# BENEFICIAL EFFECTS OF LOW CHROMIUM III CONCENTRATIONS IN IRON DEFICIENT MAIZE PLANTS

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## RESUMEN

### EFECTOS BENEFICIOSOS DE BAJAS CONCENTRACIONES DE CROMO III EN PLANTAS DE MAIZ DEFICIENTES EN HIERRO

Se ha estudiado la influencia de una concentración baja de Cr III (1 $\mu$M) sobre el crecimiento, el contenido en clorofilas y carotenoides y la ultraestructura de cloroplastos de plantas de ***Zea mays***. Las plantas fueron cultivadas hidropónicamente con soluciones nutritivas sin Fe (deficiencia de Fe severa) o con una concentración subóptima de Fe (10 $\mu$M Fe; deficiencia moderada de Fe). Se observó un significativo efecto beneficioso del Cr III en las plantas con suministro subóptimo de Fe. La concentración de clorofilas y carotenoides en las hojas jóvenes se incrementó significativamente en presencia de Cr III y la ultraestructura de los cloroplastos mejoró notablemente. El Cr III a bajas concentraciones no ejerció efecto alguno, ni beneficioso ni tóxico, en las plantas sin suministro de Fe, que sufrían severos síntomas de deficiencia de Fe. Se sugiere que el efecto beneficioso del Cr III podría estar relacionado con una mayor disponibilidad de Fe biológicamente activo.

Palabras clave: Cromo. Elemento beneficioso. Deficiencia de hierro. Ultraestructura de cloroplastos. Microscopía electrónica de transmisión.

## SUMMARY

The influence of a low Cr III concentration (1 $\mu$M) on growth, chlorophyll and carotenoid contents and chloroplast ultrastructure of ***Zea mays*** plants grown in nutrient solution without Fe supply (severe Fe deficiency) or with a suboptimal Fe concentration (10 $\mu$M, moderate Fe-deficiency) was investigated. Chromium significantly enhanced the performance of plants exposed to suboptimal Fe concentrations. Chlorophyll and carotenoid concentrations in young leaves were significantly increased and chloroplast ultrastructure was substantially improved. No effect of Cr, neither beneficial nor toxic, was observed in plants without Fe supply which showed severe Fe-deficiency symptoms. It is suggested that the beneficial effect of low Cr may be due to an increase of the availability of biologically active Fe.

Key words: Chromium. Beneficial alement. Iron deficiency. Chloroplast ultrastructure. Transmission electron microscopy.

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## INTRODUCTION

Chromium is an essential element for men and animals but, up to date, no essential function for chromium has been described in plants (Barceló *et al.*, 1987). Nevertheless, low Cr concentrations may have a beneficial influence on plant performance and growth stimulating effects of Cr have been described, among others, in grapes (Dobrolyubskii and Viktorova, 1974), potatoes (Bertrand and de Wolf, 1968), orange trees and avocados (Haas and Brusca, 1961). The favourable influence of low Cr concentrations have been attributed to positive influences of Cr on mineral nutrition, water relationships and biomass partitioning (Pratt, 1966; Barceló *et al.*, 1986). Also the antifungal properties of Cr may play a role in increased crop production in presence of low Cr concentrations (Huffman and Allaway, 1973). Recently we reported on the beneficial influence of Cr III on growth (Bonet *et al.*, 1991) and chloroplast ultrastructure (Poschenrieder *et al.*, 1991) in Fe-deficient bean plants. Chromium increased the o-phenantroline-extractable leaf Fe concentration, but results on catalase activity were not in line with the hypothesis that the beneficial effect of low Cr concentrations was due to a Cr-induced increase of biologically active Fe in plants (Poschenrieder *et al.*, 1991). Here we report results on the effects of low Cr III supply on chloroplast ultrastructure in *Zea mays* plants grown under severe and moderate Fe-deficiency conditions.

