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Ultrastructure of Mineral Deficient Leaves of Hevea *III. Quantitative Considerations*

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A quantitative evaluation of the structural details in the palisade and spongy cells of Hevea *leaves, deficient in minerals, was carried out and subjected to statistical analysis. Six properties were studied: cell width, cell length, cell size, number of chloroplasts in a cell area of 100 μm^2, size of chloroplasts and number of grana per chloroplast.*

Palisade and spongy cell size appeared to be depressed generally but the reduction of spongy cell size was only significant in -K treatments, in fact, the size was significantly increased in P deficient leaves. Deficiencies in many of the minerals tended to reduce significantly the number of chloroplasts per unit area, but only in the palisade cells; in the spongy cells, significant reduction was shown only by Ca deficiency. It also appeared that the absence of K or Ca affected the size of the chloroplasts significantly. Deficiency of all the major elements reduced the number of grana per chloroplast in the palisade cells, except in the case of magnesium.

The effects of mineral deficiencies on the ultrastructure of *Hevea* leaves grown in sand culture have been studied in detail[1,2]. The leaves were examined one year after the commencement of the experiment.

Alteration in the chloroplast ultrastructure was observed with each deficiency and this was mostly affecting the lamellar development. Nitrogen, phosphorus or sulphur deficiency showed specific changes in the chloroplasts. Grana with limited lamellae stacks and whose wide spaces represented excessively swollen outer loculi were observed from N-deficient chloroplasts. Phosphorus-deficient chloroplasts showed loosely aligned extended lamellar systems while in S deficiency, the chloroplasts tended to show large multiples of granal stacks which were separated into a couple of short stacks, due to the swelling of the loculi.

Even though K and Mn deficiency showed abundant plastoglobuli in the chloroplasts, the differences in the chloroplasts could be distinguished by the structural details of the grana. Manganese-deficient chloroplasts, in addition, appeared highly vacuolated and the grana were in continuity with each other while K deficiency showed chloroplasts with fewer grana and limited stacks. Calcium deficiency was distinctive in that the chloroplasts were small and displayed enlarged starch loci and electron-transparent stroma matrix. Lack of structural integrity was the characteristic of Mg-deficient chloroplasts. Changes seen in the other deficiencies were general and these might have been induced by other factors as well.

Evaluation of some cell properties as affected by mineral deficiencies was also undertaken but these observations were

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not subjected to statistical analysis[1,2]. This paper quantitatively evaluates these ultrastructural details in an attempt to provide a further insight into the importance of these minerals in the growth of *Hevea*. So far there have been no such studies.

EXPERIMENTAL

Micrographs of palisade and spongy cell structure from the previous studies[1,2] were examined and the following properties evaluated: cell width, cell length, cell size, chloroplast number per 100 μm^2, chloroplast size and number of grana per chloroplast section.

Six samples of each of both palisade and spongy cells per treatment were studied. However, in some of the treatments, all the six samples were not available, therefore histograms were made from available mean data. The results were analysed for variance.

RESULTS

Palisade Cell Properties (Tables 1 *and* 2)

Cell width. A mean cell width of 7.82 μm was observed with very highly significant treatment differences. In the ranked treatment means, –P showed the highest value and together with –Zn, and –Ca showed higher values than the control plants, –P and –Zn were the only two treatments which showed significant differences from the control plants.

TABLE 1. ANALYSIS OF VARIANCE OF LEAF PALISADE CELL PROPERTIES

Analysis	Cell width (μm)	Cell length (μm)	Cell size (μm^2)	No. of chloroplasts in cell area of 100 μm^2	Size of chloroplast (μm^2)	No. of grana per chloroplast
	d.f. m.s.	d.f. m.s.	d.f. m.s.	d.f. m.s.	d.f. m.s.	d.f. m.s.
Between treatment	11 25.82	8 196.00	8 5 952.26	11 43.97	11 30.02	10 65.95
Within treatment	60 2.26	45 17.12	45 1 719.48	60 2.66	60 4.72	55 8.30
F ratio	11.43***	11.45***	3.46**	16.54***	6.36***	7.94***
s.d.	1.50	4.14	41.47	1.63	2.17	2.88
Mean	7.82	29.83	175.04	5.96	6.46	9.24
c.v. (%)	19.22	13.87	23.69	27.36	33.60	31.18
s.e.	0.61	1.69	16.93	0.67	0.89	1.18

