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CORRELATIONS OF THE CHARACTERS OF PETIOLAR STOMATA WITH LEAF RETENTION AFTER THE INCIDENCE OF *PHYTOPHTHORA* LEAF FALL DISEASE IN *HEVEA BRASILIENSIS* (WILLD EX.) ADR. DE JUSS MUELL. ARG.

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The characters of petiolar stomata and the leaf retention percentage after the incidence of *Phytophthora* leaf fall disease in *Hevea brasiliensis* (Willd. ex Adr. Juss) Muell. Arg. was studied in six *Hevea* clones. Considerable differences between disease resistant and susceptible groups of clones were observed for leaf retention percentage, no. of stomata/10 mm^2 length of stomatal aperture and aperture index. Leaf retention percentage had negative correlations with no. of stomata /10mm^2 ($P<0.01$), length of stomatal aperture ($P<0.05$) and the aperture index ($P<0.01$). Partial and multiple correlations showed the importance of the frequency of petiolar stomata and the aperture index as criteria for selection of disease resistant clones. About 68 per cent variation in leaf retention after the incidence of *Phytophthora* leaf fall disease could be explained by the characters of petiolar stomata.

Key words – *Hevea brasiliensis*, Petiolar stomata, *Phytophthora* leaf fall disease, Disease resistance.

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INTRODUCTION

The size and density of stomata in *Hevea brasiliensis* are clonal characteristics (Senanayake and Samaranayake, 1970; Premakumari et. al, 1979; Premakumari and Panikkar, 1984). Abnormal leaf fall caused by *Phytophthora* is a major disease in *Hevea* causing considerable yield drop, possibly due to the high leaf fall (Radhakrishna Pillay and Chee, 1968) and prophylactic spraying of the foliage with Bordeaux mixture or oil based copper oxychloride dispersed in diluent spray oil is the recommended control measure (Radhakrishna Pillay et al, 1980). Based on the observation that entry of the pathogen is through the stomata (Thankamma et al, 1975) the petiolar stomata of budwood plants of disease resistant and susceptible *Hevea* clones has already been studied in detail (Premakumari et al, 1979). Further observations on the organographic and clonal variability of stomatal features of tapping trees of different clones (Premakumari and Panikkar, 1984) indicated the role of petiolar stomata as a factor influencing the occurrence of *Phytophthora* leaf fall disease. The present work aims to ascertain the degree of relationship of frequency, aperture length and aperture index of the stomata on the petiole with leaf retention after disease incidence.

EXPERIMENTAL

The study was conducted on six clones of *Hevea brasiliensis* belonging to the 1954 hand pollination progenies planted at the Experiment Station of the Rubber Research Institute of India. Of these, three clones were fairly resistant to *Phytophthora* leaf fall disease, whereas, the other three were susceptible under unsprayed conditions. Three trees were chosen in each clone. For observations on stomatal characteristics, five branches were chosen at random on each tree. One leaf was collected from each branch when the leaves had hardened after refoliation following wintering. All the samples were collected between 10 AM and 11 AM on the same day and the leaves collected belonged to comparable nodal position on the twig. Samples of the petioles were taken two centimetres away from the nodal union. Epidermal peelings of the abaxial half were taken and stained following standard methodology (Purvis et al, 1966).

The number of stomata per ten square millimetre was counted. The length and width of the aperture of three stomata per peeling were recorded from each sample using a screw eyepiece micrometer and the tree means were assessed. The aperture index was calculated from the frequency of stomata and the length of the aperture (Premakumari et al, 1979).

For observations on leaf retention, five branches selected at random from each tree were labelled after refoliation and hardening of leaves following wintering. Only the branches which had a minimum of hundred leaves were chosen for this purpose. Leaf count was taken before the incidence of *Phytophthora* leaf fall disease. After the incidence of disease, leaf count was taken again and the percentage leaf retention was assessed.

