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Effects of excess copper on the photosynthetic pigments in rice plants

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Abstract. Rice (*Oryza sativa* L. cv. Safari) plants were grown over a 30 day period in nutrient solution containing concentrations of Cu varying from 0.002 to 6.25 mg/l. The shoot content of Cu, Mn, Fe and Mg was measured as well as the photosynthetic pigments. Cu concentration tended to increase in the shoot with increasing levels of the metal in nutrient solution; however, Mn and Fe concentrations showed a progressive decrease above the 0.05 mg/l Cu treatment. The shoot Mg concentration exhibited little variation with the different Cu treatments. Except for the deficiency treatment (0.002 mg Cu/l), chlorophyll and carotenoid contents decreased, on a fresh weight basis, with increasing levels of copper in the medium. Phytoene, phytofluene, and protochlorophyll (ide) were measured in rice shoots as well as in isolated protoplasts incubated with Mn and Fe. Phytoene and phytofluene concentrations decreased with increasing copper concentrations in the rice shoots. However, in isolated protoplasts incubated with varying Mn concentrations, an increase in their concentrations was found within the range of 50-250 μM Mn. Protochlorophyll (ide) concentrations decreased above the 0.01 mg/l Cu treatment in rice shoots. Yet, in isolated protoplasts incubated with varying Fe concentrations, an increase in their concentrations was found within the range of 4-6 mM Fe. The observed decrease of pigment content is explained by the roles of Fe and Mn in chlorophyll and carotenoid biosynthesis.

Key words: Carotenoids; Chlorophylls; Copper toxicity; Rice.

Introduction

It is well known that chloroplast pigment content varies characteristically among plant species (Takagi *et al.*, 1990; Thompson, 1949) and also varies with growth conditions, such as in the presence of excess amounts of most metals (Barcelo *et al.*, 1986; Barua and Jana, 1986; Baszynski *et al.*, 1982; Droppa *et al.*, 1984; Fernandes and Henriques, 1991; Sandmann and Bögar, 1980; Singh, 1988; Stiborová *et al.*, 1986). Barua and Jana (1986) reported that high levels of Hg, As, Pb, and Cr decrease chlorophyll (Chl) content in *Spinacea oleracea*. Barcelo *et al.* (1986) suggested that as Cr toxicity caused inhibition of Fe and Zn transport in *Phaseolus vulgaris*, Chl and carotenoid decreases induced by Cr might ultimately result from a decrease in these latter metals. While Singh (1988) observed that toxic concentrations of Zn and Mn decreased Chl contents in *Indigofera glandulosa*, Stiborová *et al.* (1986) found that Zn toxicity had little effect on the Chl content of *Hordeum vulgare*. With respect to Cu, the literature contains conflicting results. While Baszynski *et al.* (1982) reported a small increase on Chl and carotenoids in tolerant *Spinacea oleracea* exposed to high Cu concentrations, other workers (Barua and Jana, 1986; Fernandes and Henriques, 1991; Sandmann and Bögar, 1980; Stiborová *et al.*, 1986) found a decrease in these pigment levels. These contradictory results and the rel-

Abbreviations: Chl = chlorophyll; PChl (ide) = protochlorophyll (ide); PT = phytoene; PTF = phytofluene.

ative meagre information on the effects of excess Cu on the content of photosynthetic pigments in higher plants has prompted this study. The objective is to obtain a better understanding of these aspects on rice plants exposed for 30 days to varying Cu concentrations in a hydroponic growth medium.

Materials and Methods

Rice (*Oryza sativa* L. cv. Safari) seeds were treated as described before (Lidon and Henriques, 1991) and were germinated in moistened filter paper at 28°C for 3 days. The seedlings were hydroponically grown for 30 days in cylindric 21 pots at 35-37/25-27°C day/night temperatures and under 250 μE PAR $m^{-2}s^{-1}$ irradiance over a 12 h-day period as already described (Lidon and Henriques, 1991a). The nutrient solution used was that of Yoshida *et al.* (1976) containing Cu concentrations below the normal (0.002 mg/l), normal (0.01 mg/l) and toxic (0.05; 0.25; 1.25 and 6.25 mg/l) levels. Other nutrients were used at the following concentrations: N (40 mg/l); P (10 mg/l); K (40 mg/l); Ca (40 mg/l); Mg (40 mg/l); Mn (0.5 mg/l); Mo (0.05 mg/l); B (0.2 mg/l); Zn (0.01 mg/l); and the hexahydrated $FeCl_3$ (2 mg/l). The solution was adjusted to pH 5.5 daily and the volume brought to the original value with nutrient solution. The whole solution was renewed every 5 days.