d.f. = degrees of freedom
m.s. = mean squares
*P < 0.05
**P < 0.01
***P < 0.001

TABLE 2. RANKED TREATMENT MEANS OF LEAF PALISADE CELL PROPERTIES

Cell width (μm)	Cell length (μm)	Cell size (μm^2)	No. of chloroplasts in cell area of 100 μm^2	Size of chloroplasts (μm^2)	No. of grana per chloroplast
–P 13.31	–Mn 37.76	–Mn 231.62	–Cu 11.51	–S 10.86	–B 14.67
–Zn 10.14	–S 37.52	–S 218.06	–Mg 8.56	–Cu 8.77	–CP 13.00
–Ca 8.54	–Cu 33.23	–N 190.22	CP 8.52	–P 8.45	–Cu 12.67
CP 7.92	–Mg 30.43	–Ca 163.20	–Fe 7.31	–B 7.23	–Mg 11.50
–K 7.54	–B 30.23	–Cu 162.87	–B 6.69	–Zn 6.88	–N 8.33
–S 7.42	–Fe 28.38	–B 162.64	–Mn 5.96	–Fe 6.69	–S 8.17
–N 7.10	–Ca 25.93	–Zn 155.43	–S 5.46	–Mn 6.36	–Zn 8.17
–Mn 6.49	–N 24.21	–Mg 152.70	–Zn 4.73	CP 6.19	–Fe 7.76
–Cu 6.41	–Zn 20.96	–Fe 138.63	–Ca 4.22	–Mg 4.83	–Mn 7.67
–Mg 6.37			–N 3.27	–N 4.41	–K 6.33
–Fe 6.29			–K 2.71	–Ca 4.04	–Ca 3.50
–B 6.29			–P 2.56	–K 2.88	

CP = control plants

Cell length. A mean cell length of 29.83 μm was observed with very highly significant treatment differences, –Mn topped the list, being closely followed by –S, –Ca, –N and –Zn which were at the bottom of the list, showed significant differences only with the top three treatments. No data was available for the control plants.

Cell size. A mean cell size of 175.04 μm^2 was observed, –Mn tops the list and –Fe is at the bottom of the list. The cell sizes of –Mn and –S showed significant differences from the rest of the treatments. No data was available for the control plants.

Number of chloroplasts in unit area. The unit area chosen was 100 μm^2 of the cell area. The mean chloroplast number was found to be 5.96, –Cu topped the list followed by –Mg and control. Only –Cu showed a significantly higher value than control; however, those below –Mn showed significantly lower values than the control plants.

Size of chloroplasts. A mean size of 6.46 μm^2 was observed. Even though the top three in the list: –S, –Cu and –P respectively did not show significant differences in their sizes, only –S was significantly higher than control. Also only –K showed a significantly lower value than the control plants, despite –Mg, –Ca treatments having lower values than control.

Number of grana per chloroplast. The mean number of grana per chloroplast was 9.24 with highly significant treatment differences; –B treatment showed the highest number of grana, followed by control. In fact, the rest of the treatments showed lower values than the control

plants. However, –B, –Cu and –Mg treatments did not show significant differences from control; the rest do. No data was available for –P treatment. *Figure 1* summarises the various values of the palisade cell properties obtained in comparison with control.

Spongy Cell Properties (Tables 3 *and* 4)

Cell width. The mean cell width was observed to be 12.0 μm with very highly significant treatment differences; –P treatment showed the biggest cell width and –Fe the smallest; –P, –Zn, –Cu and –Ca treatments showed bigger cell width than control. Of all the treatments studied, only –P showed a significant difference from control.

Cell length. The mean cell length was 17.13 μm; –P treatment topped the list and –K was at the bottom with the control plants occupying the middle position. Treatment differences were only significant at 1% level. All the treatments, however, did not show any significant differences from control.

Cell size. A mean cell size of 164.13μm^2 was observed; –P treatment which showed the biggest cell size was also the only one significantly higher than control. Those treatments which had lower values than the control plants were, however, not significantly different with the exception of –K treatment.

TABLE 3. ANALYSIS OF VARIANCE OF LEAF SPONGY CELL PROPERTIES

Analysis	Cell width (μm)		Cell length (μm)		Cell size (μm^2)		No. of chloroplasts in cell area of 100 μm^2		Size of chloroplasts (μm^2)		No. of grana per chloroplasts	
	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.
Between treatment	10	24.93	10	30.98	10	14 755.24	11	11.52	11	29.73	10	83.81
Within treatment	55	6.09	55	14.53	55	3 448.16	60	2.87	60	2.96	55	8.52
F ratio	4.09***		2.13*		4.28***		4.02***		10.03***		9.77***	
s.d.	2.47		3.81		58.72		1.69		1.72		2.92	
Mean	12.00		17.13		164.13		4.70		6.31		9.59	
c.v. (%)	20.58		22.26		35.78		36.05		27.28		30.44	
s.e.	1.01		1.56		23.98		0.69		0.70		1.19	

