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Variations in Leaf Morphology and Anatomy between Clones of Hevea

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Variations in the leaf morphology and anatomy of eleven Hevea *clones were examined. Highly significant clonal differences were shown in the stomatal density but the leaf area measurements between clones, however, reached significance only at lower levels. A number of leaf morphological and anatomical parameters* viz. *cell number in upper epidermis, leaf thickness, palisade thickness, spongy layer thickness, mean number of cells per unit length of palisade section and spongy section also revealed significant clonal differences. Nevertheless, no obvious relationship was observed between these structural properties and yield or vigour of these clones.*

This technique can also be used for studying the mode of inheritance of these properties in cultivars and their progenies. However for such a study to be meaningful, correlation between these structural parameters and the efficiency of photosynthesis should first be examined.

The plant factors which control the rate of passage of water through the plant are to a great extent those that control the rate of transpiration. Of the shoot characters held responsible for rapid transpiration, leaf characteristics such as stomatal frequency and dimensions and surface area are important[1].

Considerable variation in stomatal distribution was shown to exist over the surface of single leaves and between leaves of the same variety in the four apple varieties that Slack[2] examined. Significant differences were also established between varieties in stomatal number per unit area. Carpenter and Smith[3] studied the stomatal frequency and size of about fifty species of Appalachian hardwoods and found no relationship between the frequency and shade tolerance.

There is little information on stomatal distribution in *Hevea* leaves. Rao[4] illustrated the occurence and nature of the reticulate cuticle on the lower leaf epidermis and mentioned the distribution of stomata on the lower epidermis. Senanayake[5] examined the abaxial foliar characteristics of three species of *Hevea* and found that stomatal densities per unit area differed in the experimental plants. Later, Senanayake and Samaranayake[6] examined twenty five cultivars of *Hevea brasiliensis* and showed intraspecific variation in stomatal density per unit area. Stomatal characters of *Hevea* have also been examined in relation to their possible use in breeding and selection of disease tolerant clones[7].

The current study examines the variation in morphological characteristics such as number of stomata per unit area, surface area of leaflet, number of upper epidermal cells per unit area, and anatomical characters such as leaf thickness, palisade thickness, spongy layer thickness, number of cells per unit area in the palisade region, number of cells per unit area in the spongy region and number of cells per unit area of leaf section.

The data obtained are examined for their inter-relationship between properties

of vigour and yield of the clones selected for the study.

MATERIALS AND METHODS

Three leaflets from three different mature leaves were selected at random from five trees each of eleven clones during July/August. After measuring the surface area of the leaflet by tracing on sectional pad, a small portion of each leaflet was sampled for light microscopy. Portions of the same leaflets were examined by replicating the lower epidermis with replicating tape and shadow casting the replica with palladium gold. The shadow cast replicas were mounted on glass slides in Canada balsam and examined conveniently. *Figure 1* shows a typical preparation.

The upper epidermis was prepared by a different technique. A piece of leaf about 5 mm square was cut and treated in chlorox for two days. The upper epidermis was detached from the leaves and brushed to remove adhering parenchyma cells. It was then washed carefully in distilled water a number of times, stained by the Safranin/Fast green technique and mounted in Canada balsam. A typical result is shown in *Figure 2*.

For sectioning, the sampled pieces were fixed in 1% osmium tetroxide for 24 h, dehydrated in ethanol series and embedded in methacrylate. Light micrographs were prepared from sections. A typical

