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Ultrastructure of Mineral Deficient Leaves of Hevea *II. Effects of Micronutrient Deficiencies*

SAMSIDAR BTE HAMZAH and J.B. GOMEZ

Effects of micronutrient deficiencies on the ultrastructure of Hevea *leaves were examined a year after commencing the experiment. The palisade cell size and the number of chloroplasts per cell section were considerably reduced with Fe and Zn deficiencies. The size of the spongy cell was reduced only with Mn deficiency. This deficiency also revealed less chloroplasts per palisade section. Although ultrastructural changes were observed in the chloroplasts with deficiency of each element they were more general than distinctive except for Mn. The roles of micronutrients in plants are also discussed.*

Micronutrients are essential to plant growth but unlike the macronutrients, they are only required in very small quantities. Compared to effects of macronutrients, fewer detailed studies have been made on effects of micronutrient deficiencies on the ultrastructure of leaves.

Bogorad *et al.*[1] studied the ultrastructure of chloroplasts from iron-deficient *Xanthium* leaves. The principal changes were mainly in the lamellar structure. The lamellae were reduced in number and occurred in groups of two or three. The interlamellar regions were more granular and coarse and the fine lamellar stacking of normal chloroplasts was lacking. Laulhere and Mache[2] observed that iron-deficient pea leaves lacked chloroplastic ribosomal RNA and the ultrastructure of the plastids resembled those of the leucoplasts. The chlorophyll per gramme dry matter, of chlorotic pea leaves suffering from iron deficiency, was also found to be half as much as the normal leaves[3].

In Mn-deficient spinach leaf, Possingham *et al.*[4] observed that only the chloroplasts were markedly changed, the other cell organelles remained unaltered. As the deficiency progressed, the grana increased in size and the intergranal connections disappeared.

Reed[5] investigated the effects of Cu and Zn deficiencies on the leaf structure of tomato plants. With Cu deficiency, the palisade cells became separated, then shrunk and ultimately disappeared as a result of lysis of the contents. With Zn deficiency, palisade cells were longer, spongy cells were more compact and plastids were smaller.

Rajaratnam[6] observed the effects of B deficiency in oil-palm seedlings grown in sand and water cultures. The deficiency caused morphological changes and decreased the yield. The leaf area decreased with increasing severity of the deficiency.

This paper examines the effects of micronutrient (Mn, Fe, B, Zn, Cu) deficiencies on the ultrastructure of both the palisade and spongy cells of *Hevea* leaves. This complements earlier observations on the effects of macronutrient deficiencies[7].

MATERIALS AND METHODS

Leaf samples were taken from plants grown under the same conditions as

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earlier described[7]. The elements withheld were Mn, Fe, B, Zn and Cu. Seedlings for B, Zn and Cu deficiency studies were planted in glass urns; while those for Mn and Fe deficiency studies were planted in earthenware pots coated with bituminous paints.

RESULTS

Plants given the complete nutrient solution *i.e.* the controls have been described earlier[7].

Manganese Deficiency

The palisade cells measure up to 42 μ long and the spongy cells, 15 μ across. Both types of cells contain parietal cytoplasm with large central vacuoles with or without tannin deposits *(Figure 1)*.There are about ten chloroplasts per palisade cell section and five to seven per spongy cell section.

The chloroplasts are mostly ellipsoidal, measuring about 2–5 μ long and 1.5–2 μ wide in the palisade cells and 2–4 μ long and 0.5–2.5 μ wide in the spongy cells. They are characterised by the presence of large numbers of plastoglobuli *(Figure 2)* The grana are randomly oriented, limited in granal lamellae and seem to be in continuity with one another *(Figure 3)*. The granal compartments which tend to swell also give the chloroplasts a vesiculated appearance. Stroma lamellae are virtually absent in the palisade cell. Starch loci have not been detected in both types of cells.

