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Physiological Effects of Copper on Some Biochemical Parameters in *Zea mays* L. Seedlings

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Abstract: Growth parameters and biochemical changes were studied in roots and leaves of 15 day old maize grown in a nutrient solution containing various copper concentrations (0, 25, 50, 75 and 100 µM). An accumulation of H_2O_2 was observed in roots and shoots. The leaf chlorophyll a, b and carotenoid contents decreased with increasing Cu concentration. The results demonstrated adverse effects of Cu on N metabolism and plant growth. Cu exposure elevated Cu concentration and decreased Nitrate Reductase (NR) activity in the roots and shoots. However, Cu exposure increased total free amino acid content in the leaves.

Key words: Copper, *Zea mays*, nitrate, nitrate reductase, free amino acid, chlorophyll

INTRODUCTION

Plants take up Cu and its accumulation has been reported in leaves, stems, fruits, seeds and roots which increases with increase in the exogenous Cu level (Pinochet *et al.*, 1999; Xiong and Wang, 2005). Although Cu is an essential micronutrient for normal plant metabolism, playing an important role in a large number of metalloenzymes, photosynthesis-related plastocyanin and membrane structure, it has been reported to be among the toxic heavy metals (Wong and Bradshaw, 1982; Li and Xiong, 2004). Excessive Cu accumulated in plant tissue can be toxic to plants, affecting several physiological and biochemical processes and growth (Fernandes and Henriques, 1991). Copper treatment brings change in nitrogen metabolism with a reduction of total nitrogen (Llorens *et al.*, 2000). Cu exposure results in increase of free amino acids (Mazen, 2004) and inhibition of nitrate reductase activity (Luna *et al.*, 1997). The presence of Cu significantly decreases chlorophyll content and inhibits growth (Ralph and Burchett, 1998; Fargasova, 2001).

Most of the *in vitro* studies using isolated chloroplasts or excised leaves have reported a direct effect of copper on the photosynthetic electron transport chain (Sandman and Böger, 1980; Szalontai *et al.*, 1999). PSII seems to contain binding sites for excess Cu^{2+} both on the oxidizing and reducing side (Patsikka *et al.*, 2001).

The aim of this study was to investigate the effect of copper toxicity on nitrate reductase activity, H_2O_2, protein, nitrate, chlorophyll contents and level of hill reaction in Cu-treated maize plant.

MATERIALS AND METHODS

Seeds of *Zea mays* L. were surface sterilized with 0.1% $HgCl_2$ for 10 min, rinsed thoroughly with running water and germinated in a greenhouse on moist filter paper in Petri dishes for 3 days. The seedlings were randomly placed in polyethylene pots (10 plants per pot) filled with 300 mL of a non-aerated modified Hoagland's nutrient solution (Ouzounidou *et al.*, 1994).

A randomized block, experimental design with five Cu treatments (0, 25, 50, 75 and 100 µM Cu) and three replicates was used. Copper was supplied as $CuSO_4.5H_2O$. The plants were grown for 15 days in a growth chamber (16 h light/8 h dark) under a light intensity of 15 W m^{-2} at plant level with a day/night temperature regime of 27/22°C and relative humidity of 85% (Ouzounidou *et al.*, 1994). After 15 days, grown seedlings were taken as the reference material for the experiments done in the presence of added copper.

The H_2O_2 level was colorimetrically measured as described by Jana and Choudhuri (1981).

The soluble protein content was colorimetrically measured as described by the method of Bradford (1976). Protein levels were expressed as mg g^{-1} fresh weight.

The chlorophyll a, b and carotenoid content were colorimetrically measured as described by Dere *et al.* (1998). The amount of these pigments was calculated according to the formulas of Lichtenthaler and Wellburn (1985).

The nitrate content, nitrate reductase (EC 1.6.6.1) activity and total free amino acid content were colorimetrically measured as described by Xiong *et al.* (2006).

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The PSII electron transfer activity (hill reaction) was colorimetrically measured as described by Patsikka *et al.* (2001). Photoinhibition was monitored by measuring the rate by which the reduce dichlorophenol-indophenol (DCPIP).

All experiments process was conducted in the Urmia university plant physiology laboratory (of September to November 2006).

RESULTS

A significant reduction in shoot and root soluble proteins were observed in Cu-treated plants (Fig. 1).