Shoot Cu, Mn, Fe and Mg were measured at the end of the 30 day experimental period using a Perkin -Elmer model 3030 atomic absorption unit, after sucessive digestion of the samples in a nitric:perchloric (5:2, v/v) mixture followed by treatment with a nitric:sulfuric:perchloric (10:1:10 v/v/v) mixture (Jackson, 1958; Ohki, 1975).

Chl determination was based on subsamples of leaf tissues and followed the method of Arnon (1949).

Extraction and quantitative determination of α -carotene β-carotene, violoxanthin, lutein, lutein-5, 6 -epoxyd antheroxanthin, neoxanthin and zeaxanthin from isolated chloroplasts were carried out according to Hager and Meyer-Bertenrath (1966).

Phytoene (PT) and phytofluene (PTF) extraction and quantification followed, with some minor modifications, the methods of Wilkinson and Ohki (1988) and Hager and Meyer-Bertenrath (1966), respectively. Leaf samples (0.3 g) were extracted with acetone (85%), and total volume was reduced to about 25 ml using a rotatory evaporator (20-30°C dark). The acetone extract was washed three times with n-pentane. Carotenoid components of the extract were separated using 0.3 mm layer of $CaCO_3$, MgO and $Ca(OH)_2$ TLC plate made up with 30% KOH. After TLC plate activation and cooling in a desiccator, the TLC plates were developed with n-octane:acetone:chloroform (60:50:40, v/v/v). The top 2 cm of the chromatogram were scraped into n-pentane. PT and PTF were quantitated spectrophotometrically according to Davies (1965) using $\varepsilon_1^{1\%}$ for PT (1250) (285 nm) and for PTF (1350) (348 nm).

Protochlorophyll (ide) [PChl (ide)] extraction and quantitative determination followed the method of Siegelman and Schopfer (1971) with some minor modifications. Leaf samples (5 g) were extracted in an Omni -Mixer for 3 minutes with 2.5 g of Polyclar AT and 40 ml of extraction medium (50 mM tricine, 50 mM KOH, 2 mM $MgSO_4$, 1 mM EDTA, 0.06% (v/v) Triton X-100, 25% glycerol; pH 8.6). The temperature was maintained below 7°C by immersing the grinding container in an ice bath. The homogenate was clarified by filtration through a 15-cm milk filter disk and centrifuged at 78000xg at 0-4°C for 1 h. A lipid layer was aspirated from the top of the centrifuge tubes and the supernatant solution was used for PChl (ide) quantification. The determination of PChl (ide) concentration was carried out spectrophotometrically in the dark, at 10°C, measuring the absorbance at 639 nm of a solution containing about 5 mg protein per ml.

Leaf protoplast isolation followed the method described by Rubinstein (1978) with the modifications introduced by Kelly and Weskich (1988). Light microscopy revealed that 91% of the protoplasts excluded Evans blue dye and were, therefore, presumed to be intact. Isolated protoplasts (about 4 mg protein/ml) were incubated for 4 h at 25°C with 10 mM $MgCl_2$ and varying concentrations of Mn (0.0025, 0.025, 25, 50, 250, 1000 and 1500 μM). PT and PTF were extracted using 2 ml acetone (100%), concentrated, washed with n -pentane, separated by TLC and quantified as already described. Protoplast incubation (about 5 mg protein/ml) was also carried out for 4 h at 25°C with 50 mM tricine-KOH buffer system (pH 8.6) containing 2 mM $MgSO_4$ and varying concentrations of Fe (2, 4, 6, 8, 10, 12 mM). PChl (ide) was quantified after further treatments as already described.

Protein was estimated by the method of Bradford (1976) using bovine serum albumin as standard.