d.f. = degrees of freedom
m.s. = mean squares
*P < 0.05
**P < 0.01
***P < 0.001

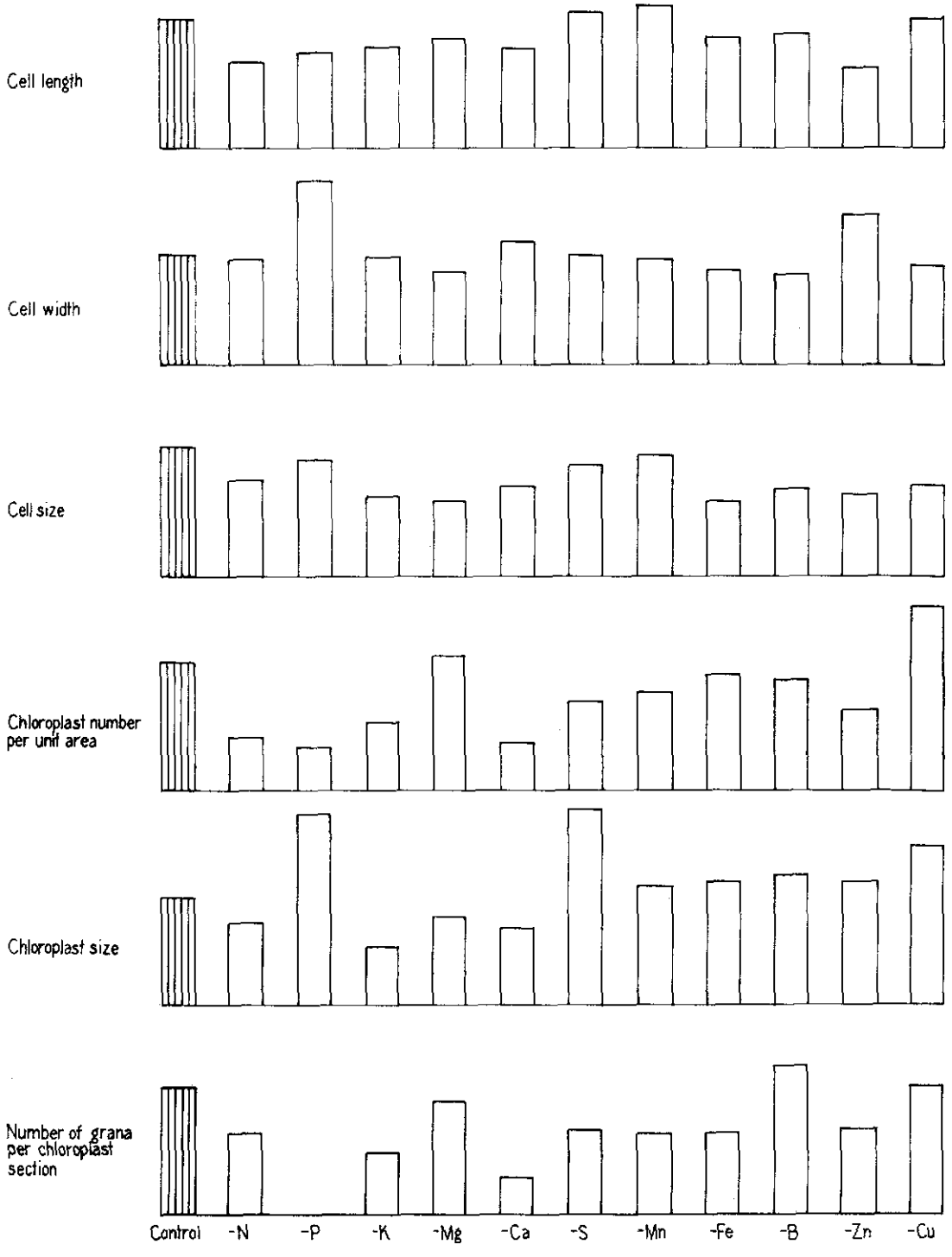

Figure 1. Histogram of leaf palisade cell properties.

