PLANT RESPONSE TO COPPER AND ZINC HYDROXIDESULPHATE AND HYDROXIDECARBONATE USED AS AN ALTERNATIVE COPPER AND ZINC SOURCES IN MINERAL NUTRITION

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ABSTRACT

We examined the possibility to apply the copper and zinc mixed hydroxide salt as an alternative source of copper and zinc in the nutrient media. Some elemental levels, morphological and photosynthetic chracteristics of lettuce plants grown in the nutrient solution containing mixed hydroxide salt of copper and zinc were compared to control plants grown in the nutrient media where the copper and zinc were as $CuSO_4$ and $ZnSO_4$. Growth of the hydroxidesulphate treated plants was inhibited compared to the control and hydroxidecarbonate treated plants. The chlorophill concentration, photosynthetic activity were lower in hydroxidesulphate treated plants, and their chloroplasts were filled with swelling thylacoidal membranes. The hidroxidecarbonate created better conditions for lettuce plants growth.

Key words*:* heavy metals, nutrient solutions, Zn - hydroxidesulphate, Zn - hydroxidecarbonate, chlorophyll content, photosynthetic activity, *Lactuca sativa*.

INTRODUCTION

Copper and zinc are required by biological system as structural and catalytic components of proteins and enzymes, and as cofactors, and are necessary to normal growth and development. A common characteristic of heavy metals in general, is that when in excess, they are strongly phytotoxic. Therefore the pollution by these metals cause growth inhibition, they become toxic to cells and cause death of plants (Steffens 1990). Heavy metal ions at physiological concentrations appear to inhibit ATP-synthesis, and in consequence the energy-metabolisms of plants (Teige et al. 1990). A Cu - mediated lipid peroxidation results in a loss of chloroplast membrane function, and has also been proposed as a mechanism of inhibition by this metal. De Vos_et al. (1991) suggested that the primary effect of copper toxicity is the damage of the permeability of root cells, causing loss of ions and other solutes (Wainwright and_Woolhouse, 1977). Copper and zinc are taken up by the same uptake system, therefore Cu becomes toxic also when outcompeting Zn (Kabata-Pendias and Pendias, 1984).

Toxic or lethal levels of heavy metals are experienced by plants growing near mining or smelting operation, industrial and municipal soil waste disposal sites, on some natural soil types, and on some agricultural soils (Steffens_1990). The present study was undertaken to investigate the possibility that new mixed hydroxide salts of Cu and Zn could be utilized as sources of these microelements in the nutrient media. These compounds have low solubility and good tolerance of the plants, and can guarantee the necessary concentrations, avoiding excessive accumulation (Arrambarri et al. 1987).

Photosynthesis, the ultrastructure of chloroplasts, copper and zinc uptake and distribution in the plants as well as their effect on growth were studied.

MATERIALS AND METHODS

Plant material

Nine-day-old lettuce (*Lactuca sativa*) seedlings ("Julta Gumurjinska") were grown in aerated nutrient slution containing (mM): 2.5 $Ca(NO_3)_2.4H_2O$; 1.33 KH_2PO_4; 2.08 KNO_3; 3.5 $MgSO_4.7H_2O$; 3.5 KCL with the addition of microelements according to Hoagland and Arnon (1950) in a naturally illuminated greenhouse. Copper and zinc were applid as $CuSO_4$ and $ZnSO_4$ (after Hoagland and Arnon, 1950) in the controls, and as hydroxidesulphates and hydroxidecarbonates in the experimental groups. The hydroxide salts have the following structure: mixed hydroxidesulphate - $Zn_4(OH)_6SO_4.5H_2O$, $Cu_4(OH)_6SO_4$, $Cu_3(OH)_4SO_4$; mixed hydroxidecarbonate - $(ZnCu)_5(OH)_6(CO_3)_2$, $Zn_5(OH)_6(CO_3)_2$ and were produced after the method of continuous precipitation from sulphate solutions of copper

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and zinc with $NaCO_3$ and NaOH. The plant material was divided into 6 experimental groups according to the chemical form of copper and zinc in the nutrient solution: (1) Control (Cu and Zn after Hoagland and Arnon, 1950); Cu and Zn as mixed hydroxidesulphate in Cu/Zn ratio: (2), 2.03; (3), 1.01; Cu and Zn as hydroxidecarbonate in Cu/Zn ratio: (4), 1.02; (5), 1.98; (6), 0.53. The plants were cultivated to maturity.