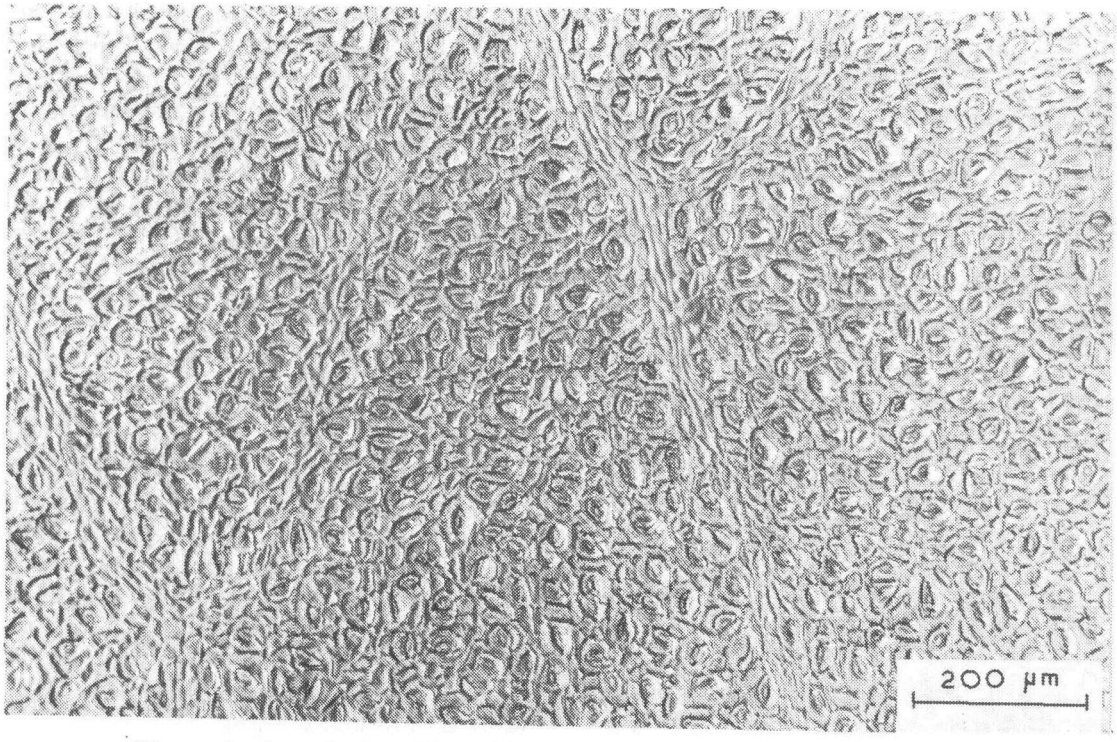

Figure 1. A typical replica of lower epidermis (adaxial). Mag. ×112.