TABLE 4. RANKED TREATMENT MEANS OF LEAF SPONGY CELL PROPERTIES

Cell width (μm)	Cell length (μm)	Cell size (μm^2)	No. of chloroplasts in cell area of 100 μm^2	Size of chloroplasts (μm^2)	No. of grana per chloroplast
–P 16.36	–P 19.56	–P 238.93	–Mg 6.37	–S 10.96	–Mg 15.83
–Zn 14.75	–Zn 19.56	–Zn 234.55	–B 6.30	–P 9.01	–Cu 13.67
–Cu 13.15	–Cu 19.24	–Cu 211.45	–K 5.95	–Cu 8.27	–B 13.67
–Ca 12.75	–S 18.92	–Ca 189.37	–Mn 5.61	–Mn 6.43	CP 11.50
CP 12.27	–N 18.80	CP 161.99	–Cu 5.22	–Zn 6.29	–S 10.00
–S 11.26	CP 17.60	–Mn 148.61	–Fe 5.19	CP 6.19	–N 9.67
–B 11.02	–Ca 16.32	–S 146.27	CP 4.72	–B 6.05	–Mn 8.17
–K 10.90	–Fe 15.23	–Fe 145.59	–S 4.62	–Mg 5.69	–Zn 6.67
–Mn 10.82	–Mn 15.15	–N 131.52	–N 4.13	–N 5.44	–Fe 6.00
–N 10.54	–B 14.87	–B 115.81	–Zn 3.28	–Fe 4.67	–K 5.50
–Fe 9.78	–K 13.15	–K 81.34	–P 3.09	–Ca 3.84	–Ca 4.83
			–Ca 1.88	–K 2.88	

CP = control plants

Number of chloroplasts in unit area. The mean number of chloroplasts per 100 μm^2 was 4.70. Even though –Mg had the highest number of chloroplasts, it was not significantly different from control, which occupied about the middle position in the list. Only –Ca treatment showed a significantly lower value than the control plants.

Size of chloroplasts. The mean size of the chloroplasts was found to be 6.31μm^2; –S treatment topped the list, followed by –P. These two treatments showed significantly higher values than control. The last two treatments at the bottom of the list *i.e.* –Ca and –K respectively, were also significantly different from the control plants.

Number of grana per chloroplast. A mean value of 9.59 grana per chloroplast was observed; –Mg treatment which topped the list, did not show any significant differences from –Cu and –B treatments, it was however significantly higher than the control plants. Treatments from –Zn down the list *i.e.* –Fe, –K and –Ca showed significantly lower values than control. *Figure 2* summarises the various values of the spongy cell properties obtained in comparison with control.

DISCUSSION AND CONCLUSION

Based on the *F* ratio tests, the leaf properties showed highly significant differences between treatment variances in both the palisade and spongy cells. The palisade cell size and the spongy cell length were significantly different only at 5% and 1% respectively.

From the histograms it appears that *(Figures 1* and *2)* the palisade cell size was depressed by the absence of any one of the minerals studied and the spongy cell size was reduced by the deficiency in some of the minerals only. Deficiency in some of the minerals like P, Zn, Cu or Ca also

