Effect of copper on growth in cucumber plants (*Cucumis sativus*) and its relationships with carbohydrate accumulation and changes in ion contents

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Abstract

Twenty-day-old cucumber plants were submitted to copper stress during 5 days. Leaf expansion rather than dry weight accumulation was the first target of copper inhibition. Despite of a higher copper accumulation in the second leaf (SL) rather than in the first (FL), photosynthesis decrease was more pronounced in the latter. Since leaf expansion declined, leaves became a weak sink and this might account for the observed accumulation of carbohydrates in leaves. This accumulation could induce a feedback inhibition of photosynthesis. The significant accumulation of starch and sucrose did not occur in roots and seemed to be confined to leaves. Copper was sequestered primarily in the roots. The ion distribution (calcium, potassium and magnesium) between roots and leaves of cucumber plants suggested that the uptake and the upward translocation of these ions could be altered by copper excess. The decrease in potassium content may have played a crucial role in the inhibition of leaf expansion. Moreover, in leaves, the observed decline of magnesium content could contribute to the reduction in net assimilation rate and to the accumulation of assimilates in leaves.
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Keywords: Copper stress; Cation contents; Cucumber; Carbohydrate allocation

1. Introduction

Copper is an ubiquitous pollutant in the environment due to the emission and atmospheric deposition of metal dust released by human activities. In addition, soils may contain elevated levels of copper because of its widespread use as a pesticide, land application of sewage sludges as well as mining and smelting activities. Copper as well as other heavy metals enter plants mainly through the root system and may cause a range of morphological and physiological disorders [1]. Copper is an essential element because it is involved in a number of physiological processes such as the photosynthetic and respiratory electron transport chains [2] and as a cofactor or as a part of the prosthetic group of many key enzymes involved in different metabolic pathways, including ATP synthesis [3]. Higher plants take up copper from the soil solution mainly as Cu^{2+}. In excess, the absorbed copper can be considered as a toxic element leading to growth inhibition [4]. Many studies have been carried out on the effect of copper excess on growth, mineral nutrition and metabolism of plants. Copper excess reduces growth [5], photosynthetic activity [6] and the quantum yield of PSII photochemistry assessed by chlorophyll fluorescence [7]. Lidon et al. [6] suggested that the primary sites of copper inhibition are the antenna chlorophyll molecules of PSII. Copper excess may also result in membrane damage [8] and suppression of enzyme activities [9]. For most crop species, the critical level for copper toxicity in leaves is above $20–30 \, \mu g \, g^{-1}$ dry weight [10,11]. Others studies have been carried out to elucidate the mechanisms of photosynthesis inhibition by copper [7,12,13]. Photosynthesis inhibition depends both on the leaf growth stage and on the copper concentration used [12] and it may result from an altered source–sink relationship which leads to an accumulation of carbohydrates [14]. Copper excess commonly inhibits cell elongation [15,16] a complex process depending on cell turgor pressure, synthesis of wall components, as well as on growth regulator contents. There are two major requirements for cell elongation:

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an increase in cell wall extensibility and solute accumulation to create an internal osmotic potential [17]. The precise role of copper in growth inhibition remains unclear. The aim of the present work was to get more information on the cause of growth inhibition by copper, and to assess the relationships between the decrease in leaf area, leaf photosynthesis and ion partitioning. This was addressed by measuring leaf gas exchange, carbohydrate and mineral (K, Cu, Ca and Mg) contents in cucumber plants submitted to copper stress.

2. Materials and methods

2.1. Plant material

Cucumber (*Cucumis sativus* L. cv. Vert long maraicher, Caillard France) seeds were germinated for 24 h on wet filter paper in Petri dishes at room temperature. Germinated seeds were transplanted into pots filled with commercial sand (800 g) and kept under controlled conditions at day/night temperature of 28/21 °C, 65% RH with 14 h photoperiod and 180 μmol m^{-2} s^{-1} PAR. Each pot contained one seedling. Plants were watered once a day with tap water, at 80% of the field capacity, during the first week until the first leaf (FL) appeared and then with the following modified Hoagland nutrient solution (pH 5.2) until the appearance of the third leaf (22 days after sowing): Ca(NO$_3$)$_2$ (2.5 mM); NH$_4$NO$_3$ (1 mM); KH$_2$PO$_4$ (0.5 mM); MgSO$_4$ (0.5 mM); KCl (2.5 mM); NaFeEDTA (0.064 mM); H$_3$BO$_3$ (0.025 μM); MnCl$_2$ (10 μM); ZnCl$_2$ (1.5 μM); CuCl$_2$ (0.5 μM) and Na$_2$MoO$_4$ (0.2 μM).

2.2. Application of copper stress

After the appearance of the third leaf, 4 μg of copper chloride per gram of sand per day were added to the Hoagland solution for 5 days, leading to a final Cu supply of 20 μg g^{-1} sand (Cu treatment). The control plants were watered with nutrient solution without additional copper. Eight replicates were performed for each treatment. At the end of the copper treatment, the seedlings were 27 days old.

2.3. Net photosynthesis and stomatal conductance

Net photosynthetic rate (*A*) and stomatal conductance (*g*) were measured on both the first and second leaves (SL) of each plant using a LI-6400 infrared gas analyser (LiCor Nebraska, USA). Average leaf temperature, leaf-to-air difference in water mole fraction, ambient CO$_2$ mole fraction and photon flux density were, respectively: 25 °C, 11 mmol mol^{-1}, 370 μmol mol^{-1} and 500 μmol m^{-2} s^{-1}. The leaf was enclosed in a ventilated chamber and CO$_2$ and H$_2$O exchanges were monitored until steady-state values of *A* and *g* were obtained. Ci was calculated according to the equation of Von Caemmerer and Farquhar [18].