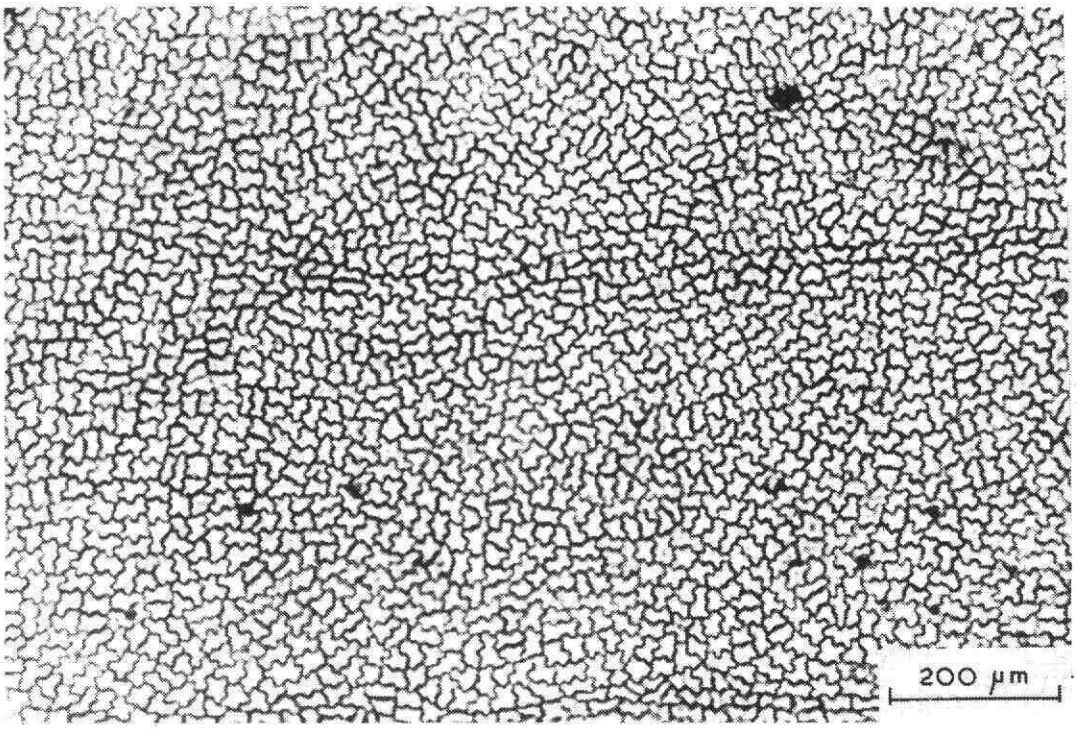

Figure 2. A typical preparation of upper epidermis (adaxial). Mag. × 112.

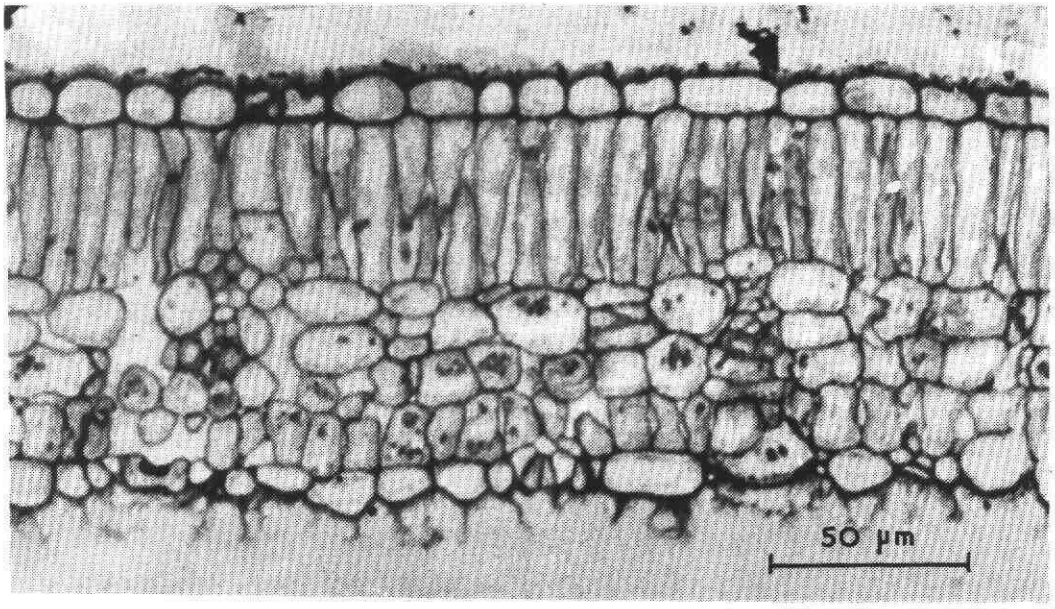

Figure 3. A typical leaf section showing palisade and spongy tissue. Mag. × 512.

result is shown in *Figure 3*. Measurements of leaf thickness, palisade thickness, spongy thickness, number of palisade cells in 0.2 mm of leaf section and number of spongy cells in 0.2 mm of leaf section were made from the light micrographs obtained. The data obtained were statistically analysed.

RESULTS

Tables 1 and *2* present the data on surface area of leaflets. The clones approach significant difference at 0.1% probability. The values range from 120.53 per square centimetre in PR 255 to 74.27 per square centimetre in ES 4. PR 255 appears to be different from the others except ES 7,

