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Cu-ions mediated changes in growth, chlorophyll and other ion contents in a Cu-tolerant *Koeleria splendens*

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Abstract

The effects of Cu^{2+} on growth, chlorophyll and other ion contents of *Koeleria splendens* originated from Cu-contaminated soil have been investigated in nutrient solution. The most evident Cu^{2+} effects concern the root growth, especially the root length. Since in plants grown under lower Cu^{2+} concentrations (4 and 8 μM) root elongation, biomass, chlorophyll, Mg^{2+}, Fe^{2+}, Ca^{2+} and K^+ content were increased compared with the control, the development of an adaptive mechanism of *K. splendens* to Cu^{2+} is suggested. High Cu^{2+} concentration (160 μM) caused a significant reduction in root length and biomass as well as a decreased rate of chlorophyll biosynthesis. The reduction of growth can be correlated with the toxic effect of Cu^{2+} on photosynthesis, root respiration and protein synthesis in roots. 160 μM Cu^{2+}-treatment had a negative influence on the concentrations of Ca^{2+}, Fe^{2+}, Mg^{2+} and K^+ and a positive influence on the Cu^{2+} concentration in the plant tissues. Loss of nutrients similar to the senescence response suggests that excess of Cu^{2+} leads to the progressive senescence of the plants. Our results demonstrate the existence of an adaptive mechanism of *K. splendens* under low Cu^{2+} concentrations, while high Cu^{2+} quantities cause disturbances in plant function.

Key words: calcium, copper, iron, magnesium, potassium, root biomass, root length

Introduction

Copper, as essential biological element, interferes with numerous physiological functions. It is a constituent micronutrient of the protein component of several enzymes, mainly of those participating in electron flow, catalysing redox reaction in mitochondria, chloroplasts, cell wall and in the cytoplasm of plant cells (*e.g.* Lolkema and Vooijs 1986). However, excessive concentrations can be toxic to plants (Jensen and Adalsteinsson 1989, De Vos *et al.* 1991). The term "toxic concentration" is used in the literature for a heavy metal concentration that significantly inhibits metabolic activity without inducing plant death. Intact plants exposed to this

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concentration stay alive, but with decreased growth and development (Clijsters and Van Assche 1985).

Cu-ions may be actively absorbed, absorbed via passive diffusion due to ion activity gradients, or even inadvertently accumulated by an active mechanism by which a macronutrient cation is generally absorbed. Their toxicity can induce deficiency of other essential elements and as a consequence inhibition of element-ion dependent reactions (Ouzounidou *et al.* 1992). Copper at concentrations higher than 1 μM is a potent inhibitor of photosynthetic electron transport and is responsible for the degradation of the chloroplast inner structure and the decreased pigment content (Baszynski *et al.* 1988, Mohanty *et al.* 1989, Ouzounidou 1994a). Well-known Cu-harmful effects are inhibition of growth and alteration of the plasma membrane permeability (Ouzounidou *et al.* 1992).

Many experiments on the effect of heavy metals in plants have been undertaken in fields in which stress conditions may elicit more pronounced effects than a single stressor in a controlled environment. The objective of this study was to determine the effect of copper on growth, pigment and minerals level of *K. splendens* plants, originated from a Cu-rich soil, in controlled laboratory conditions.

Materials and methods

Plant material and cultivation: Seeds of *Koeleria splendens* C. Presl. (*Gramineae*) were collected from a metalliferous area, Gerakario, Kilkis, N. Greece. The area is characterized by Cu-Pb-Zn mineralization.

Seeds were sown on garden soil and a month after germination seven plants were transplanted to each polyethylene pot containing 300 cm^3 of nutrient solution with the following composition: KNO_3 (0.6 mM), $Ca(NO_3)_2$ (0.4 mM), $NH_4H_2PO_4$ (0.2 mM), $MgSO_4$ (0.1 mM), KCl (50 μM), H_3BO_4 (25 μM), FeNaEDTA (20 μM), $MnSO_4$ (2 μM), $ZnSO_4$ (2 μM), $CuSO_4$ (0.5 μM), and $(NH_4)_6Mo_7O_{24}$ (0.5 μM), (initial pH 5.5 ± 0.1). The growth solution also contained Cu^{2+} at different concentrations (0, 4, 8, 16, 32, 80 and 160 μM $CuSO_4$). Solutions were renewed three times weekly. All pots (seven treatments, in three replicates in time) were placed in a growth chamber with 80 ± 5% relative humidity, 16 h photoperiod (irradiance 15 W m^{-2}) and temperature of 25 ± 1 °C. After a 15-d period of Cu^{2+} treatment plants were harvested and root length and fresh mass of plants were measured.