Atomic absorption determination of elements

Dry plant material (1g) from roots and leaves was separately ashed at 550^0C. The dried residue was brought to standard volume with 20% HCL. Cu, Zn, Fe, and Ca and Mg were determined directly by atomic absorption spectrometry (Karl Zeiss, Jena, Germany) at 324 nm using an air-acetylene flame.

Electronic microscopy

Segments (1-2 mm) from the mesophyll tissue of the second completely formed leaf of lettuce plants from all groups were fixed in 5% glutaraldehyde in Nacacodylate buffer, pH 7.2 at 4°C for 3 h, and washed and postfixed in 1.3% (w/v) OsO4 in the same buffer for 12 h. The samples were dehydrated through a graded ethanol series (25, 50, 75, 96, 100%); ethanol-propylene oxide (1:1, v/v); propylene oxide and propylene oxide-Durcupan ACM (1:1,v/v), and embedded in Durcupan ACM (Fluca AG, Buchs, Switzerland). Ultrathin section were cut with a LKB ultramicrotome, stained with uranil acetate and lead citrate (Reynolds, 1963) and observed in a Zeiss EM 109 transmission electron microscope at 80 KV.

Determination of chlorophyll

Leaf discs (1 cm^2) were incubated in 80% acetone for 1 h for complete extraction of chlorophyll. Total chlorophyll was determined from the absorbance at 665 nm and 649 nm and the extinction coefficients given in Arnon (1949).

Photosynthesis rate measurement

The rate of photosynthesis ($mgCO_2/m^2sec$) was measured by a portable photosynthesis apparatus (LI-600, Li-Cor, Lincoln, NE, USA). Leaves of 5-6 plants were placed in a 0.25-L chamber. Quantum flux density was 870 µmol m^2 s^{-1}, provided by a 500 W incadescent lamp (ZLN - 500 W, EVZ, Bulgaria) with reflector. Leaf temperature was $27^0C\pm2^0C$.

RESULTS AND DISCUSSIONS

The concentrations of the elements Cu, Fe, Zn in the control and experimental plants are shown in table 1.

The concentrations of Cu, Zn and Fe followed the order Fe>Zn>Cu in the leaves, Fe>Zn>Cu in the roots in the control plants. The concentrations of the elements were higher in the experimental groups and were within the same order. In the plants treated with hidroxidesulphate the uptake of the above ions was higher than that of the control plants as well as those treated with hydroxicarbonate. In the plant treated with hidroxidecarbonate with Cu/Zn ratio 0.53 the concentrations of Cu, Zn and Fe were similar to that of the control plants. Copper level in the leaves of the same variant was 1.2 times higher than that of the control leaves, whereas the concentration in the root was 3 times higher than the control. This suggests that an active mechanism in the

Table 1. Concentrations of Cu, Zn, Fe in the leaves and roots from control and treated *Lactuca sativa* plants. The concentrations are expressed in mg (1000 g dry weight)$^{-1}$ as means of 3 experiments with SE

Variants	Leaves			Roots		
	Cu	Zn	Fe	Cu	Zn	Fe
1	7.9±1.3	108± 8.5	220±14.5	29± 3.4	187±17.8	1406±142
2	21.9±2.8	180±17.1	268±17.8	300±26.6	701±58.0	1536±195
3	10.8±1.4	132±14.5	203±25.0	242±24.0	633±62.0	2045±112
4	13.5±1.2	189±16.5	316±33.0	197±14.0	303±28.3	2482±228
5	18.8±1.2	168±16.0	264±20.5	183±16.0	263±21.0	2095±259
6	10.0±1.2	151±15.1	243±21.0	87± 7.6	213±17.5	1820±168

root hinders the entrance of these elements into the others parts of the plants (Jarvis, 1980; Jensen and Adalsteinsson 1989).

The growth parameters of lettuce are depedent on the form in which Cu and Zn were added to the nutrient solution (Figures 1, 2 and 3).

