# ORGANOGRAPHIC VARIABILITY OF STOMATAL CHARACTERS IN HEVEA BRASILIENSIS MUELL. ARG. AND ITS POSSIBLE SIGNIFICANCE IN CLONAL SUSCEPTIBILITY TO LEAF FALL DISEASE.

By

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#### ABSTRACT

Density of stomata on leaf blade and petiole as well as the aperture size of the stomata on petiole, leaf blade, vein and fruit wall have been studied in six clones of Hevea brasiliensis of which three were susceptible and the other three were somewhat tolerant to abnormal leaf fall disease. The study revealed that a few stomata are present on the epidermis of petioles, veins, and fruit wall but the stomatal density is very low compared to that in the veinless portion of the leaf blade. The aperture length and aperture width of petiolar and veinal stomata were significantly high compared to those of the stomata on the leaf blade. There was a significant positive correlation between the aperture length of petiolar stomata and that of veinal stomata. The aperture length of fruit wall stomata was comparable to that of petiolar stomata, but was more wide open. It appeared that the organographic variability of stomatal characters have an influence on the organographic specificity of leaf fall disease infection. It also appeared that the aperture index of petiolar stomata was in someway related to the clonal susceptibility to abnormal leaf fall disease.

### INTRODUCTION

A review of stomatal studies in different species reveal a range of structural, functional or ontogenetic variations of the stomata of the leaf laminae. The nature and density of stomata in Hevea brasiliensis have also been studied (Bobilioff, 1923; Chen et al., 1981; Panikkar, 1974; Senanayake et al., 1970). Attempts to study the entire stomatiferous area of a plant, however, are very rare. Nevertheless such investigations will yield useful information on the nature and extent of variation of the stomata of the different stomatiferous parts as well as their functional significance. This piece of investigation has been attempted with a view to ascertaining the nature of the stomata on the leaf blade, vein, petiole and fruit wall of Hevea brasiliensis and also their probable significance in relation to abnormal leaf fall disease.

#### MATERIALS AND METHODS

The studies were conducted on six clones of Hevea brasiliensis namely, RRII 101, RRII 102, RRII 105, RRII 106, PR 107 and Tjir 1, planted in 1966 in a single tree single plot clone trial with forty replications. Three trees were selected randomly from each clone. Three leaves at random positions on the tree were collected from each. All the leaves collected belonged to comparable nodal positions on the twig. Fruits were collected as available. The sample collections were made when the trees were 15 years of age. Leaf bits were taken out from the middle portion of the central leaflets and peelings of the lower epidermis were prepared. Samples of the petioles were taken 2 cm away from the node and epidermal peelings of the abaxial half were taken. Epidermal peelings

of the fruits were also taken. Standard methodology (Purvis et. al., 1966) was followed for the preparation of the peelings and for the staining.

Density of stomata per 1 mm<sup>2</sup> of the leaf blade and fruit wall and 10 mm<sup>2</sup> of the petiole was assessed. Length and width of the stomatal aperture were also measured. The aperture index of leaf blade and petiolar stomata have been worked out for which density per 1 mm<sup>2</sup> was used in both cases. The data were statistically analysed, except for the stomata on the fruit wall.

Field observations were made on the clonal response to abnormal leaf fall disease under normal prophylactic conditions during 3 years.

## RESULTS AND DISCUSSION

Stomata were present on the petiole, vein and fruit wall although their number was very low compared to that on the leaf blade. The stomatal density in the petiole and leaf blade in different clones under study are given in Table 1. In the leaf blade, stomatal density varied from 297.0 to 348.3 in an area of 1 mm<sup>2</sup>. The range of stomatal density in Hevea brasiliensis as reported by Senanayake et al., (1970) on the leaf blade is comparable to this observation. In the petiole the range was only 3.00 to 5.25 in 10 mm<sup>2</sup>.

Table 1. Clonal differences in the density of leaf blade and petiolar stomata

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Clones	Leaf blade	Petiole
	(in a unit area of 1 mm <sup>2</sup> )	(in a unit area of 10 mm <sup>2</sup> )
RRII 101	297.00 + 13.60	3.00 + 0.67
RRII 102	$343 \cdot 30 + 6 \cdot 60$	3.33 + 0.73
RRII 105	$348 \cdot 30 + 6 \cdot 30$	3.00 + 0.43
RRII 106	303.50 + 6.20	$4.63 \pm 0.28$
PR 107	332.60 + 11.10	5·25 + 0·95
Tjir 1	323·10 <del>+</del> 7·00	4.50 + 1.19

The organographic variability of the length and width of stomatal apertures and the clonal differences are given in Table 2 and Table 3, respectively. The aperture length of leaf blade stomata ranged from  $9.96~\mu m$  (RRII 101) to  $12.33~\mu m$  (RRII 105) and that of petiolar stomata ranged from  $14.10~\mu m$  (RRII 101) to  $18.60~\mu m$  (PR 107). The aperture width of the leaf blade stomata had a range of  $1.50~\mu m$  (PR 107) to  $2.20~\mu m$  (RRII 106) and that on the petiole from  $3.90~\mu m$  (RRII 105) to  $4.80~\mu m$  (RRII 101) respectively. The veinal stomata were noted to be intermediate in both the characters. The part to part difference was highly significant.