The data were statistically analysed for the relationship of the frequency, aperture length, aperture width and aperture index of petiolar stomata with leaf retention. Simple, partial and multiple correlations were examined for which standard methodology was followed (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

The means, standard errors and the coefficients of variation of the leaf retention percentage and petiolar stomatal characters with respect to the disease resistant and susceptible groups of clones are given in Table 1. The two groups showed considerable differences in the leaf retention percentage and stomatal features except aperture width. 45.5 per cent of the leaves were retained after disease incidence on the trees of clones belonging to the fairly resistant group, while the leaf retention percentage was only nine in the susceptible clones, under unsprayed conditions. Number of stomata per ten square millimetre, aperture length, aperture width and aperture index of the former group were 2.2, 17.3, 4.7 and 35.7 μm, respectively, while the respective data for the other group were 4.4, 23.2, 4.8 and 102.8 μm. Of all the traits studied, the coefficient of variation was the least for the aperture length indicating high stability.

The importance of investigating the entire stomatiferous area of a plant, with respect to the response of a plant part to a function according to its stomatal variation had been subjected to study earlier (Ramayya and Rao, 1969). The differences in leaf retention percentage and the features of the petiolar stomata of disease resistant and susceptible clones gave clear indications of the relationship among these characters, of which the aperture width did not show considerable difference among clones or

Table 1. Leaf retention percentage after *Phytophthora* leaf fall disease and the features of petiolar stomata in *Hevea brasiliensis*

Character	Disease resistant clones			Disease susceptible clones		
	Means	SE	CV%	Means	SE	CV%
Percentage of leaf retention	45.5	4.21	28	9.0	2.31	77
No. of stomata/10 mm²	2.2	0.22	35	4.4	0.35	24
Length of stomatal aperture (μm)	17.3	1.17	20	23.2	0.90	12
Width of stomatal aperture (μm)	4.7	0.45	29	4.8	0.25	16
Aperture index	35.7	3.21	27	102.8	8.66	25

between the two groups. This was in agreement with earlier studies (Premakumari et al. 1979; Premakumari and Panikkar, 1984).

Simple correlation coefficients and the first, second and third order partial correlation coefficients of the leaf retention percentage and the stomatal characters are given in Tables 2 and 3. Leaf retention percentage had negative correlations with no. of stomata / 10mm² ($r = -0.7561$, $P < 0.01$), length of stomatal operture ($r = -0.5883$ $P < 0.05$) and the aperture index ($r = -0.7824$ $P < 0.01$), although it was not correlated with aperture width. The aperture index had significant positive correlations with its factors ($r_{52} = 0.9319$, $r_{53} = 0.7419$, $P < 0.01$). However, the degree of relationship was more with no. of stomata/10mm² than with aperture length.

Table 2. Correlations of leaf retention percentage and stomatal characters

Characters		x_1	x_2	x_3	x_4	x_5
Leaf retention (%)	x_1	—	–0.7561**	–0.5883*	–0.0637	–0.7824**
No. of stomata/10 mm²	x_2	—	—	–0.4644	–0.2043	0.9319**
Aperture length	x_3	—	—	—	0.2465	0.7410**
Aperture width	x_4	—	—	—	—	–0.0692
Aperture index	x_5	—	—	—	—	—

* $P < 0.05$
** $P < 0.01$

$r_{1.2345} = 0.8233$**
$R^2 = 0.6778$

The partial correlation coefficients of leaf retention percentage with number of stomata also showed significance ($r_{123} = -0.6743$, $P < 0.01$) when the aperture length was kept constant, although this character did not show significant correlation with the aperture length when the no. of stomata/10mm² was constant. The correlations of leaf retention percentage with any of the stomatal traits were not significant when the aperture index was kept constant. The leaf retention percentage was significantly correlated with the aperture index when the aperture length was constant $r_{15?} = -0.6381$,

Table 3. Partial correlations of leaf retention percentage and stomatal characters