Element analysis: Fifteen days after exposure to $CuSO_4$, plants were divided into shoots and roots. Roots were washed in distilled water and all samples were dried for 24 h at 80 °C. Cu^{2+}, Ca^{2+}, K^+, Mg^{2+} and Fe^{2+} were determined by atomic absorption spectrophotometry (*Perkin Elmer 2390*), after wet-digesting in HNO_3-$HClO_4$ as described by Ouzounidou *et al.* (1992).

Chlorophyll determination: Chlorophyll was extracted in 80 % (v/v) acetone. Absorbance was measured at 663 and 645 nm using an *LKB Ultraspec II* spectrophotometer. For details see Ouzounidou *et al.* (1992).

Statistics: The experiment was set up in a completely randomized design with 3 replications. The significant difference between the treated and control samples was analysed by Student's t-test. The values of growth analysis presented here are means of 21 measurements, while the values of chlorophyll and elemental content are means of 3 measurements.

Results

Root growth: Exposure of seedlings to Cu^{2+} influenced root elongation dependent upon the Cu^{2+} supply (Table 1). Remarkably, in 4 and 8 μM of Cu^{2+} *K. splendens* exhibited optimal growth displaying root length by about 64 and 20 % greater than the control (18.7 cm, $P < 0.05$). Exposure to 32 and 80 μM of Cu^{2+} resulted in an inhibition of root elongation. In the higher Cu^{2+} concentration (160 μM) a strong depression, by about 45 % of the control ($P < 0.05$), of relative root length (RRL) was observed. Relative root biomass (RRB) changed under the various Cu^{2+} concentrations in the nutrient solution (Table 1). 4 and 8 μM of Cu^{2+} induced a significant increase in RRB by 52 and 30 % of the control [410 mg(f.m.) plant^{-1}], while higher Cu^{2+} treatment (160 μM) decreased the RRB by 33 % of the control ($P < 0.05$).

After 15 d of 160 μM Cu^{2+} treatment there was a decrease of surviving seedlings by about 17 % of the control.

Table 1. Chlorophyll content (mean ± S.E.), and relative root length (RRL) and relative root biomass (RRB) expressed in % of the control of *Koeleria splendens* plants grown in various Cu^{2+} concentrations.

Cu [μM]	Chlorophyll content [mg g^{-1}(f.m.)]	RRL [%]	RRB [%]
0	1.690 ± 0.05	100	100
4	2.540 ± 0.04	164	152
8	2.480 ± 0.07	120	130
16	2.100 ± 0.06	95	85
32	1.900 ± 0.05	72	81
80	1.445 ± 0.03	60	78
160	1.100 ± 0.03	55	67

Chlorophyll content: Remarkable changes in leaf chlorophyll (*a+b*) of *K. splendens* plants after 15 d exposure to various Cu^{2+} concentrations were observed (Table 1). In 4, 8 and 16 μM of Cu^{2+} chlorophyll concentrations were higher than the control by about 50, 46 and 24 %, respectively ($P < 0.05$). Symptoms of Cu^{2+} toxicity, like chlorosis and necrosis of the leaves, were observed only in the higher Cu^{2+} concentration (160 μM). In this concentration chlorophyll content revealed a reduction equal to 35 % of the control (Table 1, $P < 0.005$).

Elemental status: After 15-d exposure to various Cu^{2+} concentrations the total amount of copper taken up and translocated was lower in shoots than in roots of *K. splendens* seedlings (Fig. 1*A*). The root Cu^{2+} content in the solution containing 160 μM of Cu^{2+} was about 15 times higher than in the above-ground parts. The application of increasing Cu^{2+} concentrations in the growth solution induced changes