Table 2. Part to part difference in the size of stomatal aperture

Leaf blade	Vein	Petiole
10.94	14-15	16.88
1.93	3.16	4.26
	10.94	10.94 14.15

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Clones	Leaf blade	Aperture length $\mu_{\rm m}$	m Petiole	Leaf blade	Aperture width µm	Petiole
RRII 101	9.96 + 0.40	11.50 + 0.55	14.10 + 1.16	1.90 + 0.13	2.50 + 0.15	4.80 + 0.50
<b>RRII 102</b>	11.60 + 0.77	11.50 + 0.84	14.80 + 1.36	2.00 + 0.47	3.40 + 0.46	4.70 + 0.47
<b>RRII 105</b>	12.33 + 0.70	12.80	14.31 + 0.74	2.00 + 0.13	3.10 + 0.17	3.90 + 0.36
RRI 106	11.30 + 0.24	16.30 + 0.66	17.70 + 0.71	2.20 + 0.15	3.10 + 0.42	4.40 + 0.20
PR 107	10.60 + 0.14	16.30 + 1.13	18.60 + 1.12	1.50 + 0.08	4.40 + 0.80	4.10 + 0.29
7年1	10.50 + 0.29	13.90 + 0.71	17.73 + 0.99	2.00 + 0.19	3.80 + 0.45	4.40 + 0.27

A positive correlation between the aperture length of veinal stomata and that of petiolar stomata was highly significant and no other significant correlations were observed (Table 4). The aperture index of leaf blade and petiolar stomata in different clones are furnished in Table 5. The aperture index of petiolar stomata ranged from 4 23 (RRII 101) to 9.77 (PR 107) and that of leaf blade stomata ranged from 2940.30 (RRII 101) to 4284.10 (RRII 105).

Table 4. Correlation in the length and width of stomatal apertures in the leaf blade vein and petiole

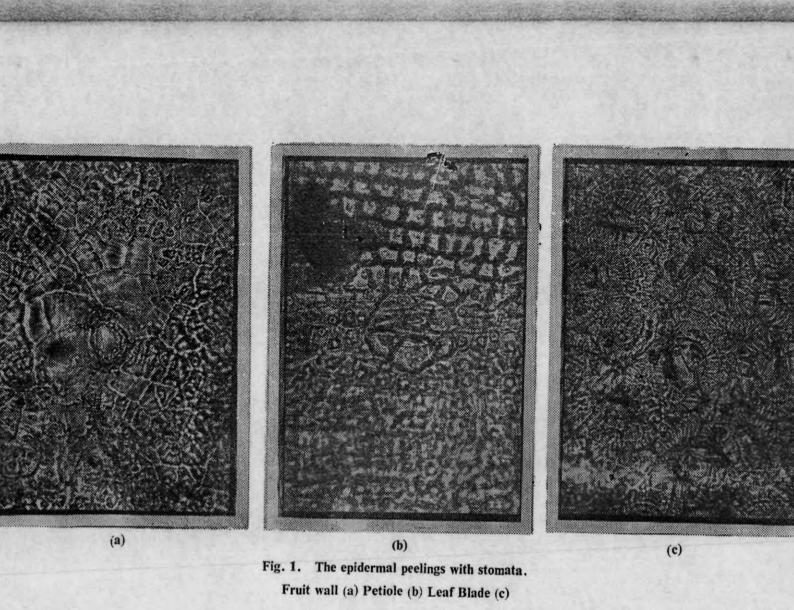
Characters	Aperture length	Aperture width
Leaf blade vs. vein	0.05	0.05
Leaf blade vs. petiole	0.14	0.34
Vein vs. petiole	0.75**	0 02
** P 0:01		

Table 5. Clonal differences in leaf blade and petiolar stomatal aperture index

Clones	Tolerant to 1	eaf fall disease	Susceptible Clones	to leaf fall	disease
	Petiolar stomatal index	Leaf blade stomatal index		Petiolar stomatal index	Leaf blade stomatal index
RRII 101	4.23	2940 · 30	RRII 106	8 · 21	3429 60
RRII 102	4.93	3982 · 30	PR 107	9.77	3525 60
RRII 105	4.29	4284 · 10	Tjir 1	7.97	3392 · 26
	4:48 <u>+</u>	3735·60 <u>+</u>		8·65 <u>+</u>	3449·30 <u>+</u>
	0.22	259 · 70		0.56	39 · 70

The fruit wall stomata were characterised by long apertures comparable to petiolar stomata and the opening was very wide (Fig. 1).