Results

Above the 0.01 mg/l Cu concentration level, shoot elongation progressively decreased (Lidon and Henriques, *in press* [a]). The shoots of the 1.25 mg/l Cu treatment also showed marked chlorosis, and those of the 6.25 mg/l Cu treatment became almost completely necrotic, an observation that agrees with the effect of excess Cu on *Agrostis gigantea* and *Hordeum vulgare* reported by Hogan and Rauser (1981) and Stiborová *et al.* (1986), respectively.

The concentrations of Cu, Mn, Mg and Fe in shoots (on a dry weight basis) at 30 days following germination are shown in Table 1. The Cu concentration in the shoot increased progressively with increasing levels of the metal in the solution. The increase was rather gradual between the 0.002 and the 1.25 mg/l treatments but showed a sharp increase in the 6.25 mg/l treatment. This was probably due to the loss of the regulatory mechanisms responsible for translocation of Cu to the shoot (Lidon and Henriques, *in press* [b]). Mn and Fe concentrations decreased markedly after the 0.05 mg/l Cu treatment. This result seems to agree with Harrison *et al.* (1983) but not with Bowen (1969, 1981) and Brown *et al.* (1972). Between the 0.002 and 0.05 mg/l Cu treatments, the shoot Mn and Fe concentrations did not exhibit similar trends. Indeed, although the Fe content increased with increasing Cu levels in the medium, the Mn content remained relatively constant. The Mg concentrations in the shoot were similar in all treatments and did not show any visible correlation to Cu levels.

From these data it is apparent that the plants are able to regulate the amount of Cu that is translocated from the root to the shoot, attempting to avoid reaching the toxicity levels inhibitory to metabolism. However, after the 0.05 mg/l Cu treatment increasing Cu concentrations induce a decrease of Mn and Fe contents. It seems that high Cu concentrations act as antagonists of Mn and Fe accumulation in rice shoots. Also, since the shoot Mg concentrations were relatively constant within these treatments, it seems that in rice plants the mechanism(s) of Mg and Cu accumulation are not linked.

Data of Chl and carotenoid content from 30-day Cu-treated rice plants (Table 2, 3) showed that on a fresh weight basis, except for the deficiency treatment (0.002 mg Cu/l), both groups of pigments tend to decrease with increasing levels of Cu in the medium in a similar way. This result seems to agree with the

Table 1. *Concentrations of Cu, Mn, Fe and Mg in rice shoots*
Each value is the mean ± (S. E.) based on three replicates of three independent series.

Cu treatments (mg Cu/l)	Metal concentrations in rice shoots			
	Cu μg/g (dw) ± (S. E.)	Mn μg/g (dw) ± (S. E.)	Fe μg/g (dw) ± (S. E.)	Mg mg/g (dw) ± (S. E.)
0.002	17.5± (1.2)	797.5± (31.2)	355.0± (25.1)	6.0± (0.5)
0.01	21.5± (1.6)	725.0± (47.1)	377.5± (24.7)	5.6± (0.4)
0.05	27.0± (2.1)	810.0± (32.2)	492.5± (30.2)	5.6± (0.3)
0.25	46.5± (3.3)	767.5± (27.5)	335.0± (23.6)	5.3± (0.3)
1.25	95.0± (6.9)	255.0± (15.7)	106.5± (6.9)	5.1± (0.3)
6.25	508.0± (21.1)	77.0± (5.1)	88.5± (5.8)	6.5± (0.5)

Table 2. *Chlorophyll (Chl) contents of leaf tissues*
Each value is the mean ± (S. E.) based on three replicates of three independent series.

	Cu treatment (mg Cu/l)					
	0.002	0.01	0.05	0.25	1.25	6.25
Chl *a* mg/g (fw) ± (S. E.)	2.194± (0.20)	2.625± (0.19)	1.865± (0.15)	1.236± (0.11)	0.471± (0.03)	0.221± (0.02)
Chl *b* mg/g (fw) ± (S. E.)	0.773± (0.05)	0.831± (0.06)	0.706± (0.05)	0.471± (0.04)	0.179± (0.01)	0.097± (0.006)