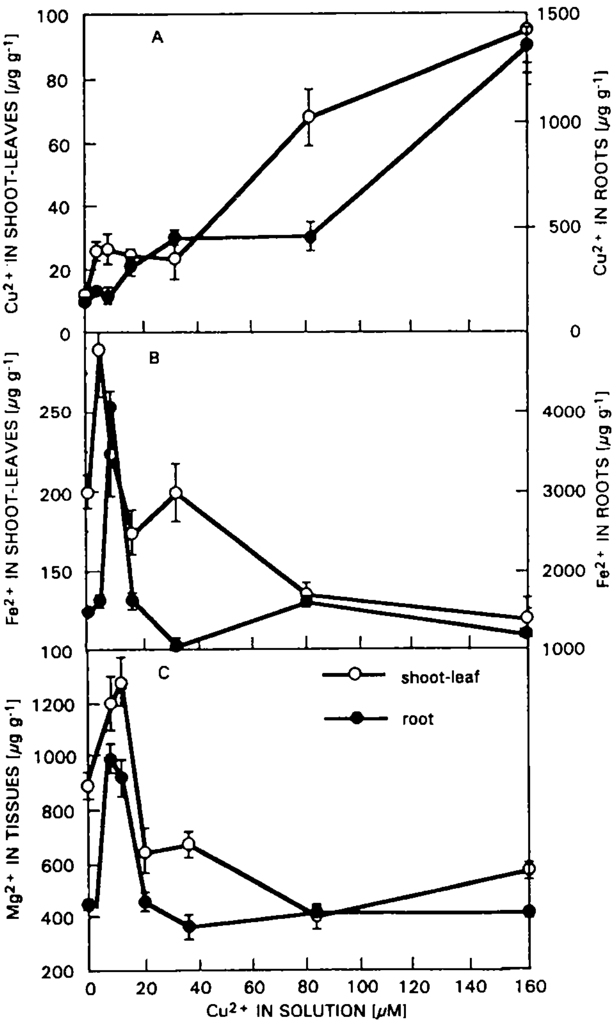

Fig. 1. Cu^{2+} (*A*), Fe^{2+} (*B*) and Mg^{2+} (*C*) concentrations in roots and shoots of *K. splendens* plants as a function of various Cu^{2+} concentrations. Values are means of 3 replicates, S.E. is given by vertical bars.

on elemental distribution in roots and shoots of the plants. The Fe^{2+} concentration in both roots and shoots increased during low Cu^{2+} treatments (4 and 8 μM Cu^{2+}) (Fig. 1*B*). The highest Fe^{2+} concentrations occurred in 4 and 8 μM of Cu^{2+} for shoots and roots, respectively. On exposure to 160 μM of Cu^{2+}, Fe^{2+} content was decreased by 60 and 20 % in shoots and roots, respectively, compared to the control ($P < 0.05$). Similar pattern was observed for Mg^{2+} concentration in plant tissues (Fig. 1*C*). Low Cu^{2+} treatments (4 and 8 μM) enhanced Mg^{2+} content by 2 and 1.5 times in roots and shoots, respectively. However, 160 μM Cu^{2+} supply induced Mg^{2+} reduction both in roots and shoots by 9 and 36 %, respectively.

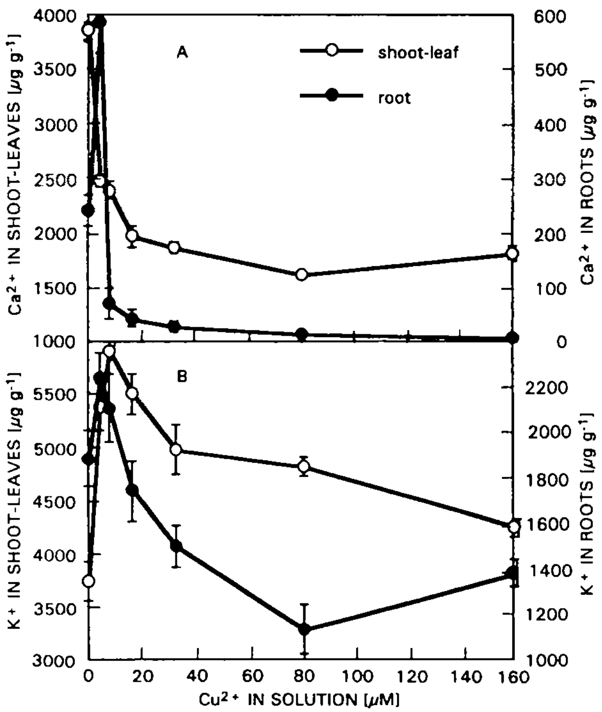

Fig. 2. Ca^{2+} (*A*) and K^+ (*B*) concentrations in roots and shoots of *K. splendens* plants as a function of various Cu^{2+} concentrations. Values are means of 3 replicates, S.E. is given by vertical bars.

The Ca^{2+} concentration in roots increased in 4 μM Cu treatment, by 2 times more than in the control ($P < 0.05$, Fig. 2*A*). In contrast, in the above-ground parts a progressive reduction of Ca^{2+} content was observed with increasing Cu^{2+} supply. Consequently, in 160 μM Cu^{2+} a significant decrease in Ca^{2+} content of 96 % and 60 % of the control in roots and shoots ($P < 0.005$), respectively, was observed (Fig. 2*A*). The K^+ concentration in roots and shoots displayed a significant ($P < 0.005$) increase by about 18 and 43 % at 4 μM Cu^{2+}, but at the highest Cu^{2+} concentration there was no change in K^+ content in the shoots with respect to the control. By contrast, K^+ concentration dropped sharply in the roots ($P < 0.005$) after high Cu treatment (Fig. 2*B*).