Figure 2 shows the endogenous H_2O_2 level roots and shoots of maize seedlings in the presence and absence of $CuSO_4$. It is clear that $CuSO_4$-treated roots in 100 μm concentration of Cu had higher H_2O_2 level than controls plant and shoots of other cu-treated plant.

Present results demonstrated Cu adverse effects on N metabolism. Cu exposure decreased nitrate content (Fig. 3) and nitrate reductase activity (Fig. 4) but total
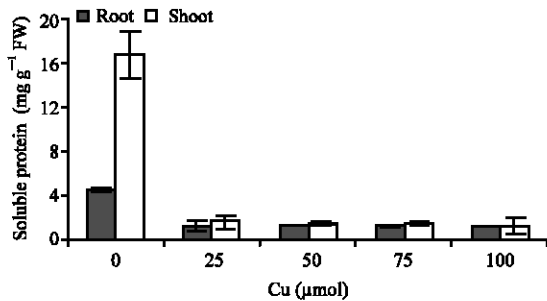

Fig. 1: Changes in soluble protein content in shoots and roots of the maize plant in the different Cu concentrations. Vertical bars represent standard errors
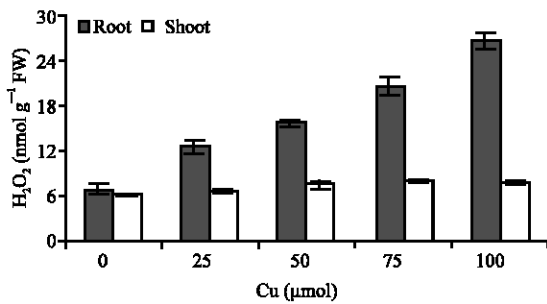

Fig. 2: Changes in H_2O_2 content in shoots and roots of maize plant in the different Cu concentrations. Vertical bars represent standard errors
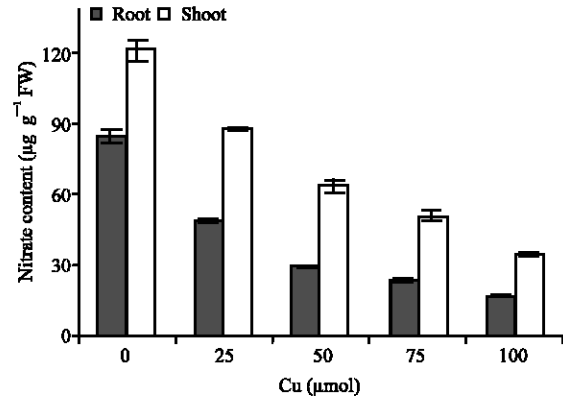

Fig. 3: Changes in nitrate content in shoots and roots of maize in the different Cu concentrations. Vertical bars represent standard errors
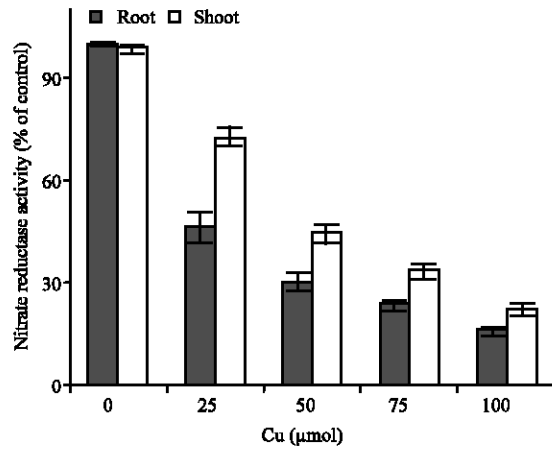

Fig. 4: Changes in nitrate reductase activity in shoots and roots of maize in the different Cu concentrations. Vertical bars represent standard errors
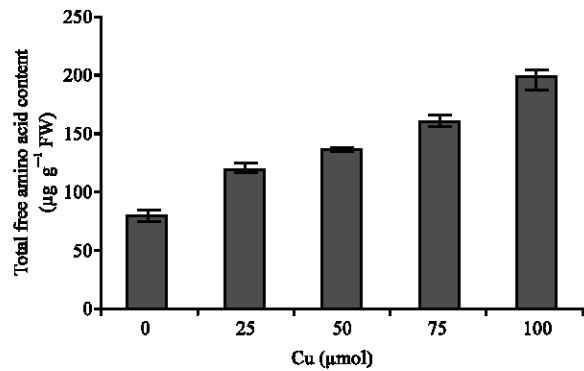