works of Barua and Jana (1986), Sandmann and Bögar (1980), and Stiborová *et al.* (1986), but not with the work of Baszynski *et al.* (1982) on the effect of excess Cu on tolerant *Spinacea oleracea*. The pigment increase observed from the 0.002 to the 0.01 mg/l Cu treatments was pratically insignificant for total carotenoids but was more pronounced for Chl. On a fresh weight basis, the decrease of carotenoids was not identical for the different components (Table 3). Although α-carotene and zeaxanthin concentrations decreased progressively from the 0.002 mg/l Cu treatment up to the 6.25 mg/l Cu treatment, β-carotene, neoxanthin, anteroxanthin, lutein, lutein-5, 6-epoxyd, and violoxanthin concentrations decreased only after the 0.01 mg/l Cu treatment. On a Chl basis (Table 4), the concentration of α-carotene, β-carotene, violoxanthin, lutein, lutein-5, 6-epoxyd, antheroxanthin, neoxanthin, and zeaxanthin in isolated chloroplasts revealed that although the α-carotene concentration decreased after the 0.01 mg/l Cu treatment the zeaxanthin and lutein concentrations increased. The β-carotene concentration decreased from the 0.002 mg/l to the 6.25 mg/l Cu treatment. The neoxanthin concentration decreased up to the 0.25 mg/l Cu treatment, but higher concentrations resulted in β-carotene increase up to the 6.25 mg/l Cu treatment. Lutein -5, 6-epoxyd, anteroxanthin and violoxanthin all increased when toxin Cu levels were present in the nutrient solution.

The isoprenoid biosynthetic system produces carotenoids, Chl, gibberellins, sterols and several quinones that are required for growth and photosynthesis (Wilkinson and Ohki, 1988). Our data (Table 5) clearly indicate that in long-term experiments increasing Cu concentrations affects the isoprenoid biosynthetic system of rice plants. This observation partly agrees with the suggestion of Droppa *et al.* (1984). Indeed, on a fresh

Table 3. *Carotenoid contents on a fresh weight basis*
Each value is the mean ± (S. E.) based on three replicates of three independent series.

Carotenoids [mg/kg (fw)] ± (SE)	Cu treatment [mg Cu/l]					
	0.002	0.01	0.05	0.25	1.25	6.25
Neoxanthin	29.25± (2.5)	32.26± (2.9)	23.97± (1.8)	13.80± (1.1)	7.27± (0.5)	3.97± (0.3)
Violoxanthin	22.01± (2.2)	24.76± (2.3)	21.50± (2.0)	11.19± (1.0)	9.57± (0.7)	6.71± (0.4)
Lutein-5,6-epoxyd	22.85± (2.1)	24.63± (1.8)	22.07± (1.9)	11.30± (1.1)	9.85± (0.9)	6.91± (0.5)
Anteroxanthin	23.40± (2.2)	26.62± (2.4)	23.12± (2.3)	11.84± (1.0)	10.18± (0.8)	7.46± (0.6)
Zeaxanthin	92.00± (8.5)	91.89± (8.6)	76.48± (6.8)	51.88± (4.4)	29.70± (1.4)	24.51± (2.3)
Lutein	93.70± (8.9)	96.50± (7.5)	80.98± (7.4)	54.80± (5.1)	30.16± (2.7)	24.83± (2.1)
β-carotene	80.10± (7.6)	92.13± (6.9)	66.69± (6.2)	42.23± (4.1)	14.15± (1.2)	5.62± (0.3)
α-carotene	69.01± (6.3)	60.36± (5.4)	58.47± (5.1)	37.81± (3.2)	13.44± (1.3)	5.14± (0.4)
Total	432.32	449.15	373.28	234.85	124.32	85.15

Table 4. *Carotenoid contents on a chlorophyll (Chl) basis*
Each value is the mean±(S. E.) based on three replicates of three independent series.