Iron Deficiency

Sizes of both the cells are reduced; the palisade cells measure 30 μ long and the spongy cells 20 μ across. The parietal cytoplasm is meagre in these cells with the vacuoles containing either tannin or lipid globules. The nucleus is, however, centrally located in the palisdae cells. The chloroplast number is only reduced in the palisade cells, i.e. ten to twelve chloroplasts per cell section and there are about seven to eight chloroplasts per spongy cell section.

The chloroplasts in the palisade cells are more elongated than ellipsoidal when compared to those found in the spongy cells. The sizes range from 2–6 μ long and 1–2 μ wide in the palisade and 2–4 μ long and 1–2 μ wide in the spongy cells. There are several starch vesicles in the chloroplasts but their presence is not consistent, they may vary from twelve to zero in the sections of palisade cells alone.

The lamellar system is poorly developed. The granal stacks are generally reduced and very often the lamellae extend across nearly the whole length of the chloroplast *(Figure 4)*. Swellings of the loculi and intergranal frets are also common *(Figure 5)*. In the spongy cells and electron-transparent stroma matrix, which is free of lamellae, is commonly observed *(Figure 6)*.

Boron Deficiency

The tubular palisade cells measure up to 35 μ long and have parietal cytoplasm containing up to fifteen chloroplasts. The spongy cells are mostly isodiametrical measuring 20 μ across and have several chloroplasts; six to eight per cell section. Tannin deposits are occasionally observed in the vacuoles of both types of cells.

The chloroplasts are ellipsoidal and measure 3–5 μ long and 1.5–2.5 μ wide in the palisade cells and 3.5–5 μ long and 1–2 μ wide in the spongy cells. The lamellar system is well developed but does not fill up the stroma space which is otherwise occupied by granular material;