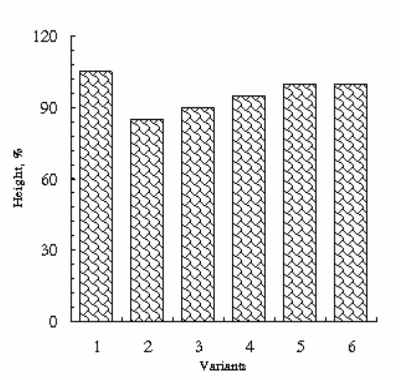

Figure 1. Change in the height of control and treated *Lactuta sativa* plants. The data are from three experiments

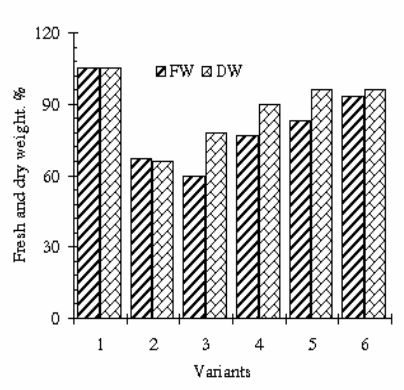

Figure 2. Changes in the leaves fresh and dry weight of control and treated *Lactuta sativa* plants. The data are from three experiments

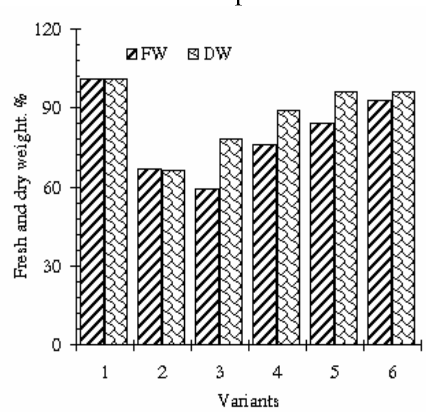

Figure 3. Changes in the root fresh and dry weight of control and treated *Lactuta sativa* plants. The data are from experiments

There was significantly more inhibition in the growth of plants cultivated in the nutrient solution containing mixed hidroxidesulphate compared to the control plants and the plants cultivated in the presence of mixed hydroxidecarbonate.

The plant growth was inhibited, manifested by decreased height to 86%, dry leaf weight to 73%, fresh leaf weight to 72%, dry root weight to 59%, fresh root weight to 77%. The growth of lettuce plants treated with mixed hydroxidecarbonate was changed in more narrow limits. The growth parameters of plant of variant 6 (Cu/Zn ratio 0.53) were very near to those of the control variant. Determination of the chlorophyll content of plants is often carried out to assess the impact of environmental stress, as changes in pigment concentration are linked to visual symptoms of plant illness and photosynthetic plant productivity (Parekh et al., 1990). Chlorophyll biosynthesis was changed by hydroxidesulphate treatment in lettuce plants (Figure 4). In the plants treated with Cu/Zn = 1.01 hydroxidesulphate the chlorophyll concentration was strongly inhibited. The chlorophyll *a* reached to 52% and chlorophyll *b* to 58% in comparison to control plants. The hydroxidecarbonate creates more favourable conditions for the chlorophyll synthesis. The concentration of chlorophyll varied in the range of 0.34 to 0.42 mg/g FW for chlorophyll *a*, and from 0.19 to 0.23 mg/g FW for chlorophyll *b*. The corresponding values of the control variant were 0.45 mg/g FW and 0.26 mg/g FW, respectively.

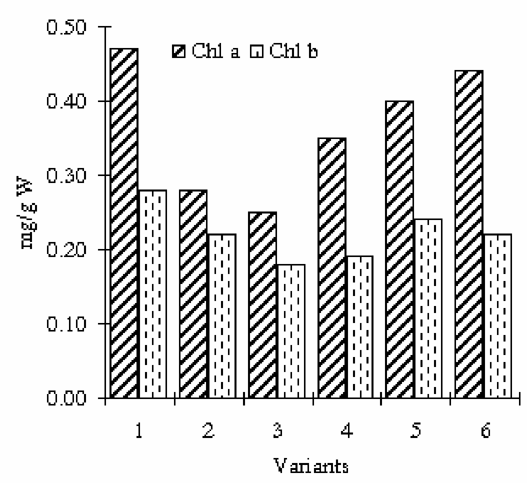

Figure 4. Chlorophyll content (mg/g FW) in the leaves of control and treated *Lactuta sativa* plants. The data are from three experiments

The excess of Cu can induce Fe-deficiency chlorosis (Foy et al., 1978). The data obtained for the Fe concentration in control and experimental plants were the same (Table 1), thus the differences in the chlorophyll content can not be attributed to Fe concentration.