Carotenoids [μg/mg (Chl)] ± (SE)	Cu treatment [mg Cu/l]					
	0.002	0.01	0.05	0.25	1.25	6.25
Neoxanthin	9.86± (0.6)	9.34± (0.7)	9.32± (0.8)	8.09± (0.6)	11.18± (1.0)	12.49± (1.1)
Violoxanthin	7.42± (0.5)	7.16± (0.6)	8.36± (0.7)	6.55± (0.6)	14.70± (1.2)	21.13± (1.8)
Lutein-5,6-epoxyd	7.70± (0.7)	7.13± (0.7)	8.58± (0.8)	6.62± (0.6)	15.13± (1.4)	21.75± (2.1)
Anteroxanthin	7.88± (0.7)	7.70± (0.7)	8.99± (0.7)	6.94± (0.6)	15.64± (1.4)	23.48± (2.2)
Zeaxanthin	31.01± (3.1)	26.59± (2.5)	29.75± (2.8)	30.40± (3.0)	45.64± (4.5)	77.20± (7.2)
Lutein	31.58± (3.1)	27.92± (2.7)	31.50± (3.0)	32.11± (3.1)	46.35± (4.2)	78.21± (7.2)
β-carotene	26.99± (2.4)	26.66± (2.5)	25.94± (2.4)	24.74± (2.3)	21.74± (1.9)	17.70± (1.5)
α-carotene	23.26± (2.2)	23.48± (2.1)	22.74± (1.9)	22.15± (2.1)	20.66± (2.0)	16.18± (1.4)
Total	145.70	135.98	145.18	137.60	191.04	268.14

weight basis, a 15-fold decrease in PTF was observed as well as an 8-fold decrease in PT following an increase in Cu from 0.002 to 6.25 mg/l Cu (Table 5). Because PT synthetase activity specifically requires Mn (Maudinas *et al.*, 1977; Wilkinson and Ohki, 1988) and because Mn concentration in rice shoots showed a sharp decrease between the 0.002 and the 6.25 mg/l Cu treatments (Table 1), the isolated protoplasts were incubated for 4 h with increasing Mn concentrations without any further addition of geranylgeranyl pyrophosphate. PT and PTF content increased in the Cu -treated protoplasts within the range of 50-250 μM Mn (Table 6). It can be concluded, therefore, that Mn-deficiency in rice shoots, triggered by excess Cu, limits PT synthetase activity much more than its substrate concentration. Indeed, it is particularly remarkable that high activities of this enzyme were found in the 1.25 mg/l Cu treated protoplasts. However, it must be pointed out that Mn concentrations below and above the 50-250 μM Mn range limit this enzyme activity.

The PChl (ide) content of the 30-day Cu-treated rice plants showed a 2.5 fold decrease on a fresh weight basis between the 0.01 and the 6.25 mg/l Cu treatment (Table 5). Incubation of isolated protoplasts from all treatments showed that varying the Fe supply in the range of 4-6 mM increased the PChl (ide) concentration particularly in the 0.25 and 1.25 mg/l Cu treatments (Table 7). This observation is similar to the report by Castelfranco (1983). However, higher Fe concentrations decreased PChl (ide) concentrations. From these data it can be concluded that Fe deficiency induced by excess Cu specifically inhibits PChl (ide) synthesis.

Discussion

It has been shown that carotenoid accumulation and Chl synthesis are related (Frosch and Mohr, 1980; Malhotra *et al.*, 1982; Misra and Biswal, 1981), since both groups of pigments share the isoprenoid biosynthetic pathway (Goodwin, 1965; Wilkinson and Ohki, 1988). Although several authors (Fernandes and Henriques, 1991) have reported that a peroxidation mechanism triggered by excess Cu can induce Chl destruction, which in turn leads to a decrease of carotenoids, our data indicate that carotenoid synthesis is also indirectly affected by the high Cu treatments. Between the 0.05 and the 6.25 mg/l Cu treatments, the Fe content of the shoot showed a 5-fold decrease (Table 1) and the amount of PChl (ide) showed a 2.5 fold decrease between the 0.01 and the 6.25 mg/l Cu treatment (Table 5). Yet, a partial recovery in the PChl (ide) concentration occurred in isolated protoplasts incubated with 4-6 mM Fe especially above the 0.05 mg/l Cu treatment (Table 7). As Chl biosynthesis requires Mg and Fe (Castelfranco, 1983), we suggest that although the shoot Mg concentration was not limiting for Chl synthesis, this process must be inhibited at the conversion of Mg -protoporphyrin IX (6-methyl ester) to PChl (ide). Indeed, as isocyclic ring formation *in vivo* requires Fe (Castelfranco, 1983), the observed antagonism between Cu and Fe contents in rice shoots must, at least partly, induce this inhibition. Between the 0.05 and the 6.25 mg/l Cu treatments the shoot Mn content showed a 10 -fold decrease (Table 1) and PT and PTF contents also decreased (Table 5). Because a partial recovery of PT and PTF concentrations occurred in isolated protoplasts incubated with 50-250 μM Mn (Table 6), we suggest that Mn-deficiency, triggered by excess Cu, induced on a fresh weight basis the decrease of carotenoids (Table 3) through the inhibition of PT synthetase activity, as reported by Knotz *et al.* (1977) and Maudinas *et al.* (1977). Additionally, as the shoot Mg content did not vary significantly with the different

Table 5. *Concentrations of PTF, PT and PChl (ide) in rice shoots tissues*
Each value is the mean ± (S. E.) based on three replicates of three independent series.

