be arrested in such cases.

3.3. Statistical analysis

Data on the different parameters under study were subjected to statistical analysis to find out the significance of variation (Table 3).

Computed t test and chi square (χ^2) values									
SPECIES	Size of stomata*		Size of epidermal cells*		Size of cystolith*		Palisade ratio**	Stomatal frequency**	Stomatal Index**
	L	B	L	B	L	B			
<i>Ecbolium linneanum</i> Kurz. var. <i>laetevirens</i>	8.36	4.22	4.55	10.06	24.54	8.43	0.0122	5.0841	2.7522
<i>Eranthemum capense</i> L.	8.67	2.95	8.13	2.62	123.79	1.03	0.0242	21.2625	0.9926
<i>Justicia betonica</i> L.	14.85	28.59	5.77	4.77	57.20	13.70	0.0860	19.4595	2.3292
<i>J. gendarussa</i> Burm.f.	3.45	3.05	4.03	3.95	1.51	2.39	0.0011	1.2267	0.0923
<i>Rhinacanthus communis</i> Nees	7.39	5.47	13.72	6.72	146.05	2.00	0.0014	42.3200	3.1103
<i>Ruellia tuberosa</i> L.	11.42	4.27	8.83	11.37	22.56	4.01	1.2085	115.2941	1.1628
<i>Strobilanthes ciliatus</i> Nees	4.19	2.97	9.72	6.12	236.11	11.06	0.4424	1.7882	3.2732

* Computed t test values; ** Computed chi square test values; L – Length; B - Breadth

3.3.1. Students 't' test

The length and breadth of stomata, epidermal cells and cystolith under normal and polluted conditions were analysed on the basis of students 't' test. In the majority of cases, significant variations were obtained for each parameter. Under polluted conditions, the changes appeared to be insignificant in the following cases.

- Breadth of the epidermal cell in *E. capense* L.
- Length of the cystolith in *J. gendarussa* J Burm.f.
- Breadth of the cystolith in *J.gendarussa* Burm.f. and *R.communis*Nees.

3.3.2. Chi-square test(χ^2 test)

The parameters such as palisaderatio, stomatal frequency and Stomatal Index under natural and stress induced conditions were tabulated by this method. No significant change occurred in the palisade ratio and Stomatal Index.

On the contrary, stomatal frequency showed significant variation under pollution stress, except for *S.ciliatus* Nees (Table 3).

The study exemplifies well the fact that Stomatal Index and palisade ratio are more reliable than other criteria such as stomatal frequency, size of epidermal cells, size of stomata and size of cystolith.

3.3.3. Percentage of variation

The percentages of variation in the epidermal features were tabulated (Table 4). The variations in the length and breadth of stomata are maximum in *J. betonica* L., and minimum in *J. gendarussa* Burm.f.

Species	%of variation in the size of the stomata		%of variation in the size of the epidermis (L& B)		%of variation in the size of the cystolith (L& B)		% variation in palisade ratio	%of variation stomatal frequency	%of variation - stomatal index
	L	B	L	B	L	B			
<i>Ecbolium linneanum</i> Kurz. var. <i>laetevirens</i>	-20.29	-24.03	-24.77	-33.45	-41.22	-30.47	-2.43	10.29	24.98
<i>Eranthemum capense</i> L.	-26.32	-17.38	-34.12	-27.64	-65.54	-4.14	-2.01	22.50	11.15
<i>Justicia betonica</i> L.	-40.79	-54.12	-35.02	-28.00	-45.64	-41.35	-5.21	16.21	16.46
<i>J. gendarussa</i> Burm.f.	-5.38	-11.35	-5.91	-17.06	-2.07	-15.36	-0.56	4.34	1.50
<i>Rhinacanthus communis</i> Nees	-31.26	-21.25	-35.00	-28.00	-39.12	-9.65	-0.70	23.00	17.23
<i>Ruellia tuberosa</i> L.	-38.96	-26.33	-41.39	-42.38	-29.04	-27.99	-12.54	41.17	9.85
<i>Strobilanthes ciliatus</i> Nees	-13.91	-15.38	-35.23	-35.44	-50.06	-30.26	-9.31	6.37	20.39

L – Length; B – Breadth

The increase in the Stomatal Index is maximum in *S. ciliatus* Nees and is minimum in *J. gendarussa* Burm.f. Regarding the stomatal frequency, minimum deviation is observed in *J. gendarussa* Burm.f., while the maximum deviation is encountered in *R. tuberosa* L. The mean palisade ratio value of *J. gendarussa* is least deviated by the stress from pollution while the percentage of variation is maximum in *R. tuberosa* L. The later species is also characterised by the maximum deviation in the size of the epidermal cells. The epidermal cells of *J. gendarussa* Burm.f. show minimum deviation due to environmental stress. In *S. ciliatus* Nees., the cystolith shows maximum deviation in length. The percentage of variation in the length of cystolith is minimum in *J. gendarussa*. With regard to the reduction in the breadth of the cystolith, maximum variation is shown by *J. betonica* L., while *R. communis* Nees, shows minimum variation. The study indicates that, of the seven species under study, *J. gendarussa* Burm.f., appears to be the most tolerant species while *J. betonica* L., and *R. tuberosa* L., are highly susceptible to environmental stress.