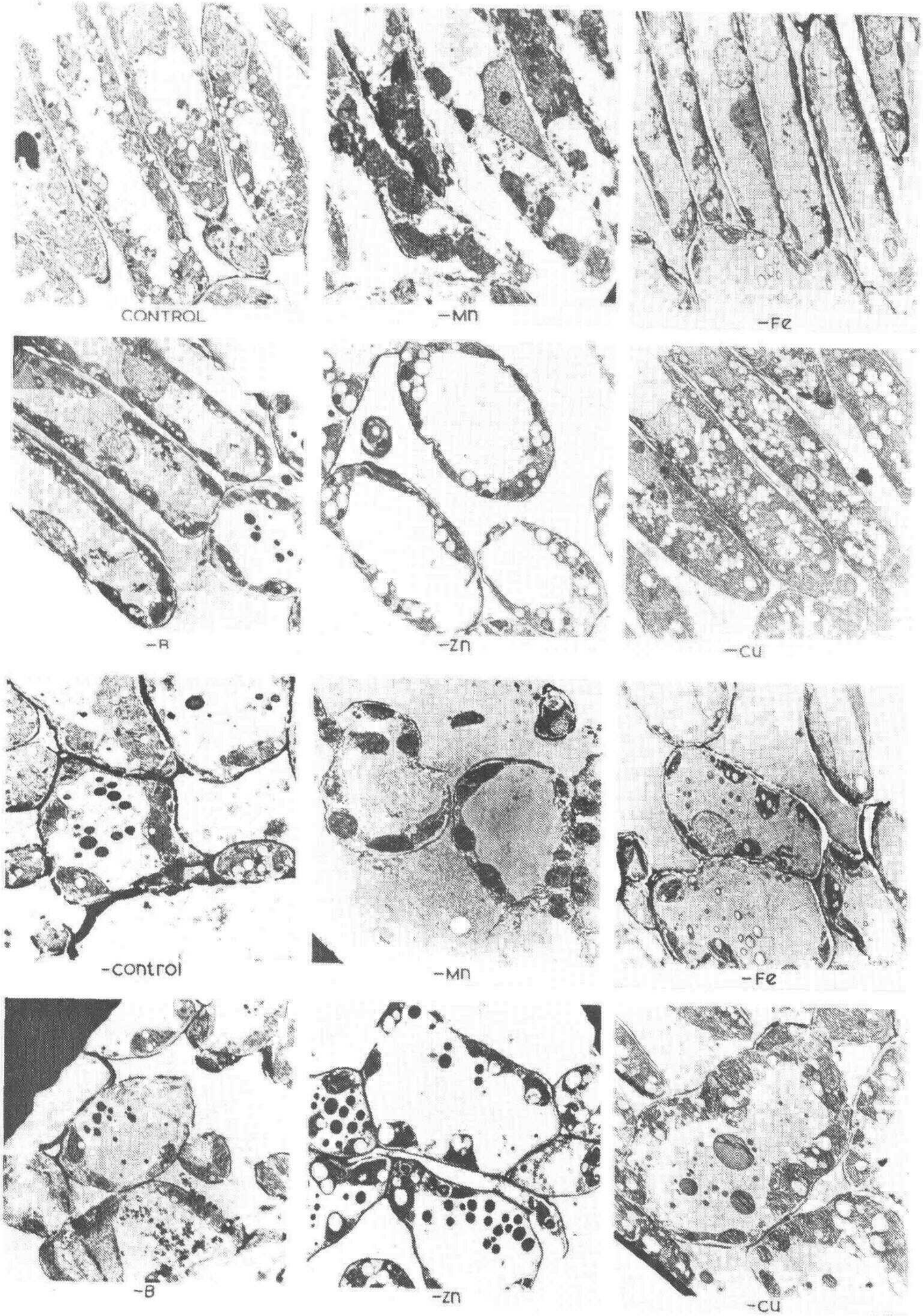

Figure 1. Representatives of palisade and spongy cells from micronutrient deficient leaves and control (complete nutrients). Mag. ×1400.

Figure 2. Chloroplast from a Mn-deficient leaf showing the large number of plastoglobuli and the vesiculated appearance in the chloroplast. Mag. ×20 000

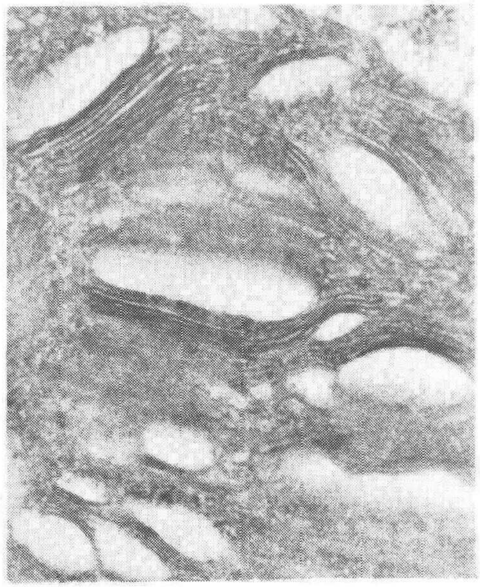

Figure 3. Grana of a Mn-deficient chloroplast showing their random orientation and continuity of the lamellae. Mag. ×40 000