There were appreciable changes in photosynthetic values (Figure 5).

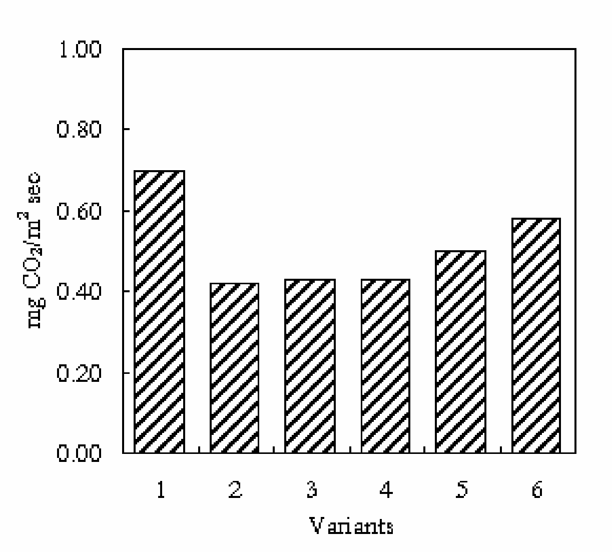

Figure 5. Rate of the photosynthesis (mg CO_2m^2sec) of control and treated *Lactuta sativa* plants. The data are from three experiments

The photosynthetic activity followed the variations in the chlorophyll concentration. The plants exposure to hydroxidesulphate resulted in a decrease in photosynthesis, reaching the lowest value at variant 3: 0.42 mg CO_2/m^2 sec compared to 0.69 mg CO_2/m^2 sec of control variant. The reduction of photosynthetic activity was also observed, in plants treated with hydroxidecarbonate, however to a lower degree (about 20%). The observed changes in photosynthetic activity could be discussed as a result of effects of the excess of Cu and Zn on the capacity of the plants for CO_2 fixation (Maksymiec et al., 1994). These results are in agreement with the observations at ultrastructural level.

The electronic microscope observations of ultrathin sections of mesophylic cells from the control variant indicated the presence of the elongated chloroplasts with a typical arrangement of the thylakoidal system in higher plants, with differentiation of the granal and stromal thylakoids, with plastoglobuli and starch grains, generally one grain per plastid (Figure 6a).