## MATERIALS AND METHODS

Seeds from *Zea mays* L. cv Adour (Fitó, S. A., Barcelona) were germinated for 7 d on perlite with distilled water. The seedlings were transplanted to continuously aerated 10 % Hoagland nutrient solution (Hoagland and Arnon, 1950) containing 0.1 M $NaSiO_3$ (Epstein *et al.*, 1988) (plastic beakers, 5 L capacity, 10 plants per beaker). Treatments with 1 $\mu$M Cr in the form of $CrCl_3$ and treatments without Cr were factorially combined with severe Fe-deficiency treatments (no Fe supply) and moderate Fe-deficiency treatments (10 $\mu$M Fe in the form of Fe-EDTA). Each Fe-Cr combination was replicated three times.

The plants were grown for 14 d in a controlled environment chamber under the following conditions: photon fluence rate 150 $\mu M\ m^{-2}\ s^{-1}$, photoperiod 16 h light, 8 h darkness; day/night temperature, 26 °C/23 °C; day/night relative humidity, 60 %/ 80 %.

At day 14 from transplantation, samples were taken from the 4th leaf for light and electron microscopy. Samples were processed by standard methods as previously described (Vázquez *et al.*, 1987). Micrographs shown in the paper are representative pictures of samples taken from three plants per treatment.

At the same time samples were taken

for growth measurements (fresh and dry weight and leaf area). The chlorophyll and carotenoid concentrations of leaves were determined on 80 % acetone extracts according to Lichtenthaler and Wellburn (1983). The Cr concentration of the acid digested ($HNO_3 : HClO_4 = 10{:}4$) plant material was determined by atomic absorption spectrometry (Perkin Elmer 703). Given results are means of three replicates per treatment. Significance of differences were determined by ANOVA.

## RESULTS

Plants grown without Fe showed severe chlorosis in young leaves. Plants receiving 10 μM Fe exhibited young leaves of a light green colour. Neither fresh and dry weight (data not shown) nor the leaf area (Table 1) was significantly affected by the Fe and Cr treatments. Chromium supply did not significantly increase the Cr concentration in the young leaves (Table 1). Plants exposed to low Fe concentrations showed significantly higher chlorophyll and carotenoid concentrations than plants without Fe supply (Table 1). In plants receiving 10 μM Fe, but not in those without Fe supply, 1 μM Cr significantly increased the chlorophyll and carotenoid concentration (Table 1).

In plants without Fe supply, severe ultrastructural effects were observed. Ameboid shaped chloroplasts with or without cytoplasma inclusions (Figs. 1, 2 and 5) were frequently observed. Inhibition of division of bundle sheet chloroplasts occurred (Fig. 3). Chloroplasts from plants without Fe supply, generally exhibited a disorganized thylakoid membrane system (Fig. 4). Light microscopy observations revealed ne-

TABLE 1

*Influence of 1 μM Cr on leaf area ($cm^2$), Cr concentration ($\mu g\ g^{-1}$ dry weight) and chlorophyll and total carotenoid concentrations ($mg\ g^{-1}$ fresh weight) in the 4th leaf of maize plants exposed to severe (no Fe supply) or moderate (10 μM Fe) Fe deficiency.*

| Treatment Fe | Cr III | Leaf area | Chromium | Chl a | Chl b | Carotenoids |
|---|---|---|---|---|---|---|
| No | No | 45.37a* | 5.14a | 0.231a | 0.072a | 0.077a |
| No | 1 μM | 43.58a | 6.26a | 0.206a | 0.058a | 0.070a |
| 10 μM | No | 39.07a | 8.28a | 0.254a | 0.076a | 0.082a |
| 10 μM | 1 μM | 39.02a | 6.34a | 0.354b | 0.298b | 0.139b |

* Values within a column followed by the same letter are not significantly different ($p > 0.05$).