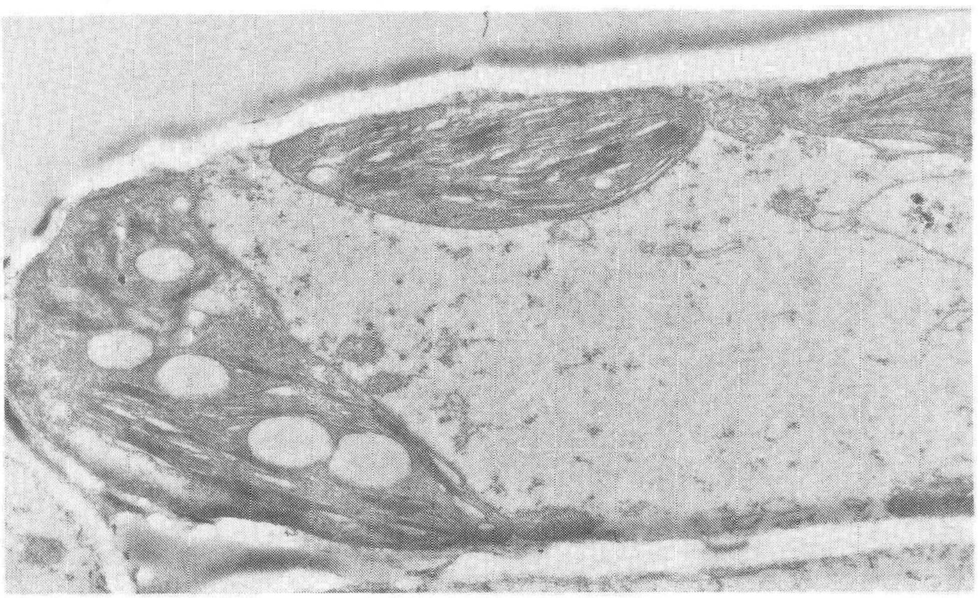

Figure 4. Chloroplasts from a Fe-deficient leaf showing granal stacks are reduced and the span of the lamellae. Mag. ×18 000.

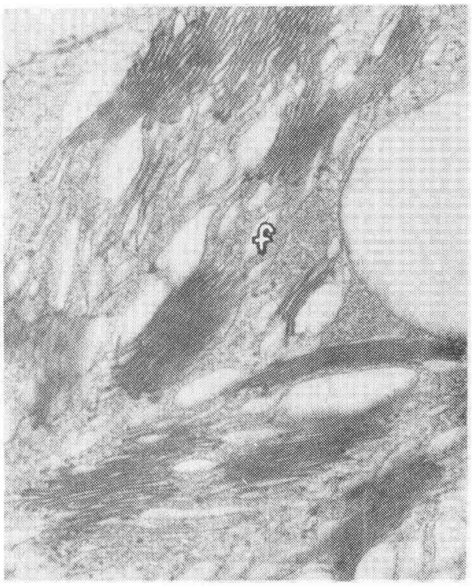

Figure 5. Chloroplast of a Fe-deficient leaf showing grana with swollen loculi and intergranal frets (f). Mag. × 40 000.

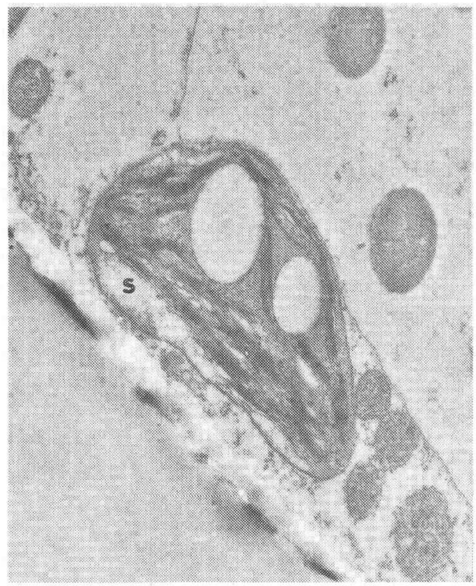

Figure 6. Chloroplast from a spongy cell of a Fe-deficient leaf showing the electron-transparent stroma matrix, free of lamellae (s). Mag. × 20 000

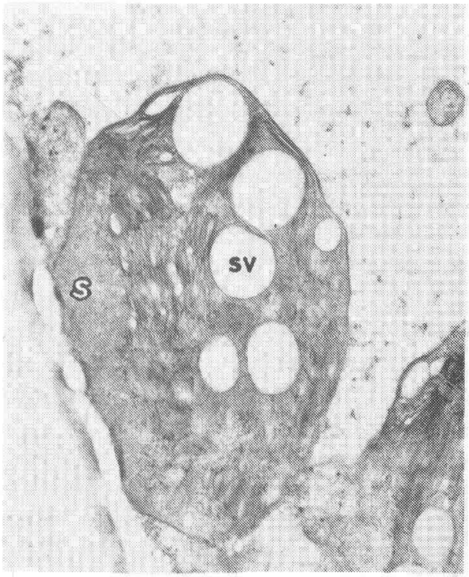

Figure 7. Chloroplast from a B-deficient leaf showing the granular, lamella-free stroma matrix (s), starch vesicles (sv) are common. Mag. × 20 000