TABLE 1. ANALYSIS OF VARIANCE TABLE FOR SURFACE AREA OF LEAFLET

Source of variation	Degrees of freedom	Mean squares
Clones	10	2 810.79 ($P<0.1$)
Error (A)	44	1 553.49***
Leaflets	2	$264.37^{N.S.}$
Leaflets × clones	20	$393.65^{N.S.}$
Error (B)	88	270.08

s.d. = 16.434
Mean = 90.32
c.v. = 18.20

TABLE 2. TABLE OF MEANS FOR SURFACE AREA OF LEAFLET

Clone	Surface area of leaflets (sq cm)
PR 255	120.53
ES 7	103.53
SS 2	97.13
RRIM 614	93.60
IRCI 10	91.60
RRIC 3	91.27
RRIM 605	86.87
RRIM 600	81.60
RRIM 501	77.73
PB 86	75.40
ES 4	74.27

s.e. = 10.177
L.s.d. = 29.09

TABLE 3. ANALYSIS OF VARIANCE TABLE FOR NUMBER OF STOMATA IN *LOWER EPIDERMIS PER SQUARE MILLIMETRE*

Source of variation	Degrees of freedom	Mean squares
Clones	10	13 087.99***
Error (A)	44	1 548.96$^{N.S.}$
Leaflets	2	946.52$^{N.S.}$
Leaflets × clones	20	862.85$^{N.S.}$
Error (B)	88	1 202.94
Error (C)	132	1 318.28

s.d. = 34.683
Mean = 310.03
c.v. = 11.19

TABLE 4. TABLE OF MEANS FOR NUMBER OF STOMATA IN LOWER EPIDERMIS PER SQUARE MILLIMETRE

Clone	*No. of stomata*
IRCI 10	369.29
RRIM 600	350.37
RRIC 3	337.20
RRIM 614	316.19
ES 4	299.31
RRIM 501	294.93
PB 86	293.59
SS 2	292.83
PR 255	292.20
ES 7	286.51
RRIM 605	277.93

s.e. = 9.375
L.s.d. = 26.25

SS 2, RRIM 614, IRCI 10 and RRIC 3. There is no significant difference between leaflets or leaflets within clones.

Tables 3 and *4* show the analysis of variance of number of stomata per square millimetre of lower epidermis. Clonal differences are very highly significant although leaflets and leaflet-clone interaction is not evident. IRCI 10 tops the list with 369 stomata and RRIM 605 is at the bottom of the list with 278 stomata in a square millimetre.

TABLE 5. ANALYSIS OF VARIANCE TABLE FOR NUMBER OF UPPER EPIDERMAL CELLS PER SQUARE MILLIMETRE

Source of variation	Degrees of freedom	Mean squares
Clones	10	571 841.80***
Error (A)	44	160 796.10***
Leaflets	2	92 625.50$^{N.S.}$
Leaflets X clones	20	50 340.14$^{N.S.}$
Error (B)	88	61 882.26

s.d. = 248.761
Mean = 1542.64
c.v. = 16.13

TABLE 6. TABLE OF MEANS FOR NUMBER OF UPPER EPIDERMAL CELLS PER SQUARE MILLIMETRE

Clone	No. of upper epidermal cells
RRIM 501	1 794.73
RRIM 605	1 775.53
ES 7	1 686.47
IRCI 10	1 604.40
PR 255	1 589.40
PB 86	1 588.47
RRIC 3	1 558.93
RRIM 600	1 492.93
RRIM 614	1 429.27
ES 4	1 315.40
SS 2	1 133.47

s.e. = 103.536
L.s.d. = 295.92

The analysis of number of upper epidermal cells is shown in *Tables 5* and *6*. Clonal differences are highly significant whereas leaflet variation and leaflet-clone interaction are not established. RRIM 501 tops the list with 1795 and SS 2 is at the bottom of the list with 1133 cells per square millimetre of upper epidermis.

The data on leaf thickness measured from sections are shown in *Tables 7* and *8*. Clonal differences are established. Leaflet variation and leaflet-clone interaction are not evident. SS 2 occupies the top of the list with 143 μ and IRCI 10 occupies the bottom of the list with 100 μ.