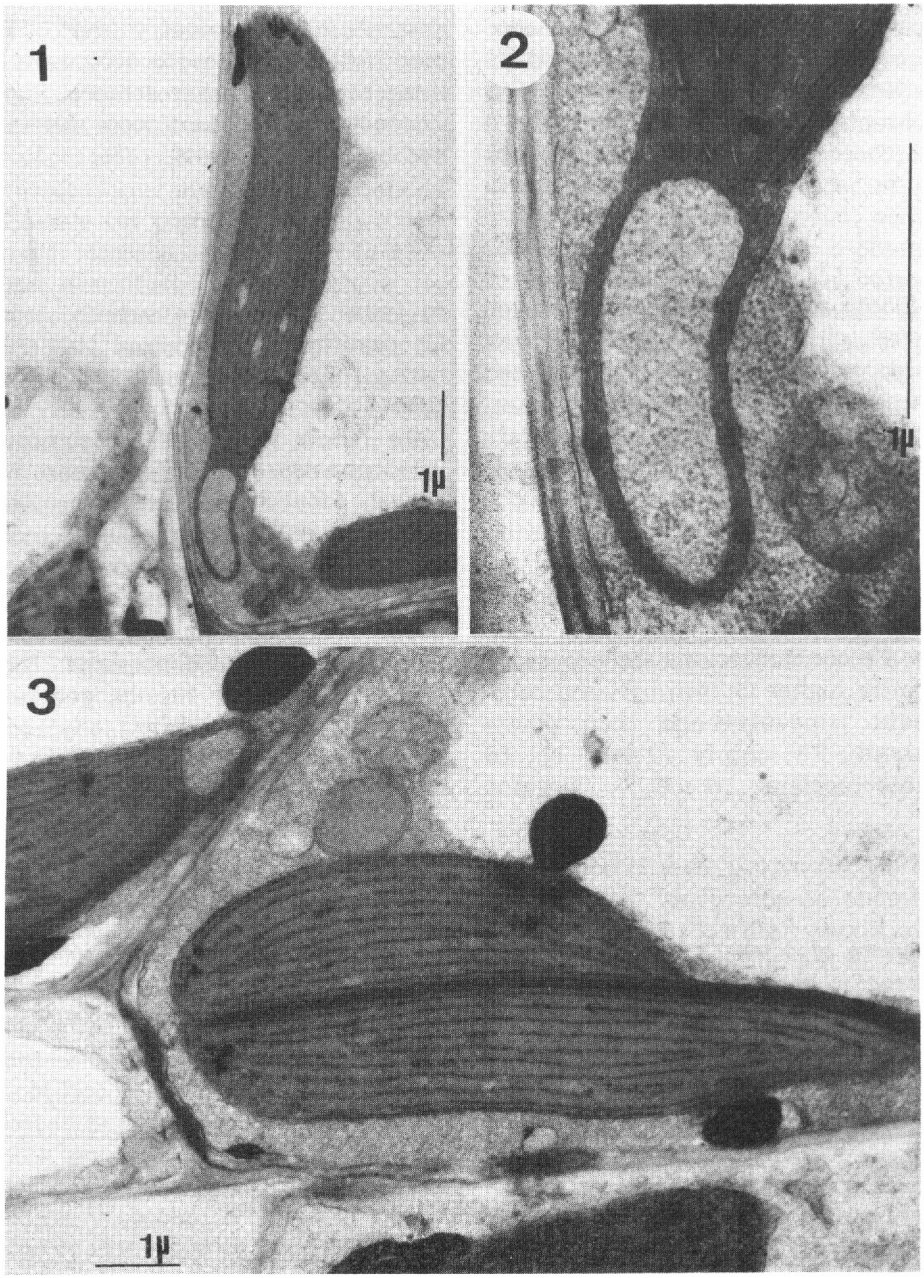


FIG. 1-3.–*TEM micrographs of chloroplasts of the 4th leaf of a maize plant grown without Fe supply.* FIG. 1: *Ameboid-shaped bundle sheet chloroplast with inclusion of a portion of cytoplasm.* FIG. 2: *Detail of the inclusion from figure 1.* FIG. 3: *Bundle sheet chloroplast which failed to divide.*