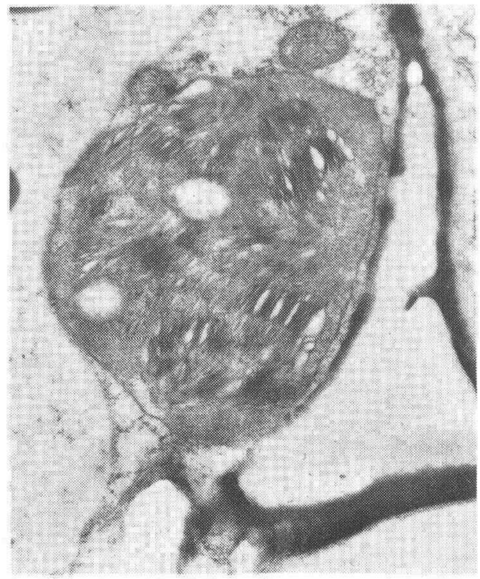

Figure 8. Spongy chloroplast from a B-deficient leaf showing grana with short lamellae which are loosely stacked, Mag. × 20 000.

often there is a lamella-free stroma matrix in the periphery of the chloroplasts in both the spongy and palisade cells *(Figure 7)*. The grana which are generally small have loosely stacked short lamellae. Swelling of the granal loculi is sometimes observed *(Figure 8)*. Stroma lamellae are better developed in the spongy than in the palisade cells *(Figure 8)*. Starch vesicles are also prominent in B-deficient chloroplasts especially in the palisade cells.

Zinc Deficiency

The palisade cells are slightly reduced in length, measuring about 20–30 μ long. The cells are loosely packed with considerable intercellular spaces. The parietal cytoplasm contains seven to eight chloroplasts per cell section. The spongy cells measure about 20 μ across. Many of them have their central vacuoles filled either with tannin or lipid droplets. Six to seven chloroplasts per spongy cell section have been observed.

The chloroplasts have characteristic large vesicles, which are sometimes filled with electron-dense starch grains *(Figure 9)*. As many as six vesicles per chloroplast section have been observed. Their presence also limits the stroma space and often brings about distortion to the chloroplasts. The grana are poorly organised with swellings in the loculi. Stroma lamellae are virtually absent.

Copper Deficiency

The palisade cells which measure up to 35 μ long and 7 μ wide contain abundant cytoplasm. One or more smaller vacuoles are seen in the cells but these are not centrally located and hence the cytoplasm is not parietal like in the spongy cells. Also tannin or lipid globules are present in the vacuoles of the latter. Spongy cells measure up to 20 μ across.

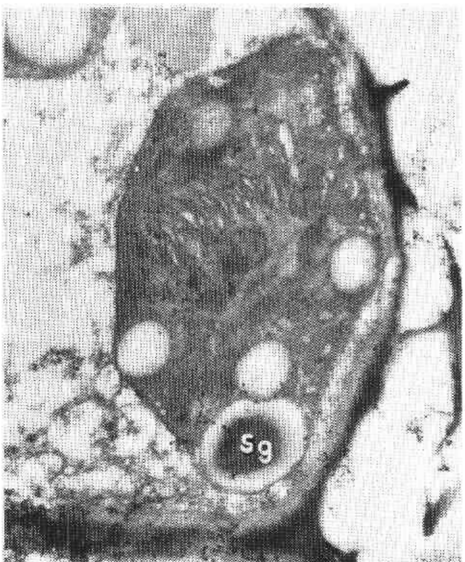

Figure 9. Chloroplast from a spongy cell of a Zn-deficient leaf showing electron-dense starch grain (sg) occupying one of the vesicles. Mag. ×20 000.