4. Discussion

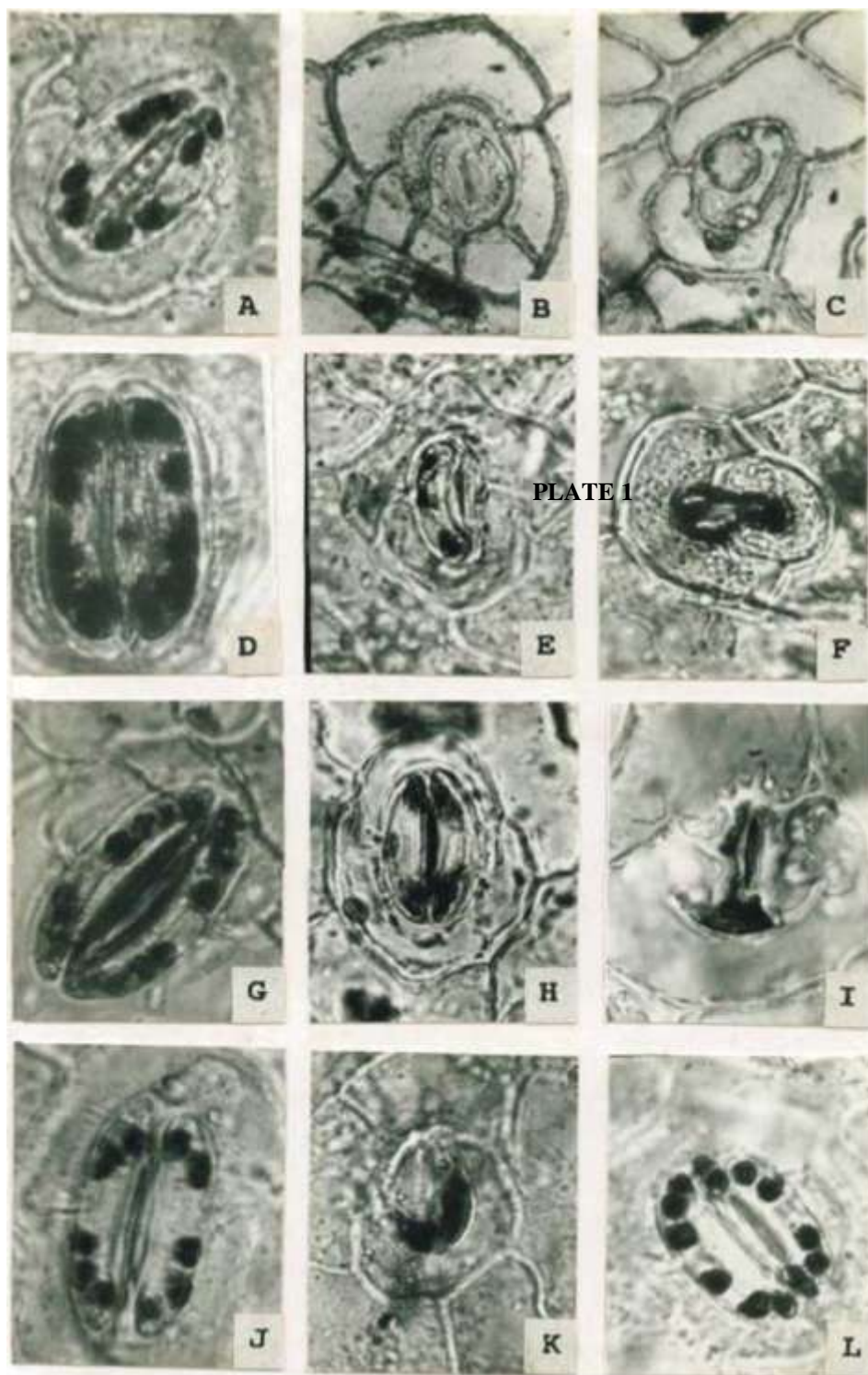
Mansfield (1973) reports that stomata provide the main route of entry for pollutants into the plant and they play a leading role in determining the overall response of plants to aerial pollutants. In the present study, the main pollutants that cause structural variations in the foliar epidermis may be H₂S and acidic fumes from the chemical laboratory and automobile exhausts from the roads. The present comparison of the epidermal features of stress induced and samples from natural habitat generally indicates a reduction in the size of stomata, epidermal cells and cystoliths. While stomatal frequency and Stomatal Index increase, palisade ratio values show insignificant changes. Jafari et al., (1979); Chakrabarty & Gupta (1981) and Inamdar & Chaudhary (1984) have shown that stomatal frequency increases due to environmental pollution. The present work is in agreement with this view. The increase in the Stomatal Index due to pollution is already established in the species of Acanthaceae viz. *Peristrophe bicalyculata* Nees (Inamdar & Chaudhary l.c). This holds true for all the taxa under study. With regard to the size of the cystolith, the length decreases in all the taxa investigated. Inamdar & Chaudhary (l.c.) have given a controversial report that the length of the cystolith increases due to pollution. However, the pollutants differ in these two cases. Patel & Devi (1985) are of opinion that the effect of pollution on foliar epidermis varies with species. In order to obtain a comparative measure of the variation of epidermal features of different taxa under stress from pollution, the significance of variation was statistically analysed, percentage of variation was also calculated.