TABLE 7. ANALYSIS OF VARIANCE TABLE FOR THICKNESS OF LEAF (MICRON)

Source of variation	Degrees of freedom	Mean squares
Clones	10	2 446.19***
Error (A)	44	512.59***
Leaflets	2	59.27$^{N.S.}$
Leaflets × clones	20	142.66$^{N.S.}$
Error (B)	88	171.29

s.d. = 13.087
Mean = 115.28
c.v. = 11.35

TABLE 8. TABLE OF MEANS FOR THICKNESS OF LEAF (MICRON)

Clone	Thickness of leaf (μ)
SS 2	143.37
RRIM 614	132.25
RRIM 600	119.63
ES 4	118.47
PB 86	112.93
RRIM 501	111.89
RRIM 605	110.87
RRIC 3	107.60
PR 255	107.43
ES 7	103.56
IRCI 10	100.11

s.e. = 5.85
L.s.d. = 16.708

Palisade layer thickness is shown in *Tables 9* and *10*. Clonal differences are very highly significant. Leaflet variation and leaflet-clone interaction are not established. SS 2 occupies the top of the list with a value of 70 μ and IRCI 10, bottom of the list with 43 μ.

Spongy layer thickness is shown in *Tables 11* and *12*. Clonal differences are significant. Leaflet variation and leaflet-clone interaction are not evident. SS 2 again tops the list with a value of 55 μ and IRCI 10 is at the bottom of the list with a value of 40 μ.

The data on number of cells per unit sectional length of the leaf is shown in *Tables 13* and *14* for the palisade layer and in *Tables 15* and *16* for the spongy

TABLE 9. ANALYSIS OF VARIANCE TABLE FOR THICKNESS OF PALISADE LAYER (MICRON)

Source of variation	Degrees of freedom	Mean squares
Clones	10	989.53***
Error (A)	44	102.82***
Leaflets	2	18.44N.S.
Leaflets × clones	20	33.35N.S.
Error (B)	88	41.15

s.d. = 6.414
Mean = 51.59
c.v. = 12.42

TABLE 10. TABLE OF MEANS FOR THICKNESS OF PALISADE LAYER

Clone	Thickness of palisade layer (μ)
SS 2	70.19
RRIM 614	61.47
RRIM 501	54.04
RRIM 605	52.01
RRIM 600	51.23
ES 4	50.20
RRIC 3	49.21
PB 86	48.23
ES 7	44.15
PR 255	43.90
IRCI 10	42.83

s.e. = 2.62
L.s.d. = 7.488

layer. Clonal differences are highly significant for both properties. Leaflet differences and leaflet-clone interaction are not evident. RRIM 614 tops the list for both properties with cell numbers of 39 and 53 respectively. However the least number is observed for ES 4 (25) for the palisade layer and for SS 2 (39) for the spongy layer.

Table 17 gives the computed values for number of stomata per leaflet. PR 255

TABLE 11. ANALYSIS OF VARIANCE TABLE FOR THICKNESS OF SPONGY LAYER (MICRON)

Source of variation	Degrees of freedom	Mean squares
Clones	10	320.7)***
Error (A)	44	113. ±0***
Leaflets	2	1 89$^{N.S.}$
Leaflets × clones	20	29.51$^{N.S.}$
Error (B)	88	45.57

s.d. = 6.751
Mean = 45.90
c.v. = 14.71

TABLE 12. TABLE OF MEANS FOR THICKNESS OF SPONGY LAYER

Clone	Thickness of spongy layer (μ)
SS 2	55.08
RRIM 614	51.35
RRIM 600	49.44
ES 4	48.22
PB 86	46.03
ES 7	43.89
PR 255	43.85
RRIC 3	43.20
RRIM 605	42.23
RRIM 501	41.31
IRCI 10	40.27

s.e. = 2.75
L.s.d. = 7.86

tops the list with 3.5 million and PB 86 is at the bottom of the list with 2.2 million.

Table 18 shows the total cell number in a 2 mm strip of leaf section excluding the epidermal regions. RRIM 614 tops the list with a value of 91 and ES 4 is at the bottom of the list with a value of 67.