There are twelve to fourteen chloroplasts per palisade cell section and five to eight per spongy cell section. These large chloroplasts (2–6 μ long and 2–3 μ wide) are so closely packed together, especially in the palisade cells, that the individuality of the chloroplasts is indistinguishable. Often their shapes are distorted due to the presence of large and numerous starch vesicles *(Figure 10)*. The latter are also seen to coalesce with one another and may even disrupt the chloroplast envelope.

Small grana are present. They consist of large multiple stacks of lamellae, often with faults, *i.e.* swelling of the loculi *(Figure 11)*. Stroma lamellae are poorly developed.

DISCUSSION AND CONCLUSION

Only deficiencies of some of the micronutrients seem to affect the growth of the

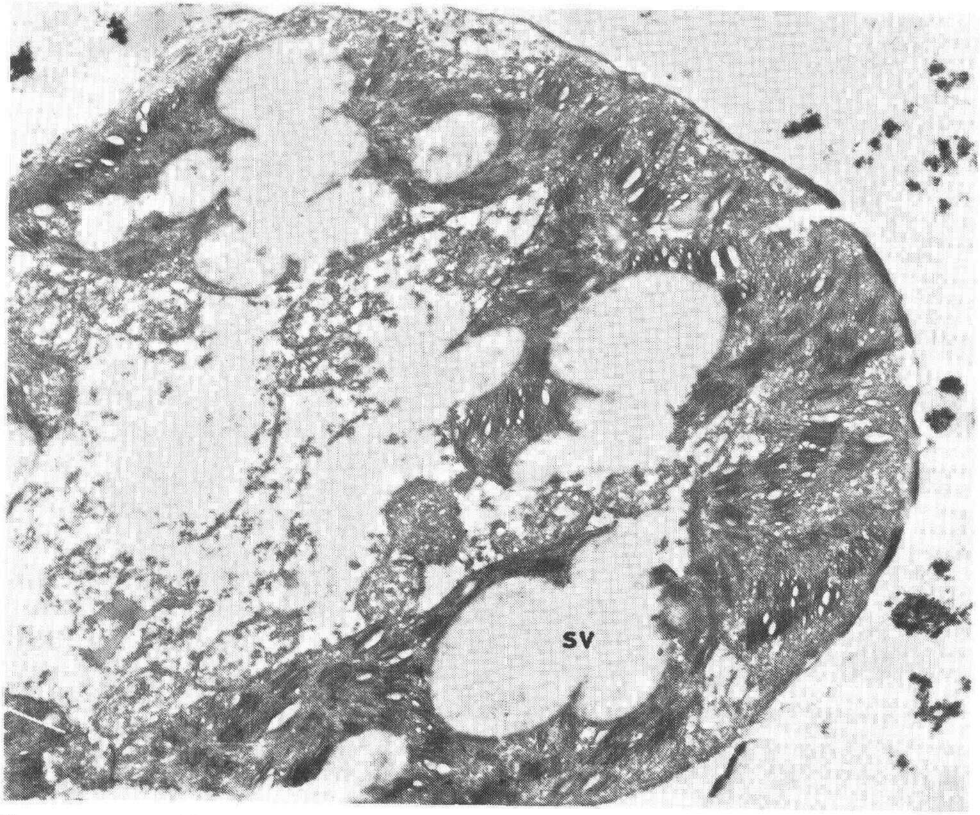

Figure 10. Palisade cell from a Cu-deficient leaf showing the closely packed chloroplasts and coalesced starch vesicles (sv). Mag. X15 000.

leaf cells. Deficiencies of Fe and Zn reduce the size of the palisade cells and the number of chloroplasts per cell section; Mn deficiency affects the spongy cells size and the chloroplast number in the palisade cell. The effect of these deficiencies on the quantity of cytoplasm is negligible.