Reports of Ahmed (1964) and Ahmad & Yunus (1981) have revealed that abnormal stomata are present in Acanthaceae and other gamopetalous families. Besides, publications of Chaphekar & Karbhari (1974), Bull & Mansfield (1974), Srivastava et al, (1975) and Capron & Mansfield (1976) have shown that when plants are exposed to Sulphur dioxide and Nitrogen dioxide, there is a reduction in the photosynthetic activity. The increased number of stomatal abnormalities, reduction in the size of mature stomata, reduction in the number and size of starch grains in the polluted plants and the arrested starch grain formation in abnormal stomata, indicate the decrease in the efficiency of the metabolic system of foliar epidermis. This is also in keeping with the views of Patel & Devi (1985) and Ahmad & Yunus (1981). The high stomatal frequency may be a compensatory mechanism

adopted by the plant to fill this gap. Gostin (2009) studied air pollution effects on the leaf structure of some species of Fabaceae. This includes cuticular and foliar anatomical response of the selected taxa to induced pollution.

5. Conclusions

The presentwork is a comparative study of epidermal features of seven species of Acanthaceae grown under natural habitat and locality under environmental stress. The stomatal Index and palisade ratio values are least affected by environmental stress. However, Stomatal abnormalities of taxa are well exemplified by plants under pollution stress. When compared to plants grown under natural habitat, the parameters such as size of stomata, epidermal cells and cystolith are affected by environmental stress. Histochemical localisation of starch is done in both types of plants. Though the results exhibit variation in the epidermal features, the ontogeny and structure of mature stomatal complex remain constant in both control plants and taxa under environmental stress. It can be concluded that the mesogenous stomatal complex though a vegetative entity, is least deviated by the environmental stress and can be considered as reliable as any other conservative taxonomic character for delimiting Acanthaceae. *Justicia gendarussa* appears to be the most tolerant species.



PL 1. Figs. A – L. Light micrographs with starch localization (Figs. A - L x 1000).

- A.** *Strobilanthes ciliatus* Nees, (P₁). Mature stomatal complex with well developed starch grains.
- B.** *S. ciliatus* Nees, (P). stomatal complex with reduced size and ill formed starch grains.
- C.** *Strobilanthes ciliatus* Nees, (P₂). Arrested stomatal development
- D.** *Justiciagendarussa* Burm.f., (P₁). Mature stomatal complex with well developed starch grains.
- E.** *G. gendarussa* Burm.f., (P). Stoma with single guard cell having lesser number of starch grains.
- F.** *G. gendarussa* Burm.f. (P). Stomatal initial showing arrested development. I₂ KI detects the presence of starch, but grain formation is arrested.
- G.** *Rhmacanthus communis* Nees, (P₁). Well developed starch grains in the mature stomata.
- H.** *R. communis* Nees (P₂). reduction in the size of the stomatal complex. The starch grains are of smaller size.
- I.** *R. communis* Nees (P). distorted guard cells with stomatal pore in the centre. The starch is detected in the guard cells but the grain formation is arrested.
- J.** *Eranthemum capense* (P₁) Mature stomatal complex with well developed starch grains.
- K.** *E. capense* L., (P). stomatocyte showing arrested development.
- L.** *L. Ecbolium linneanum* Kurz var. *laetevirens*, (P₁) Guard cells with well-developed starch grains

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