First order		Second order		Third order	
Characters	Correlation value	Characters	Correlation value	Characters	Correlation value
$r_{12.3}$	–0.6743**	$r_{12.34}$	–0.6885**	$r_{12.345}$	–0.3650
$r_{12.4}$	–0.7873**	$r_{12.35}$	–0.3724	$r_{13.245}$	–0.3113
$r_{12.5}$	–0.1194	$r_{12.45}$	–0.2128	$r_{14.235}$	–0.1863
$r_{13.2}$	–0.4092	$r_{13.24}$	–0.3182	$r_{15.234}$	0.2305
$r_{13.4}$	–0.5920*	$r_{13.25}$	–0.3558		
$r_{13.5}$	–0.0204	$r_{13.45}$	0.0727		
$r_{14.2}$	–0.3405	$r_{14.23}$	–0.2139		
$r_{14.3}$	0.1058	$r_{14.15}$	–0.2575		
$r_{14.5}$	–0.1897	$r_{14.35}$	–0.2017		
$r_{15.2}$	–0.3277	$r_{14.23}$	0.2530		
$r_{15.3}$	–0.6381**	$r_{15.24}$	–0.2392		
$r_{15.4}$	–0.7903**	$r_{15.34}$	–0.6520**		

* $P<0.05$
** $P<0.01$

$P < 0.01$) although the correlation was not significant when the no. of stomata/10mm^2 was constant. The second order partial correlations also showed significant association of leaf retention percentage with no. of stomata/10mm^2 and aperture index when the aperture length and aperture width (r_{1234} 0.6885, $r_{1534} = -0.6520$, $P < 0.01$) were constant. The third order partial correlations were not significant.

The influence of aperture length on leaf retention percentage was less pronounced since their correlation was not significant when the no. of stomata/10mm^2 or the aperture index was kept constant although the simple correlation was significnt ($P < 0.05$). However, this trait as a factor determining the aperture index and was more stable showing simple correlation with the leaf retention percentage. Hence, it could also be taken into account for selection of disease tolerant clones. However, about 68 per cent variation in leaf retention percentage could be explained by the characters of petiolar stomata, ($r_{1\cdot2343} = 0.8233$, $P < 0.01$) of which the stomatal density was the most important factor.

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REFERENCES

Panse, V. G. & Sukhatme, P. V. (1967). Statistical methods for agricultural workers. Indian Council of Agricultural Research, New Delhi.

Premakumari, D., Annamma, Y. & Bhaskaran Nair, V. K. (1979). Clonal variability for stomatal characters and its application in *Hevea* breeding and selection. *Indian Journal of Agricultural Sciences*, **49**(6) : 411–413.

Premakumari, D. & Panikkar, A. O. N. (1984). Organographic variability of stomatal characters in *Hevea brasiliensis* Muell. Arg. and its possible significance in clonal susceptibility to leaf fall disease. Proceedings of the International Rubber Conference, Sri Lanka. 1984 : 317–324.

Purvis, M. J., Collier, D. C. & Walls, D. (1966). Laboratory techniques in Botany, 2nd ed. Butterworths, London. pp. 174

Radhakrishna Pillay, P. N. & Chee, K. H. (1968). Susceptibility of *Hevea* rubber clones to leaf fall disease caused by two species of *Phytophthora*. **10** : 95–97.

Radhakrishna Pillay, P. N., George, M. K. & Rajalakshmy, V. K. (1980). Leaf and shoot diseases. In: *Handbook of Natural Rubber Production in India* (ed. P. N. Radhakrishna Pillay), Rubber Research Institute of India : 249–278.

Ramayya, N. & Jaganatha Rao (1969). Range of structural and ontogenetic stomatal variation in three species of *Oscimum*. *Current Science*, **38** : 79–82.

Senanayake, Y. D. A. & Samaranayake, P. (1970). Interspecific variation of stomatal density in *Hevea brasiliensis* Muell. Arg. *Quarterly Journal of the Rubber Research Institute, Ceylon.* **46** : 61–68.

Thankamma, L., Rajalakshmy, V. K. & Radhakrishna Pillay, P. N. (1975). Mode of entry of *Phytophthora* in *Hevea brasiliensis*. *Proceedings of the International Conference, Kuala Lumpur.* 1975 : 213–216.