Interrelationships of These Properties with Vigour and Yield of Trees

Table 19 sets out a study of the interrelationships of the various structural properties studied and the yield and vigour properties of the clones under study. The ranking of the clones in each of these pro-

TABLE 13. ANALYSIS OF VARIANCE TABLE FOR NUMBER OF CELLS IN 0.2 MM LENGTH OF PALISADE LAYER

Source of variation	Degrees of freedom	Mean squares
Clones	10	238.58***
Error (A)	44	73.82$^{N.S.}$
Leaflets	2	46.57$^{N.S.}$
Leaflets × clones	20	92.35$^{N.S.}$
Error (B)	88	69.31
Error (C)	132	70.82

s.d. = 8.325
Mean = 31.85
c.v. = 26.14

TABLE 14. TABLE OF MEANS FOR NUMBER OF CELLS IN 0.2 MM LENGTH OF PALISADE LAYER

Clone	No. of cells
RRIM 614	38.80
IRCI 10	35.93
RRIM 600	35.90
RRIC 3	33.17
PB 86	32.57
PR 255	31.27
RRIM 501	31.13
ES 7	29.70
SS 2	29.13
RRIM 605	27.57
ES 4	25.20

s.e. = 2.173
L.s.d. = 6.08

perties is given in each column and the rank correlations are given at the bottom. Judging from the significance of the rank correlations none of the structural properties of leaves examined have been found to be significant in its correlation with yield or vigour of the trees. Ceulemans *et al.*[8] however, have shown the existence of a relationship between stomatal frequency and dimension and water diffu-

TABLE 15. ANALYSIS OF VARIANCE TABLE FOR NUMBER OF CELLS IN 0.2 MM LENGTH OF SPONGY LAYER

Source of variation	Degrees of freedom	Mean squares
Clones	10	302.40***
Error (A)	44	48.61$^{N.S.}$
Leaflets	2	29.74$^{N.S.}$
Leaflets × clones	20	35.82$^{N.S.}$
Error (B)	88	43.08
Error (C)	132	44.92

s.d. = 6.564
Mean = 46.44
c.v. = 14.13

TABLE 16. TABLE OF MEANS FOR NUMBER OF CELLS IN 0.2 MM LENGTH OF SPONGY LAYER

Clone	No. of cells
RRIM 614	52.53
IRCI 10	51.97
RRIM 600	51.40
RRIC 3	48.57
PB 86	46.83
ES 7	46.37
PR 255	45.60
RRIM 501	45.50
ES 4	41.53
RRIM 605	41.33
SS 2	39.17

s.e. = 1.731
L.s.d. = 4.85

sion process, which in turn reflects the growth rate in poplars. Shimshi and Ephrat[9] have also observed that wheat cultivars having wider stomatal aperture produced higher yields without consuming more water.

Slack[2] observed that there was a high positive correlation between numbers of stomata and epidermal cell number in unit area in apples. On the other hand, significant negative linear correlation between the frequency of stomata and area of

TABLE 17. MEAN NUMBER OF STOMATA PER LEAFLET

Clone	No. of stomata
PR 255	3 521 887
IRCI 10	3 382 696
RRIC 3	3 077 624
ES 7	2 966 238
RRIM 614	2 959 538
RRIM 600	2 859 019
SS 2	2 844 258
RRIM 605	2 414 378
RRIM 501	2 292 491
ES 4	2 222 975
PB 86	2 213 669

Mean = 2 795 889
s.d. = 455 970
c.v. (%) = 16.31

TABLE 18. CELL NUMBER PER 2 MM STRIP OF LEAF SECTION

Clone	Cell no.
RRIM 614	91.33
IRCI 10	87.90
RRIM 600	87.30
RRIC 3	81.74
PB 86	79.40
PR 255	76.87
RRIM 501	76.63
ES 7	76.07
RRIM 605	68.90
SS 2	68.20
ES 4	66.73

Mean = 78.27
s.d. = 8.30
c.v. (%) = 10.60

epidermal cells had also been reported[10]. Therefore it was decided to compute the correlation coefficient between epidermal cell number and number of stomata per square millimetre. The relationship was −0.0933 which was not significant.