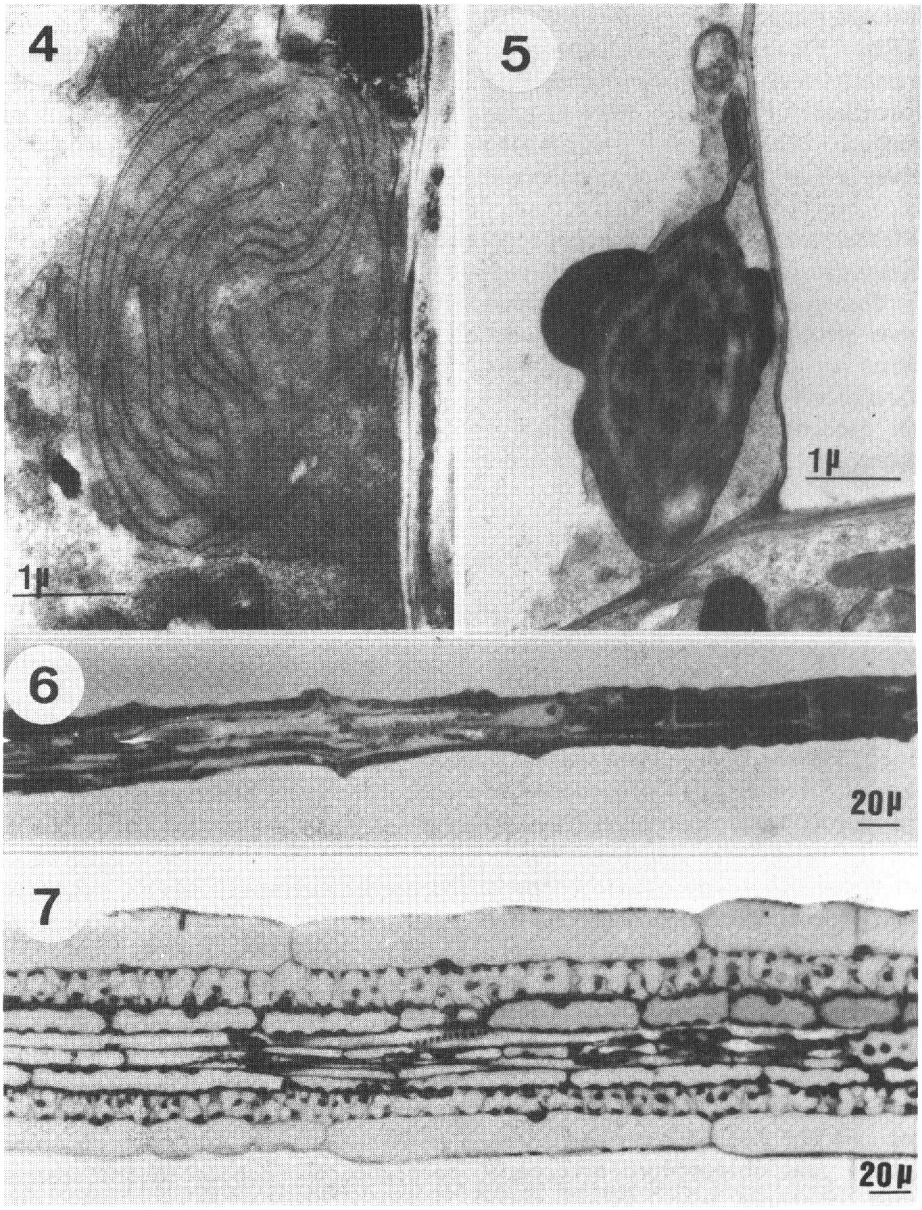


FIG. 4-7.–*Micrographs from the 4th leaf of a maize plant without Fe supply.* FIG. 4: *Swollen bundle sheet chloroplast with desorganized thylakoid membrane system.* FIG. 5: *Ameboid-shaped mesophyll chloroplast.* FIG. 6: *Light microscopy micrograph showing necrotic area next to an apparently normal area shown in* FIG. 7.

crotic areas (Fig. 6) next to apparently normally structured zones (Fig. 7). The supply of 1 μM Cr to plants without Fe supply had no significant influence neither on the leaf structure (Figs. 11, 12) nor on chloroplast ultrastructure (Fig. 8). Ameboid chloroplasts (Figs. 8, 9) and heavily damaged chloroplasts exhibiting pleomorphic forms were observed (Fig. 10).