Fig. 5: Changes in total free amino acids content in shoots of maize in the different Cu concentrations. Vertical bars represent standard errors
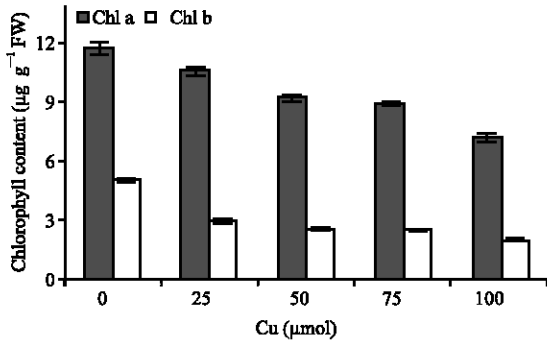
Fig. 6: Changes in total chlorophyll (a and b) content in leaves of maize in the different Cu concentrations. Vertical bars represent standard errors
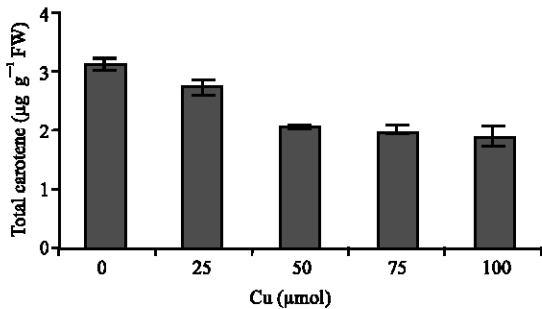

Fig. 7: Changes in total carotene content in leaves of maize plant in the different Cu concentrations. Vertical bars represent standard errors
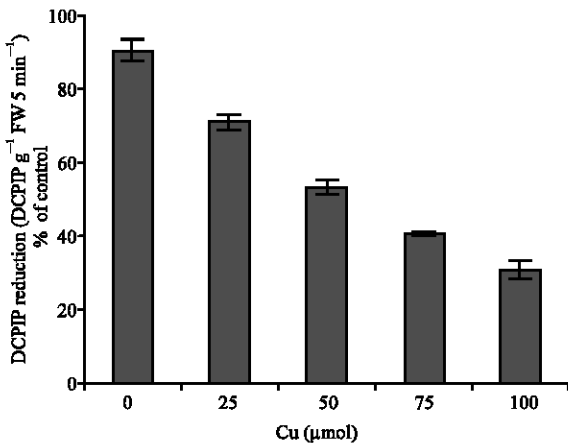

Fig. 8: Changes in DCPIP reduction in leaves of maize in the different Cu concentrations. Vertical bars represent standard errors

free amino acids content in the leaves increased by copper exposure (Fig. 5), indicating a role of free amino acids in detoxification of copper stress.

The shoots were significantly affected by Cu, which resulted in a decline of pigment content. The chlorophyll

a and b (Fig. 6) and carotenoid content (Fig. 7) were decreased in Cu-treated plants. Effect of Cu on the DCPIP reduction shows in Fig. 8. DCPIP reduction decreased with increasing Cu concentrations. It is clear that DCPIP reduction in 100 µM Cu-treated plants had about 70% lower than controls plant.

DISCUSSION

Cu^{2+} is required by biological systems as a structural and catalytic enzyme component and in the soil Cu^{2+} can be a stress factor by causing physiological responses that can decrease the vigour of the plants and inhibit plant growth (Ouzounidou, 1994).

The soluble protein content in plant cells is an important indicator of their physiological state. The reduced amount of soluble protein in the organs of the Cu-treated maize plants was most probably a result of the reduced biosynthesis or the accelerated catabolic processes, as well. This suggestion is also confirmed by Vassilev (1997) in his study on Cd and Stoeva and Bineva (2003) in their study on As (Arsenic), according to whom, the protein degradation to amino acids is in fact an adaptation of the cells to the carbohydrate deficiency. On the other hand, the accelerated catabolism is probably due to the considerable disturbances in the membrane systems, in response to the metal phytotoxicity. Present results demonstrated Cu adverse effects on N metabolism. Cu exposure decreased nitrate reductase activity and total chlorophyll content. Total free amino acids content in the leaves increased by copper exposure, indicating a role of free amino acids in detoxification of copper stress. The results suggested that Cu toxicity to the plant was at least partly via influence on N metabolism. Reduction of nitrate to nitrite catalyzed by Nitrate Reductase (NR) is considered the rate-limiting step in the assimilation of nitrate (Campbell, 1999), which eventually affects the growth and organic nitrogen status of the plants. Another direct effect of Cu exposure on NR activity decrease may lie in an increase in the enzyme breakdown induced by toxic oxygen species generated during Cu stress. Free radicals can cause the breakdown of proteins by oxidative reaction or by increasing proteolytic activity (Salo *et al.*, 1990). It suggests that Cu-mediated generation of reactive oxygen species (e.g., H_2O_2, $O_2^{\bullet}$ or $OH^{\bullet}$) could have occurred. These radicals could attack nitrate reductase and cause a decrease of its activity. In addition, NR activity decrease under Cu exposure may involve the damage of Cu to the plants' photosynthesis. In present experiments, the chlorophyll content was significantly reduced by Cu treatment. This suggests that the plants' photosynthesis could have been damaged