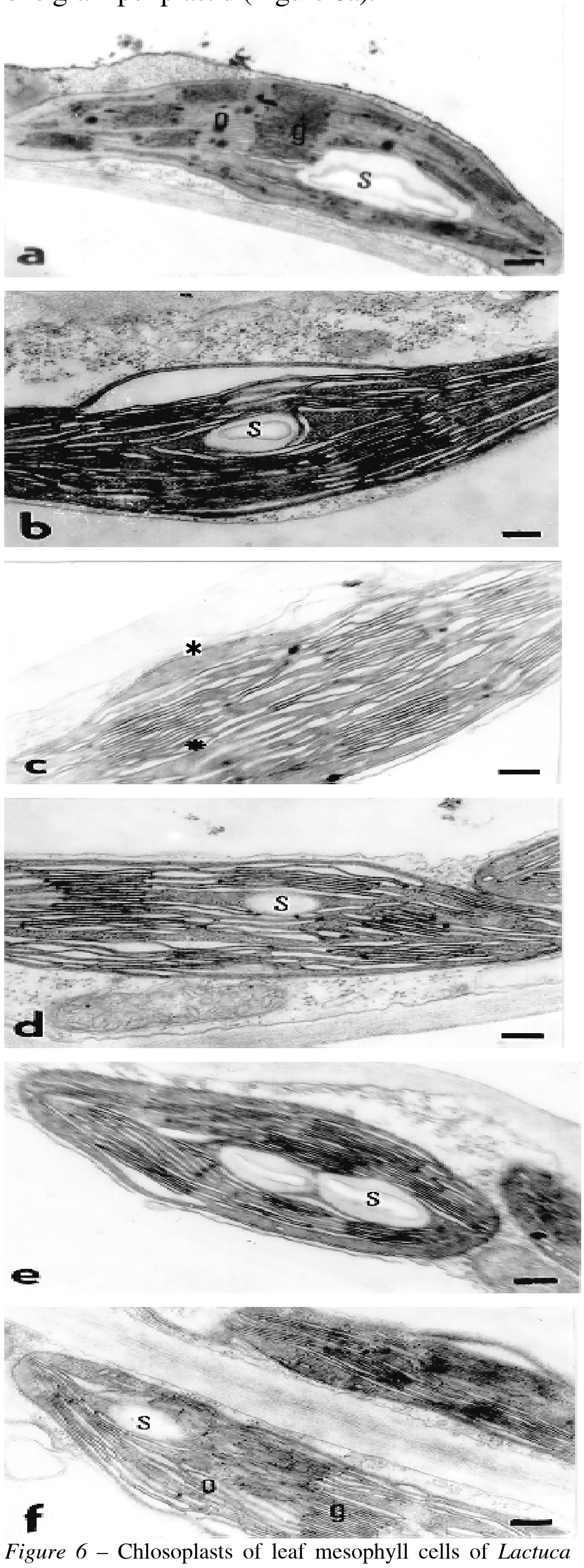

Figure 6 – Chlosoplasts of leaf mesophyll cells of *Lactuca sativa* grown in nutrient solution with different Cu and Zn sources: a - control (Cu and Zn after Hoagland and Arnon; Cu and Zn as mixed hydroxydesulphate in Cu/Zn ratio;

b – 2.03; c – 1.01; Cu and Zn as hydroxycarbonate in Cu/Zn ratio; d – 1.02; e – 1.98; f – 0.5 (Grana – g, Starck grain - s, plastoglobuli – o, swelling thylakoid membrane - ∗ are indicated. Bars = 0.2 μM)

The chloroplast ultrastructure from plants cultivated in hydroxidesulphates demonstrated alterations. The chloroplast stroma was filled with swelling thylakoid membrane (Figures 6 b, c).

The granas were difficult to distinguish from the membranous structures that seemed to the be formed as a result of degeneration of the thylakoidal system. Compared with these chloroplasts, the chloroplasts observed in hydroxidecarbonate-treated plants seemed to be relatively normal. The granas were recognizable, and appeared quite similar to the control ones (Figures. 6 d, e, f).

CONCLUSIONS

The growth of lettuce plants when Cu and Zn were applied in the from of mixed hydroxidesulphate was inhibited, but on the contrary, the same elements used as hydroxidecarbonates created a better growth condition. This was also confirmed by the rest of the parameters measured. The reduced chlorophyll concentration, photosynthetic activity and ultrastructural alterations of chloroplast indicate that this ion form is not a suitable nutrient for this plant. The hydroxidecarbonate as copper and zinc source creates more favourable conditions for lettuce plants. The exact ration between these ion forms should be precised in the future so that only the necessary concentrations be applied and environmental pollution avoided.

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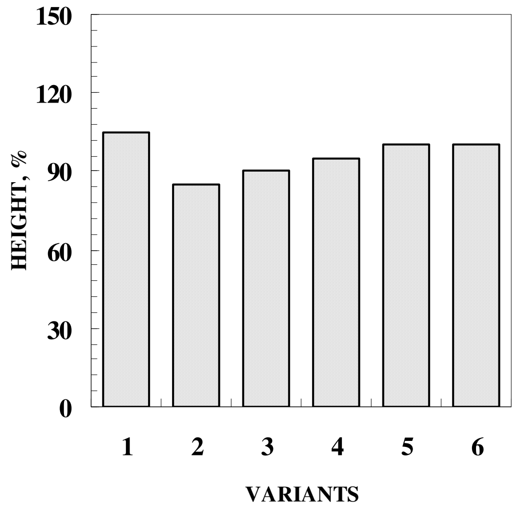