Plants grown under moderate Fe deficiency conditions generally were less damaged than those of plants grown without Fe supply. But alterations of chloroplast shape were also observed in leaves from plants with moderate Fe deficiency stress. Figures 13 to 17 show different ameboid outgrowths of chloroplasts which may contact with mitochondria (Fig. 14) and engulf mitochondria (Fig. 17) or cytoplasm portions (Figs. 15, 16).

Plants grown under moderate Fe deficiency and treated with 1 μM Cr exhibited normally shaped chloroplasts with an almost normal thylakoid membrane system (Figs. 18, 19). Comparing light micrographs from transversal leaf sections from plants grown with 10 μM Fe without Cr supply (Fig. 20) with those from plants exposed to 10 μM Fe and 1 μM Cr (Fig. 21), the higher number of chloroplasts per cell and the bigger leaf thickness are clearly visible in Cr treated plants.

## DISCUSSION

The low chlorophyll concentrations found in leaves of the maize plants indicate that plants from both treatments (no Fe supply and 10 μM Fe) suffered from Fe-deficiency. Chlorophyll a and b concentrations were significantly lower and the chl a/chl b ratios significantly higher than those found by others (Almela *et al.*, 1983) in leaves from maize plants grown under slight Fe deficiency (85 % chl a of control). Our results indicate that low Cr III concentrations have a beneficial effect on maize plants which are grown under moderate Fe-deficiency conditions (10 μM Fe). Chloroplast ultrastructure and chlorophyll and carotenoid contents were significantly improved. Chlorophyll b was the most enhanced pigment. These effects were not due to a Cr-induced growth reduction, i.e. a concentration effect, because fresh and dry weights and leaf areas of Cr-treated plants were not significantly different from those of plants without Cr supply. The failure of Cr to increase plant growth despite of the positive effect on pigment content may be due to the relatively low light intensity in our growth chamber, which may have limited photosynthesis.

The beneficial effect of Cr only occurred in plants exposed to suboptimal Fe concentrations but not in plants grown without Fe supply. In a former study on bean plants a beneficial effect of low Cr III concentrations was observed in plants without Fe supply in the nutrient solution (Bonet *et al.*, 1991; Poschenrieder *et al.*, 1991). This difference may arise from either or both the different Fe requirement and the different strategies of Fe-uptake and