TABLE 19. INTERRELATIONSHIP OF VARIOUS FEATURES OF LEAVES AND VIGOUR AND YIELD OF TREES. RANKS AND RANK CORRELATIONS

Clone	Yield mean of 14 years (g/tree/ tapping)	Yield mean of 14 years (kg/ha/yr)	Surface area of leaflet	Mean number of stomata in lower epidermis	Mean number of upper epidermal cells	Mean thickness of leaf	Mean thickness of palisade layer	Mean thickness of spongy layer
RRIM 614	1	7	4	4	9	2	2	2
PR 255	2	1	1	9	5	9	10	7
RRIM 600	3	2	8	2	8	3	5	3
RRIM 501	4	5	9	6	1	6	3	10
ES 7	5	4	2	10	3	10	9	6
RRIM 605	6	6	7	11	2	7	4	9
PB 86	7	3	10	7	6	5	8	5
IRCI 10	8	11	5	1	4	11	11	11
SS 2	9	9	3	8	11	1	1	1
ES 4	10	8	11	5	10	4	6	4
RRIC 3	11	10	6	3	7	8	7	8
Rank correlations with yield (g/tree/ tapping)		0.6820*	0.3184	−0.1454	0.2548	0.0820	0.1090	0.1180

*Significant at $P<0.05$

TABLE 19. INTERRELATIONSHIP OF VARIOUS FEATURES OF LEAVES AND VIGOUR AND YIELD OF TREES. RANKS AND RANK CORRELATIONS (CONTINUED)

Clone	Number of cells in 0.2 mm length of palisade layer	Number of cells in 0.2 mm length of spongy layer	Girth	Girth increment	Virgin bark at opening (mm)	Virgin bark at 9th year (mm)	Renewed bark at 9th year (mm)	Mean number of stomata/ leaflet	Cell number/ unit area
RRIM 614	1	1	2	1	1	1	1	5	1
PR 255	6	7	5	4	2	2	2	1	6
RRIM 600	3	3	6	3	6	6	4	6	3
RRIM 501	7	8	7	9	3	7	10	9	7
ES 7	8	6	1	2	5	3	3	4	8
RRIM 605	10	10	3	5	6	4	5	8	9
PB 86	5	5	8	8	6	7	7	11	5
IRCI 10	2	2	11	11	11	10	9	2	2
SS 2	9	11	9	7	9	9	8	7	10
ES 4	11	9	4	6	3	5	6	10	11
RRIC 3	4	4	10	10	10	11	11	3	4
Rank correlations with yield (g/tree/ tapping)	0.3634	0.3088	0.5410	0.6820*	0.6820*	0.7498**	0.7270**	0.2002	0.3910

*Significant at $P < 0.05$
**Significant at $P < 0.01$

Similarly when surface area of leaflet was correlated with epidermal cell number the relationship was 0.0538 which was again of no significance. Leaf area was also not related to stomatal number (−0.1183 not significant).

DISCUSSION AND CONCLUSION

The results of the present study are in agreement with those of Senanayake and Samaranayake[5] and Premakumari *et al.*[7] that there are significant differences in stomatal density between cultivars of *Hevea brasiliensis*. Stomatal numbers in the lower epidermis range from 6500 to 91 000 per square centimetre for a number of tree species investigated by Carpenter and Smith[3]. The values for *Hevea* appear to lie in the range of 28 000 to 37 000 per square centimetre for the eleven cases reported here, and thus are in the middle range of tree species observed elsewhere. Values for apple[2] range from 17 000 to 51 000 per square centimetre.

Intervarietal differences are established for a number of leaf characteristics examined in the present study. Although leaf area measurements show almost no clonal differences the other properties studied, *viz.* stomatal number per square millimetre, cell number in upper epidermis per square millimetre, leaf thickness, palisade thickness, spongy layer thickness, number of cells in palisade unit length and number of cells in spongy layer unit length show highly significant differences between clones. Variations between leaflets are not established; also there are no leaflet-clone interaction.

It is disappointing to find that there are no obvious relationships between these structural properties and yield as well as vigour properties such as girth, bark thickness, *etc.*

Stomatal characteristics, especially stomatal frequency have already been reported to be heritable in *Populus* and *Hordeum*[11,12] and in *Zea*, Heichel[13] showed that a simple genetic system controlled the epidermal cell and stomatal frequency. Unfortunately the choice of clones in this study does not allow a comparison of parents and progencies to examine the effects on breeding and selection, but it is suggested that such a study in the future may be rewarding.

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