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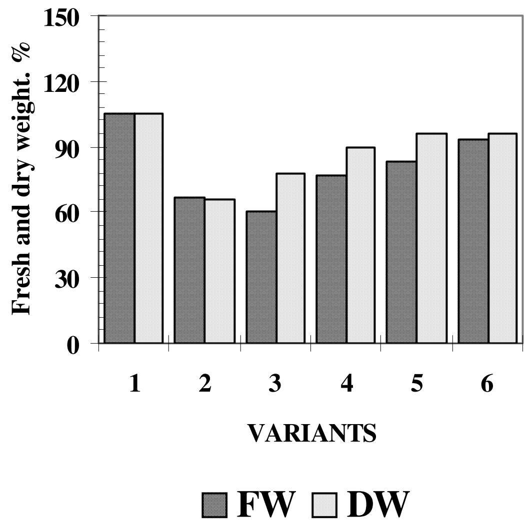

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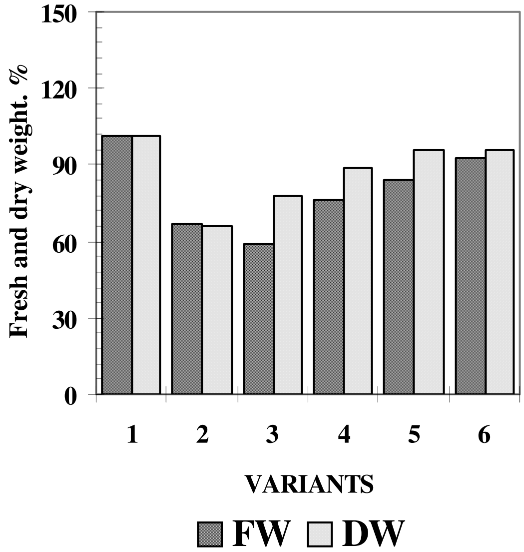

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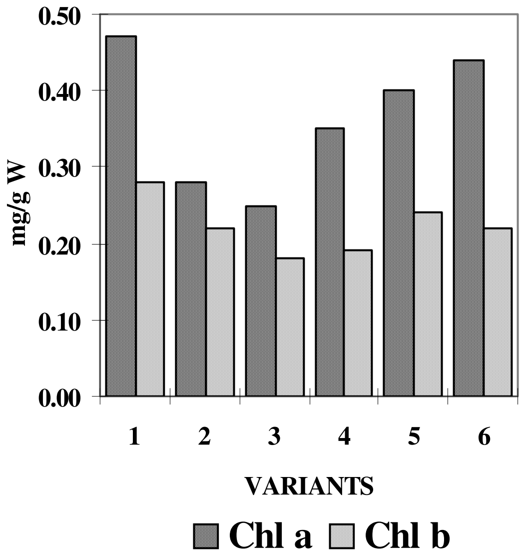

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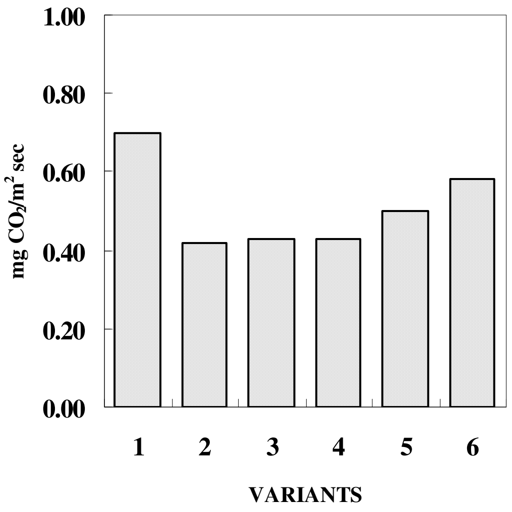