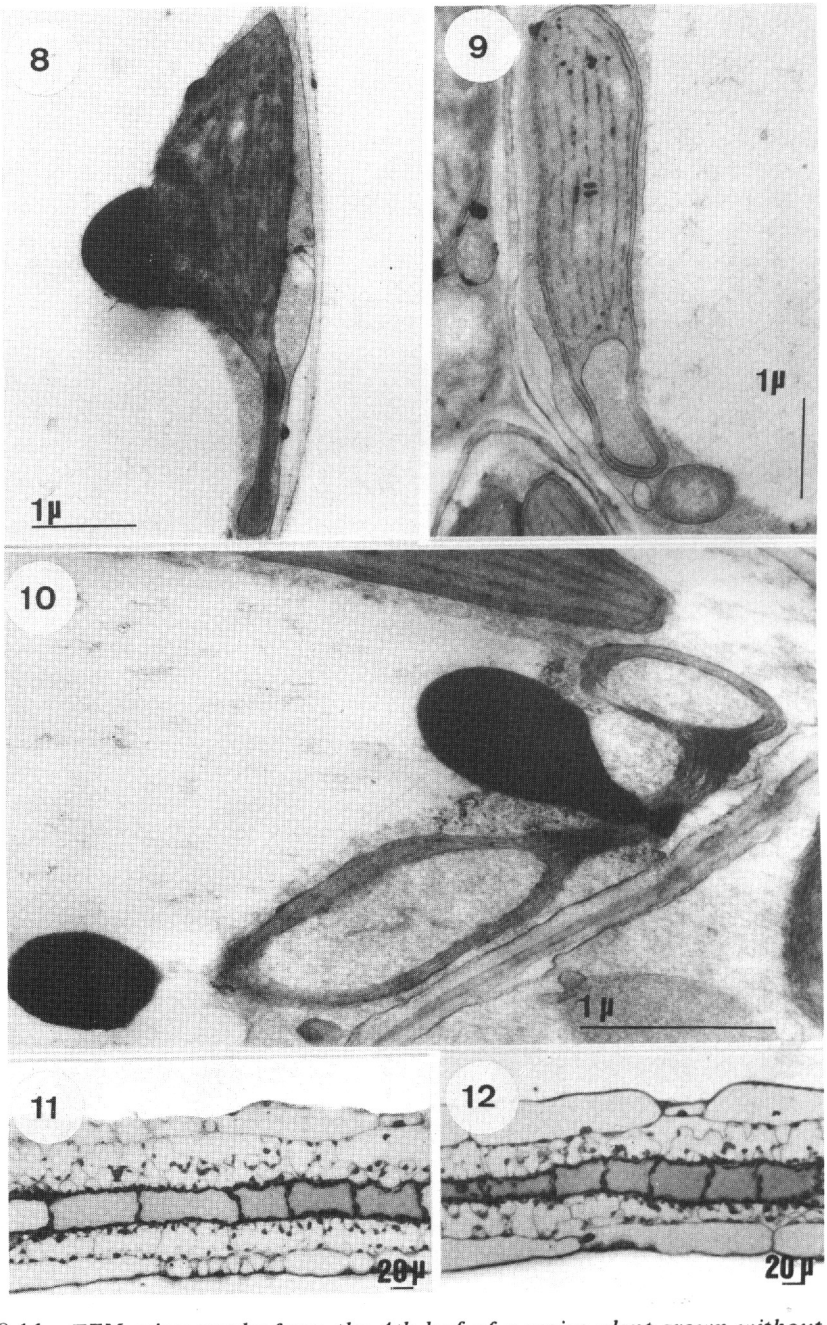


FIG. 8-11.–*TEM micrographs from the 4th leaf of a maize plant grown without Fe and 1 µM Cr.* FIG. 8: *Ameboid-shaped mesophyll chloroplast.* FIG. 9: *Ameboid-shaped bundle sheet chloroplast with inclusion of a portion of cytoplasm.* FIG. 10: *Severely damaged bundle sheet chloroplast exhibiting pleomorphic form.* FIG. 11: *Light microscopy picture of a transversal leaf section from a plant without Fe and Cr supply.* FIG. 12: *As figure 11 but from a plant treated with Cr. No influence of Cr was observed in plants without Fe supply.*