Discussion

Extension growth rates in *K. splendens* were reduced as the applied Cu^{2+} concentrations increased. Besides, in lower Cu^{2+} concentrations an enhancement of root elongation was observed. Tolerant populations, as *K. splendens* originated from Cu^{2+}-rich soils, show a high demand for copper and a more vigorous growth at elevated Cu^{2+} levels (Wu and Lin 1990, Ouzounidou 1994b). The inhibitory action of Cu^{2+} in root length growth seems to principally be due to chromosomal aberrations and abnormal cell divisions (Agarwal *et al.* 1987). Yet, the reduction of growth can be correlated with the toxic effect of Cu^{2+} on photosynthesis, root respiration and protein synthesis in roots (Ouzounidou 1994a). The optimum biomass production of *K. splendens* plants occurred under 4 and 8 μM Cu^{2+} treatment, while higher Cu^{2+} supply resulted in a decline of RRB indicating alterations of plant physiology and metabolism. Root biomass yield was revealed more tolerant to excess of Cu^{2+} than the root length. The inhibited photosynthetic activity and vitality of leaves induced by Cu^{2+} lead to the decreased biomass of *K. splendens* (Ouzounidou 1993, 1994a).

The pigment composition seemed to increase at low Cu^{2+} concentrations indicating an evolution of a probable adaptive mechanism of *K. splendens* against Cu^{2+} as it has been also reported for Cu-tolerant *Silene compacta* plants (Ouzounidou 1994b). Higher Cu^{2+} treatment induced a lower chlorophyll accumulation rate rather than a chlorophyll breakdown, as can be revealed by the initial chlorophyll content [0.630 mg g^{-1}(f.m.)]. Inhibitory effects of heavy metal on chlorophyll levels are commonly found in the literature (*e.g.* Sandmann and Boger 1980, Ouzounidou 1993, 1994b). In spite of the severe chlorophyll loss under Cu^{2+} stress significant ultrastructural and morphometric changes were not detected in leaf chloroplasts of *Thlaspi ochroleucum* (Ouzounidou *et al.* 1992).

The presence of a protective mechanism of the above plant parts against copper toxicity was observed, since only a small fraction of the total Cu^{2+} concentration was allocated in the shoots. Much of Cu^{2+} associated with plant roots may not be translocated into the shoot even when a deficiency occurs in the aerial parts. According to Allan and Jarrell (1989) Cu^{2+} has a strong affinity for several different binding sites on the cellulose discs of the cell walls. However, since the plant component of the absorption process is controlled by the plant, it becomes obvious that Cu^{2+} uptake is a function of the activity of Cu^{2+} at the absorption site outside of the plasmalemma (Baker 1990). Previously, it was shown that root cells of *T. ochroleucum* suffered severe damage by Cu^{2+} displayed ultrastructural malformations (Ouzounidou *et al.* 1992).

Cu^{2+} influences the bioelemental status in plant tissues. Decrease in chlorophyll content induced by Fe^{2+} deficiency as it was also observed by Reboredo and Henriques (1991). The detrimental effect of Cu^{2+} on Fe^{2+} uptake and translocation was possibly due to inability of Fe^{3+} to be reduced to Fe^{2+} and then to be absorbed or transported into the root (Agarwal *et al.* 1987). Under higher Cu^{2+} treatments plant tissues retained smaller amounts of Ca^{2+} and Mg^{2+} since Cu^{2+} displaces Ca^{2+} and Mg^{2+} from exchange sites and is strongly bound in the root free space (Jensen and Adalsteinsson 1989), as well as Cu-ions replace Mg-ions in the chlorophyll molecule (Agarwal *et al.* 1987). Cu^{2+} mediated K^+ loss was a result of membrane leakiness. We can assume that Cu^{2+} induce indirectly depolarization of the membrane potential as a result of depletion of electrogenic H^+-pump caused by proton uptake into the inner cellular space (Strange and Macnair 1991). The observed K^+ loss caused by excess Cu^{2+} supply is in agreement with studies in wheat (Jensen and Adalsteinsson 1989), *Mimulus guttatus* (Strange and Macnair 1991), *Silene cucubalus* (De Vos *et al.* 1991) and *Silene compacta* (Ouzounidou 1994b). According to De Vos *et al.* (1991) Cu^{2+} ions first attack sulfhydryl groups causing damage to the permeability barrier of cells, and lipid peroxidation of plasmalemma. Loss of Fe^{2+}, Ca^{2+}, K^+, Mg^{2+} similar to the senescence response (Adams *et al.* 1990) suggests that excess of Cu^{2+} leads to the progressive senescence of the leaves. In addition, Karataglis *et al.* (1991) observed that similar Cu^{2+}-induced activation of peroxidase occurred also in the senescent leaves.

Taking into account our results and the relevant references (Ouzounidou 1994a,b) we concluded that root growth either in terms of length or of biomass and chlorophyll biosynthesis rate stimulated under low Cu^{2+} concentrations in Cu-tolerant plant ecotypes; the optimum bioelemental status occurred also in lower Cu^{2+} concentrations. Part of the Cu-tolerance might result from a reduced transport of Cu^{2+} to the above ground parts.

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