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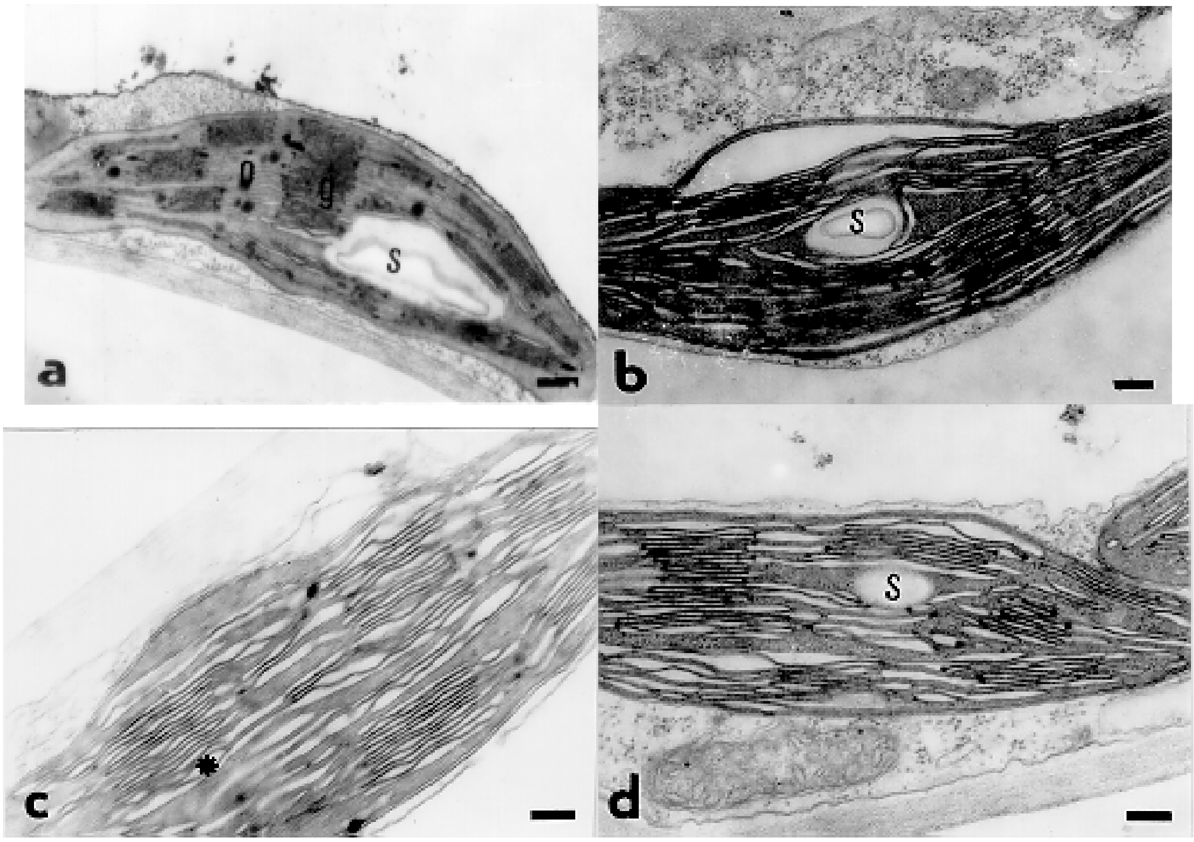

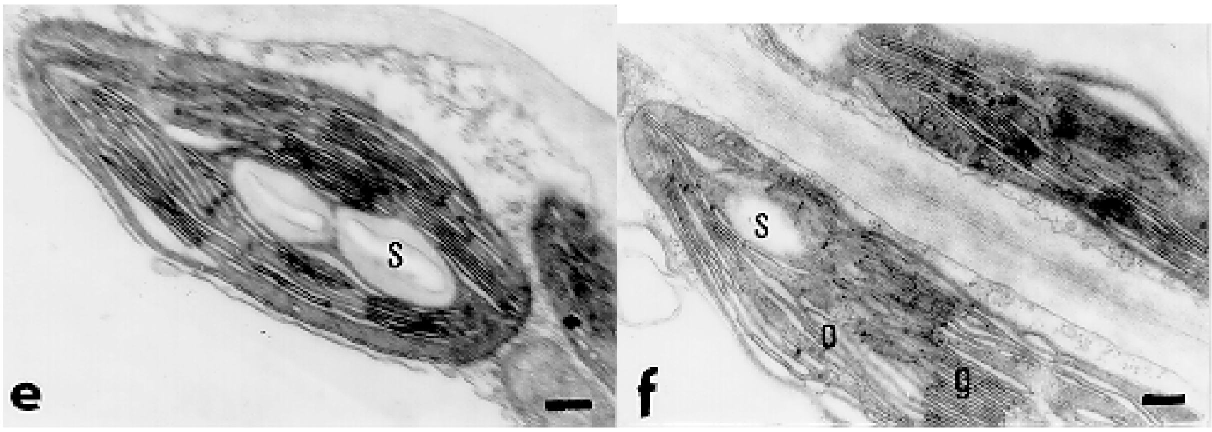

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