There are hardly any distinctive ultrastructural features that can be assigned to a particular deficiency except for Mn. The changes seen in the other deficiencies are general and may have been induced by other factors as well.

The presence of a large number of plastoglobuli within the chloroplasts is characteristic of Mn deficiency. This feature is also shown by K-deficient chloroplasts[7] but the difference between them lies mainly in the grana. Due to swelling of the granal compartments, the chloroplasts from Mn-deficient leaves have a highly vacuolated appearance. The grana are also in continuity with each other and this increases the granal size. Possingham *et al.*[4] observed similar granal size increase in Mn-deficient spinach chloroplasts. With K deficiency chloroplasts have a limited number of grana consisting of smaller number of stacks and without swellings.

Deficiency of the other micronutrients, *i.e.* Fe, B, Zn and Cu, result in chloroplasts with large and numerous starch

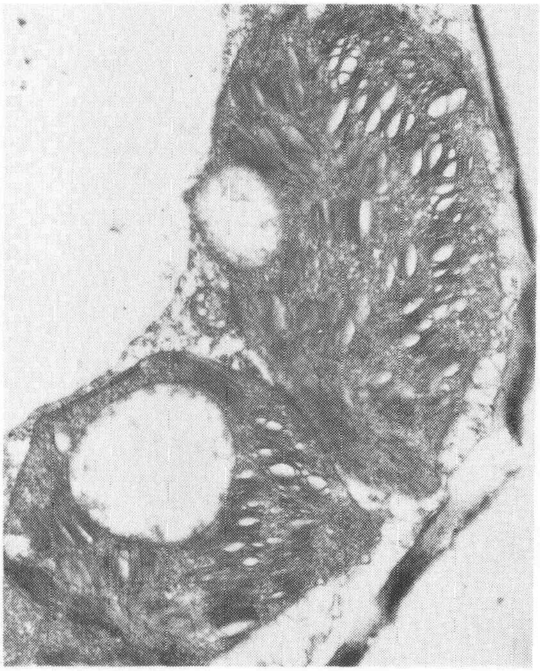

Figure 11. Chloroplasts from a Cu-deficient leaf showing grana are small and have multiple stacks, often with faults. Mag. × 20 000.

vesicles. Electron-dense starch grains are also regularly observed in some of the vesicles in Zn- and Cu-deficient chloroplasts, though more prominent in Zn-deficient chloroplasts. Large starch vesicles or loci are also the distinctive features of Ca deficiency[7] but their chloroplasts have an electron-transparent stroma-matrix, not observed in the other micronutrient deficiencies. Starch grains are also absent in this treatment.

The presence of the electron-transparent, lamella-free, zone of the stroma matrix in Fe-deficient chloroplast may be either due to the deficiency itself or the differential ability of taking up the stain. But Fe-deficient *Tradescantia* chloroplasts too, have been reported to have large electron-transparent spaces in them[8]. With B deficiency there is a similar type of zone but it is granular like the stroma.

The micronutrients, while being necessary for growth, if present in excess, may be injurious to the plants. The amount which will result in injuries varies not only with the elements but other factors like the age of the plant and the amount of other elements in the medium. Increased supply of Mg to *Hevea* plants fed with low or normal levels of Mn has been reported to produce symptoms of Mn deficiency[9].

Deficiencies in micronutrients have also been related to incidence of diseases. A striking relationship between susceptibility to Oidium attack and Zn status of the plants has been observed in *Hevea*[10]. The Zn-deficient plants are unable to produce enough of a certain metabolite which confers some degree of resistance to Oidium attack.

Many of the ultrastructural features observed in the present study indicate that micronutrients affect cellular and chloroplast architecture. The features observed indicate impairment of function follows such structural alterations. In the absence of biochemical supporting evidence, it is not possible to discuss what these effects are on the physiology of the plant.

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