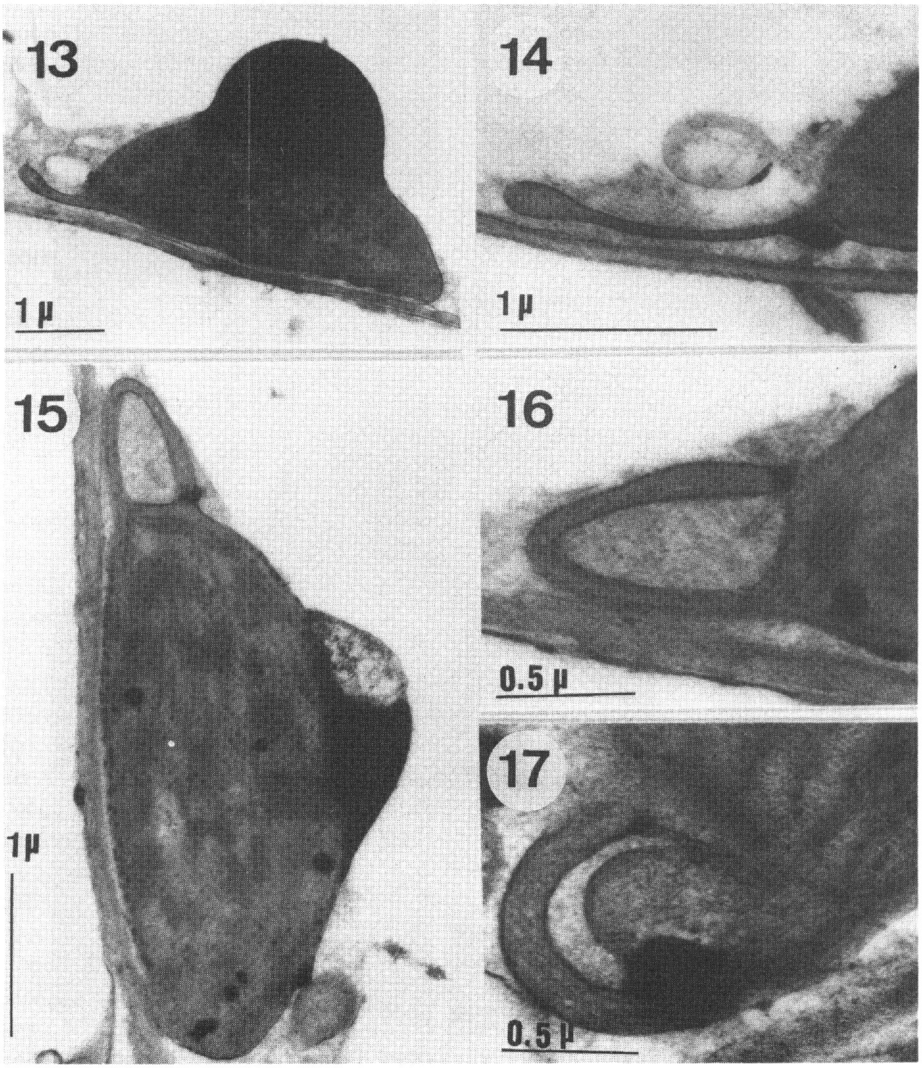


FIG. 13-17.–*TEM micrographs from 4th leaf of a maize plant grown with 10 µM Fe showing different stages of mesophyll chloroplasts engulfing mitochondria or cytoplasm.* FIG. 13: *Chloroplast with small ameboid autgrowth.* FIG. 14: *Ameboid outgrowth next to mitochondria.* FIG. 15: *Chloroplast with bent ameboid outgrowth.* FIG. 16: *Cytoplasm inclusion.* FIG. 17: *Engulfment of mitochondria.*