The clones studied had been grouped into two categories based on the field observations on abnormal leaf fall. From field observations it was found that RRII 101, RRII 102 and RRII 105 had a certain extent of tolerance to abnormal leaf fall disease, whereas the other three were highly susceptible, under normal prophylactic conditions. Earlier reports on clonal susceptibility to abnormal leaf fall disease in *Hevea brasiliensis* also support this view (Bhaskaran Nair et al., 1975; Radhakrishna Pillai and Chee, 1968).



It was also interesting to notice a part to part difference in the structure of epidermal cells. The epidermal cells of the leaf blade have sinuous walls resulting in irregular shape. It also forms a wrinkled nature due to a large number of wall outgrowths which overlap the guard cells (Fig. 1). The epidermal cells on the petiole, vein and fruit wall are somewhat regular in shape and the wall outgrowths are absent except in the region of tertiary veins.

Ramayya and Jagannatha Rao (1969) had emphasised the importance of investigating the entire stomatiferous area of a plant and the response of a given plant part to a function according to its stomatal variations. The present study brings to the fore the occurrence of stomata on the vein and fruit wall other than the leaf blade and petiole and it reveals the organographic variability in stomatal density and aperture size. Premakumari et. al., (1979) had reported the occurrence of stomata on the petiole of Hevea brasiliensis where the clonal differences in stomatal characters and their importance in clone selection were described.

In Hevea the abnormal leaf fall disease is preceded by fruit rot disease which is caused by the same organism and the leaf infection starts first on the petiole (Thankamma et. al., 1975). The nature of organographic variability in the density and aperture size of stomata and the structure and orientation of epidermal cells recall the organographic specificity of infection site of fruit rot disease and abnormal leaf fall disease and it appears to be linked with the mode of entry of *Phytophthora* spp. in Hevea brasiliensis reported by Thankamma et. al., (1975).

The clonal differences regarding the aperture index of petiolar and leaf blade stomata was another interesting feature. Of the six clones under study, those which have some extent of tolerance to abnormal leaf fall disease were characteristic with low aperture index of the petiolar stomata. The observation is reflected in Table 5 where the group mean of tolerant clones is  $4.48 \pm 0.22$  as against  $8.65 \pm 0.56$  in the susceptible group.

The present study reveals a close relationship between the organographic specificity in stomatal characters, their clonal differences, the structure and orientation of the epidermal cells in different parts and also the part to part specificity of infection as well as the clonal susceptibility to abnormal leaf fall disease. The above information has strengthened the earlier findings that the aperture index of petiolar stomata can be taken as a criterion for the selection of *Hevea* clones tolerant to abnormal leaf fall disease (Premakumari et. al., 1979).

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#### REFERENCES

BHASKARAN NAIR, V. K.; GEORGE, P. J. AND SARASWATHY AMMA, C. K. (1975). Breeding improved Hevea clones in India. Proc. int. Rubb. Conf., Kuala Lumpur 1975, pp. 45 — 54.

- Bobilioff, W. (1923). Anatomy and Physiology of Hevea brasiliensis 1, Art, Institute Orell Fussli. pp. 132 134.
- CHEN (Shiqin) etc. (1981). A study on stomata condition of rubber leaves. Chinese J. Trop. Crops. 2, 76 83.
- PANIKKAR, A. O. N. (1974). Anatomical studies in *Hevea brasiliensis* Muell. Arg. Summary of the Ph. D. work.
- PREMAKUMARI, D., ANNAMMA, Y. AND BHASKARAN NAIR, V. K. (1979). Clonal variability for stomatal characters and its application in *Hevea* breeding and selection. *Indian* J. Agric, Sci. 49, 411 3.
- Purvis, M. J., Collier, D. C. and Walls, D. (1966). Laboratory Techniques in Botany, 2nd ed. 174 pp. Butterworths, London.
- RADHAKRISHNA PILLAI, P. N. AND CHEE, K. H. (1968). Susceptibility of *Hevea* rubber clones to leaf fall disease caused by two species of *Phytophthora*, *Rubb. Bd. Bull.* 10, 95 7.
- RAMAYYA, N. AND JAGANNATHA RAO, (1969). Range of structural and ontogenetic stomatal variations in three species of Oscimum. Curr. Sci. 38, 79 82.
- SENANAYAKE, Y. D. A. AND SAMARANAYAKE, P. (1970). Intra-specific variation of stomatal density in H. brasiliensis Muell. Arg. Q. Jl. Rubb. Res. Inst. Ceylon, 46, 61—8.
- THANKAMMA, L., RAJALAKSHMI, V. K. AND RADHAKRISHNA PILLAI, P. N. (1975). Mode of entry of *Phytophthora* in *Hevea brasiliensis*. *Proc. int. Rubb. Conf. Kuala Lumpur*, 1975. pp. 213 6.

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