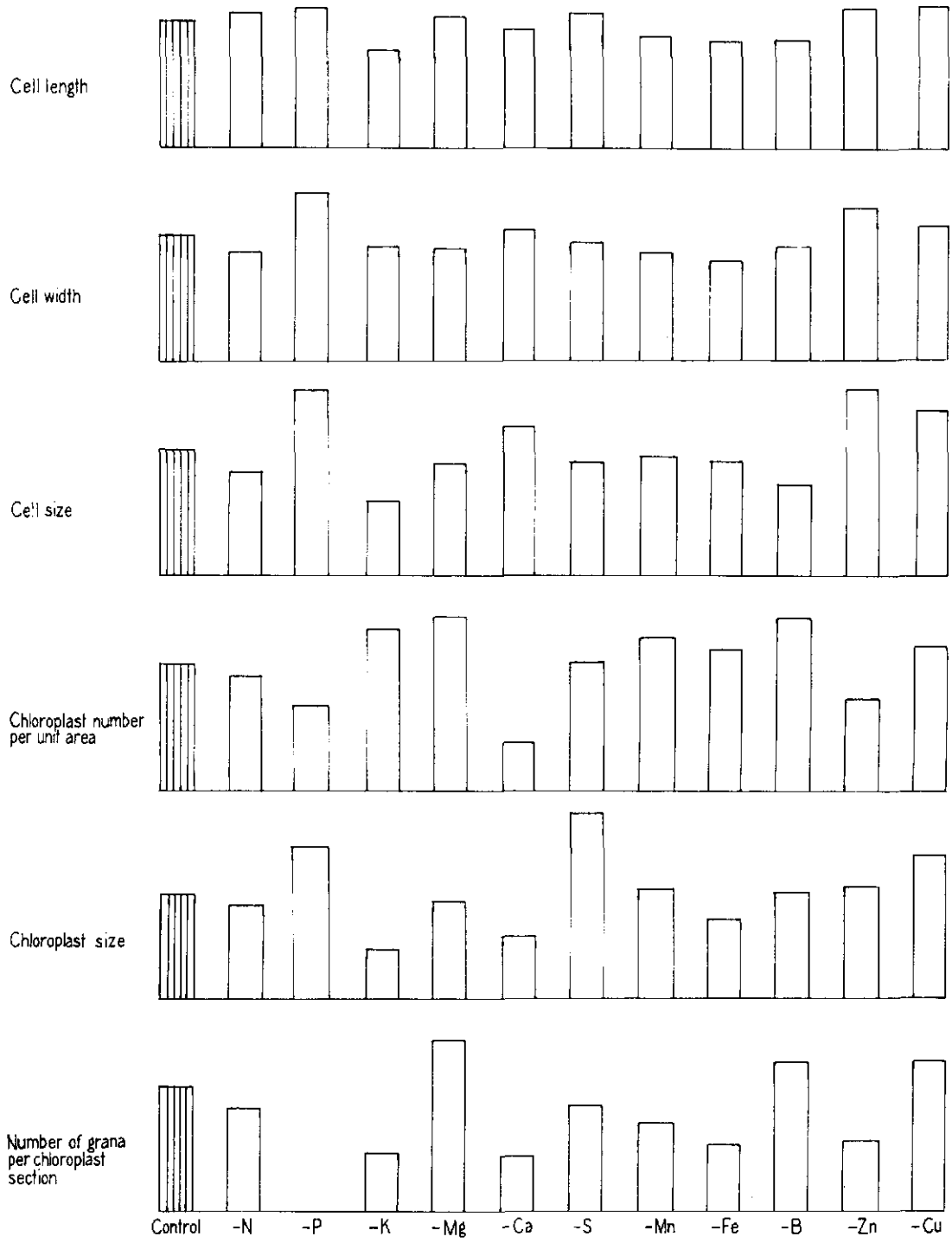

Figure 2. Histogram of leaf spongy cell properties.

increased the spongy cell size in some cases, unexpectedly. Statistical analysis however showed that deficiency in K alone significantly reduced the cell size and deficiency in P increased it. Since data on the palisade cell properties was incomplete due to sampling difficulties, comparisons were not possible.

Mineral deficiencies seemed to have a more significant effect on the chloroplast properties, especially those of the palisade cell, rather than on all the cell properties.

In the number of chloroplast per unit area of the palisade cells, all treatments except deficiencies in Fe, B and Mg showed a significant reduction. Deficiency in Cu, however, significantly increased the number of chloroplasts. In the spongy cells, the number of chloroplasts was not significantly reduced by mineral deficiencies with the exception of calcium.

The size of the chloroplasts was only significantly reduced in the palisade cells if K was deficient or if K and Ca were deficient in the spongy cells. Deficiency in S or P seemed to induce a significant increase in the size of the chloroplasts.

The number of grana per chloroplast was also significantly reduced by mineral deficiencies, especially in the palisade cells. Deficiencies in minerals except Mg, Cu and B reduced the number of grana. In the spongy cells, the number was only significantly reduced by Zn, Fe, K and Ca deficiencies. Increase in the number of grana was also observed when Mg was deficient However, increase in grana stackings was observed in N and S deficient chloroplasts of maize[3].

The observations made in this paper regarding changes in the chloroplast properties indicate that some of the mineral deficiencies would lead to subnormal photosynthesis and therefore are likely to influence growth and or rubber production. The present study was done under ideal experimental conditions where deficiency of each element could be studied. Such a situation may not necessarily occur in nature where a combination of deficiencies may be more likely, particularly in view of interaction between elements.

The demonstration that deficiency in each of the major elements except Mg reduces chloroplast number per unit area and grana number per chloroplast in the palisade cells is noteworthy. Size of chloroplasts is only reduced by K deficiency. Chloroplast properties in the spongy cells are mostly affected by Ca or K deficiencies and to a little extent by Fe and zinc.

Other studies elsewhere have also shown that cations were required to stabilise the association of membranes in the grana *i.e.* stacking of thylakoids in the grana[4]. Barr *et al.*[5] on the other hand proposed that increase in grana stacking in N- and S-deficient maize plants was due to the absence of normal amount of sulfolipids in the chloroplasts. Similar studies have not been done in *Hevea* but it appears useful to suggest that biochemistry of nutrition in *Hevea* is a worthwhile subject to pursue. From biochemical studies elsewhere, chloroplasts from mineral deficient tomato plants were also shown to have lower Hill reaction rates per unit of chlorophyll in all deficiencies except iron[6].

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