Cu treatments (mg/l)	PTF μg/g (fw) ± (S. E.)	PT μg/g (fw) ± (S. E.)	PChl (ide) Abs_{639}/10 mg Protein ± (S. E.)
0.002	15.46 ± (1.4)	45.21 ± (4.1)	0.32 ± (0.05)
0.01	14.77 ± (1.1)	44.89 ± (4.2)	0.51 ± (0.07)
0.05	14.19 ± (1.7)	44.11 ± (3.9)	0.42 ± (0.06)
0.25	8.46 ± (0.9)	31.59 ± (2.7)	0.29 ± (0.04)
1.25	4.73 ± (0.5)	22.07 ± (2.1)	0.25 ± (0.04)
6.25	1.08 ± (0.2)	5.91 ± (0.7)	0.20 ± (0.04)

Table 6. *Concentrations of PTF and PT in rice protoplasts after 4 h incubation with varying Mn concentration*
Each value is the mean ± (S. E.) based on three replicates of three indenpendent series.

Cu treatments (mg Cu/l)	Mn incubation (μM)	PTF/content mg/g (protein) ± (S. E.)	PT content mg/g (protein) ± (S. E.)
	0.0025	0.805± (0.09)	2.015± (0.22)
	0.025	0.808± (0.08)	2.110± (0.21)
	25	1.007± (0.10)	2.244± (0.19)
0.002	50	1.092± (0.12)	2.305± (0.25)
	250	1.077± (0.12)	2.219± (0.28)
	1000	0.281± (0.17)	0.557± (0.07)
	1500	0.197± (0.11)	0.220± (0.03)
	0.0025	1.221± (0.15)	2.738± (0.24)
	0.025	1.343± (0.14)	2.838± (0.22)
	25	1.302± (0.14)	3.009± (0.27)
0.01	50	1.395± (0.25)	3.041± (0.28)
	250	1.398± (0.19)	3.051± (0.27)
	1000	0.740± (0.09)	0.768± (0.06)
	1500	0.242± (0.03)	0.164± (0.02)
	0.0025	0.702± (0.08)	2.031± (0.19)
	0.025	0.501± (0.06)	2.059± (0.18)
	25	0.774± (0.09)	2.269± (0.19)
0.05	50	0.929± (0.17)	2.435± (0.21)
	250	0.869± (0.18)	2.615± (0.27)
	1000	0.630± (0.07)	0.366± (0.05)
	1500	0.338± (0.04)	0.204± (0.03)
	0.0025	0.936± (0.09)	2.348± (0.21)
	0.025	0.903± (0.10)	2.367± (0.25)
	25	1.006± (0.09)	2.536± (0.24)
0.25	50	3.247± (0.35)	3.490± (0.29)
	250	2.381± (0.24)	3.395± (0.31)
	1000	0.730± (0.08)	0.332± (0.05)
	1500	0.381± (0.05)	0.185± (0.02)
	0.0025	1.768± (0.12)	4.497± (0.35)
	0.025	1.710± (0.13)	4.556± (0.37)
	25	1.916± (0.14)	4.971± (0.39)
1.25	50	6.091± (0.31)	6.870± (0.44)
	250	4.273± (0.30)	5.943± (0.40)
	1000	1.077± (0.10)	1.076± (0.09)
	1500	0.609± (0.07)	0.687± (0.05)
	0.0025	0.735± (0.08)	1.896± (0.15)
	0.025	0.910± (0.09)	1.738± (0.14)
	25	0.909± (0.08)	2.040± (0.17)
6.25	50	1.172± (0.10)	3.236± (0.24)
	250	0.913± (0.08)	2.875± (0.22)
	1000	0.445± (0.05)	0.736± (0.08)
	1500	0.127± (0.02)	0.339± (0.04)

Table 7. *Concentrations of PChl (ide) in rice protoplasts after 4 h incubation with varying Fe concentrations*

Each value is the mean ± (S. E.) based on three replicates of three independent series.