because of the adverse effects of Cu on chlorophyll and thus supports the possibility of an indirect effect of photosynthesis damage on NR activity by Cu exposure. Our experiments indicated that NR activity in leaves was much higher than that in the roots. Two possibilities may be considered to explain these observations. The first may lie in a concentration effect of Cu the higher the Cu concentration in the roots the more serious would be the inhibition of the enzyme. The second may suggest that the reduction process from nitrate to nitrite catalyzed by NR has occurred mainly in leaves (Xiong *et al.*, 2006).

The photosynthetic pigments are some of the most important internal factors, which in certain cases are able to limit the photosynthesis rate. It was established that total carotene (Car) decreased to a lesser extent than Chl. The decreased Chl and Car may be due to the fact that there is an oxidative stress, which is a marker of the tissue aging, as result of the stress factors of the environment (Hendry and Grime, 1993). A reduction of the pigment content in the case of increasing levels of heavy metals and metalloids, was also established by Bogoeva (1998). In the present study, Cu treatment also was found to reduce total chlorophyll content. This may result from chlorophyll degradation and chlorophyll synthesis inhibition due to Cu-induced inhibition of aminolaevulinic acid dehydratase (ALA-dehydrates) (Fernandes and Henriques, 1991). Also, chlorophyll content decline could result from an indirect adverse effect of Cu exposure on N assimilation of the plants, since biosynthesis of chlorophylls is dependant on N supply from the plants' N assimilation. In this respect, Cu exposure could cause decrease of N assimilation, thus leading to a decline in chlorophyll content.

Free-radical damage induced by heavy metals by maintaining a more reducing environment in plant cells (Surasak *et al.*, 2002). Hydrogen peroxide (H_2O_2) is a constituent of oxidative plant metabolism. It is a product of peroxisomal and chloroplastic oxidative reactions (Del Rio *et al.*, 1992). H_2O_2 itself is an active oxygen species. H_2O_2 can also react with superoxide radicals to form more reactive hydroxyl radicals in the presence of trace amounts of Fe or Cu (Thompson *et al.*, 1987). The hydroxyl radicals initiate self-propagating reactions leading to peroxidation of membrane lipids and destruction of proteins (Asada and Takahashi, 1987; Bowler *et al.*, 1992; Halliwell, 1987).

Copper is essential for electron transport not only by being part of plastocyanin, but probably also as a stabilizer of the lipid environment close to electron carrier of PSII complex (Patsikka *et al.*, 2001). However, excess Cu^{2+} has also harmful effects on the donor and acceptor side of PSII (Yruela *et al.*, 1996). Cu^{2+}-induce loss of PSII activity is enhanced by light which suggests involvement of photoinhibition (Patsikka *et al.*, 1998). The ability of an exogenous electron donor to partially restore PSII activity lost by illumination of thylakoids in the presence of Cu^{2+} suggests that the damage started at the donor side of PSII (Patsikka *et al.*, 2001). In the absence of electron donation from oxygen-evolving complex, the long lived P680* oxidizes nearby components of PSII (Bumann and Oesterhelt, 1995). Furthermore Jegerschod *et al.* (1995) have suggested that the binding of the Cu^{2+} near Y_z would slow down the reduction of the oxidized primary electron donor, P680*. When the plants are grown under copper additions, the inhibitory effect of Cu^{2+} on the photosynthetic machinery is cumulative in the course of time and the extent of inhibition may also depend on the physiological stage of the leaf studied (Maksymiec and Baszynsky, 1999).

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