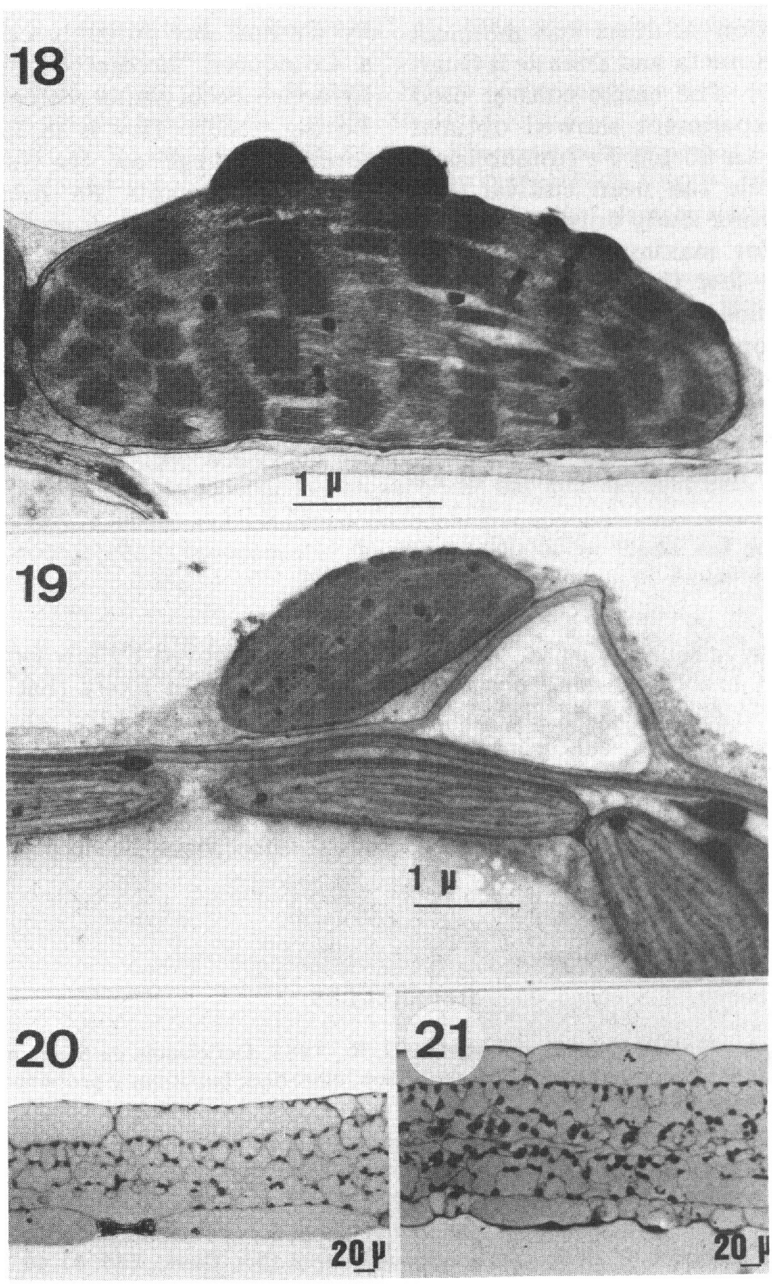


FIG. 18, 19, 21.–*Micrographs from the 4th leaf of a maize plant exposed to 10 µM Fe and 1 µM Cr.* FIG. 18: *Normally shaped mesophyll chloroplast with well organized thylakoid membrane system and abundant grana stacks.* FIG. 19: *Bundle sheet chloroplasts.* FIGS. 20 and 21: *Light microscopy pictures of transversal leaf sections from a plant without Cr supply (Fig. 20) and from a plant with Cr (Fig. 21) Note the increased number of chloroplasts per cell and the bigger leaf diameter in figure 21.*

translocation in dicot and monocot species (Romera and Díaz de la Guardia, 1991). The maize cultivar used in this experiment showed optimal growth with 30 μM Fe (unpublished data), while the bean cultivar used in our former study only required 10 μM Fe for maximum growth. The fact that low Cr III improved the performance of maize plants grown with suboptimal Fe supply, but not of those without Fe treatment suggests that the beneficial effect of Cr was not due to a partial substitution of metabolic functions of Fe by Cr but was probably caused by a Cr-induced increase of biologically active Fe in plants with moderate Fe deficiency. This is in line with former findings on the o-phenantroline extractable Fe fraction in Fe-deficient beans exposed to Cr. Although other metabolic effects of Cr, such as the increase of the concentration of free poliamines (Troyano, 1989), which are known to stabilize chloroplast membranes (Popovic *et al.*, 1979) may also play a role.

## CONCLUSIONS

We may conclude from our results, that low Cr III concentrations have a beneficial effect on maize plants grown with suboptimal Fe supply. The chloroplast ultrastructure, which is severely affected by moderate Fe-deficiency stress, is significantly improved by low Cr III concentrations. It is likely that at least in some occasions, the chromium-induced increase of productivity in field grown crops, which has been observed by several authors, was due to suboptimal Fe supply.

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