Cu treatments (mg Cu/l)	Fe incubation (mM)	PChl (ide) contents Abs_{639}/10 mg protein ± (S. E.)
	2	0.23 ± (0.05)
	4	0.25 ± (0.04)
0.002	6	0.21 ± (0.04)
	8	0.18 ± (0.03)
	10	0.21 ± (0.03)
	12	0.12 ± (0.03)
	2	0.42 ± (0.07)
	4	0.47 ± (0.08)
0.01	6	0.47 ± (0.06)
	8	0.39 ± (0.06)
	10	0.23 ± (0.05)
	12	0.17 ± (0.04)
	2	0.24 ± (0.04)
	4	0.27 ± (0.05)
0.05	6	0.26 ± (0.06)
	8	0.21 ± (0.03)
	10	0.19 ± (0.03)
	12	0.15 ± (0.03)
	2	0.18 ± (0.04)
	4	0.29 ± (0.05)
0.25	6	0.25 ± (0.05)
	8	0.21 ± (0.04)
	10	0.21 ± (0.04)
	12	0.15 ± (0.03)
	2	0.17 ± (0.03)
	4	0.29 ± (0.04)
1.25	6	0.28 ± (0.04)
	8	0.16 ± (0.03)
	10	0.17 ± (0.03)
	12	0.12 ± (0.03)
	2	0.13 ± (0.03)
	4	0.19 ± (0.03)
6.25	6	0.15 ± (0.02)
	8	0.11 ± (0.02)
	10	0.09 ± (0.03)
	12	0.09 ± (0.02)

treatments while the Mn contents showed a sharp decrease (Table 1), it is possible that the increased ratio of Mg:Mn may also facilitate the inhibition of PT synthetase, thus providing a further block of carotenoid biosynthesis above the 0.05 mg/l Cu treatment, as already reported by Wilkinson and Ohki (1988).

Droppa *et al.* (1984), working with *Beta vulgaris* submitted to Cu deficiency, suggested that the effect of this metal on photosynthetic pigment synthesis is indirect and at the terpenoid biosynthesis level. This seems to support our data. Indeed, from our data, we draw the general conclusion that under Cu excess, Chl and carotenoid contents are, at least, partly controlled by Fe, Mn and Cu interactions. It has long been recognized that different plant nutrients show complex interactions which influence their availability (Olsen, 1972). Thus our results offer evidence that in rice Fe and Mn deficiency triggered by Cu excess blocks PChl and PT synthesis, therefore, decreasing the contents of photosynthetic pigments.

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銅過量對於水稻光合作用色素之影響

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水稻(*Oryza sativa* L.)於含銅濃度 0.002 mg/l 至 6.25 mg/l 之水耕液培養 30 天後，對其莖葉內所含的銅、錳、鐵、鎂、以及光合作用色素進行測量。當水耕液銅濃度增加時，枝葉之含銅濃度亦增加，但是枝葉之錳、鐵濃度在水耕液銅濃度高於 0.05 mg/l 以上時，即逐漸降低。含鎂濃度則不呈現明顯變化。除了過低濃度(0.002 mg/l)外，單位鮮重之葉綠素及類胡蘿蔔素會隨著水耕液銅濃度之增加而降低。前述之水稻枝葉以及經錳、鐵處理過的水稻原生質體，其所含的 phytoene， phytofluene 及 protochlorophyll (ide)亦被分析。枝葉之 phytoene 及 phytofluene 濃度隨著銅濃度增加而下降，但是原生質體之 phytoene 及 phytofluene 在錳濃度 50 μM 至 250 μM 範圍內呈現增加。枝葉之 protochlorophyll (ide)在銅濃度 0.01 mg/l 以上會下降。原生質體之 protochlorophyll (ide)濃度在鐵濃度 4 mM 至 6 mM 之間呈現增加。前述色素含量之降低，可從鐵、錳在葉綠素及類胡蘿蔔素生合成中扮演的角色得到解釋。