2.4. Harvest and analysis

For each plant of the copper treatment, roots were carefully washed—three times with demineralized water—to eliminate external copper contamination. Then, leaf (FL and SL), stem, cotyledon and root dry matter were determined after lyophilization (48 h). Samples were then ground in liquid N$_2$ prior to storage at −20 °C. Leaf (FL and SL) and cotyledon areas were measured before freezing using a Li-3000A portable leaf area meter (LiCor Inc, USA). Leaf (FL and SL) and root starch concentration was measured after incubation with amyloglucosidase (from *Aspergillus niger*, Sigma, E.C. 3.2.1.3.) and the resulting glucose was assayed by a colorimetric method using glucose oxidase and peroxidase enzymes [19]. Leaf and root sucrose concentration were measured according to Van Handel [20] after destruction of other sugars with anthrone reagent by heating in alkaline medium [21].

Copper, calcium, magnesium and potassium concentrations in leaves and roots were estimated using a furnace atomic absorption spectrophotometry (Perkin Elmer) after extraction in 2 ml of 65% HNO$_3$/H$_2$O$_2$ (4:1, v/v) at 120 °C for 2 h.

2.5. Statistical analysis

Statistical analysis was based on one-way analysis of variance (ANOVA). The effects of copper treatment were considered statistically significant when $P < 0.05$. Data are presented as mean ± standard errors ($n = 8$).

3. Results

3.1. Growth parameters

Leaf, stem and root dry weights were unaffected by copper stress (Table 1). Cotyledon dry weights increased significantly in the stressed plants (Table 1) while their areas remained unaffected (data not shown). Unlike cotyledon areas, leaf areas (FL and SL) were significantly reduced (36–37%) in the stressed plants (Fig. 1A) while their dry weight were unaffected (Fig. 1B). Thus, the leaf mass area (LMA) of each leaf increased significantly in response to

Table 1
Effect of copper addition on plant dry weight (mg plant^{-1})

	Control	Cu (20 μg g^{-1})
Leaves	393.1 ± 76.4	355.9 ± 70.4
Cotyledons	37.4 ± 3.9	54.3* ± 6.4
Stem	48.8 ± 5.0	48.1 ± 2.6
Roots	157.8 ± 15.4	145.0 ± 13.3
Total dry weight	637.1 ± 68.3	603.3 ± 57.1

Data are mean ± S.E. of eight replicates per treatment. Asterisks denote significant differences (ANOVA) between control and stressed plants.
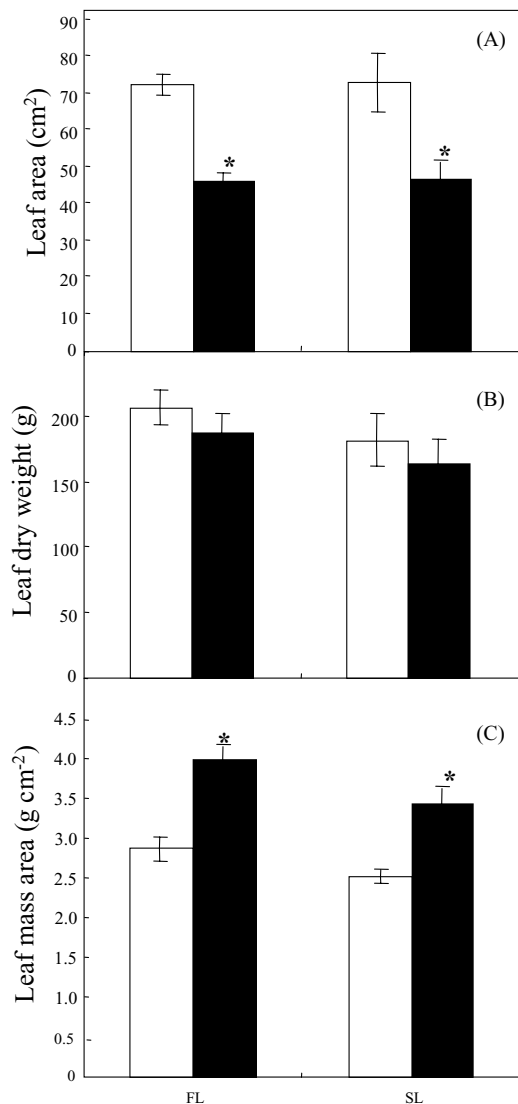
Fig. 1. Effect of copper stress on leaf area (A), leaf dry weight (B) and leaf mass area (C) in first (FL) and second (SL) leaves. Data are mean ± S.E. of eight replicates per treatment. Significant levels between control (□) and stressed plants (■) are indicated by asterisks.

copper application (Fig. 1C). Leaves of the stressed plants did not present any visible damage.

3.2. Gas exchanges

Irrespective of leaf age, net photosynthetic rates were lower in treated plants compared to control plants (Fig. 2A). The reduction of net photosynthesis was higher in FL (52%) than in SL (27%). Stomatal conductance was significantly and almost similarly affected by copper stress in FL and SL (Fig. 2B). However, C_i was only slightly reduced in the SL (Fig. 2C).

3.3. Carbohydrate contents

Starch concentration measured in first and second leaves of the stressed plants increased significantly compared to
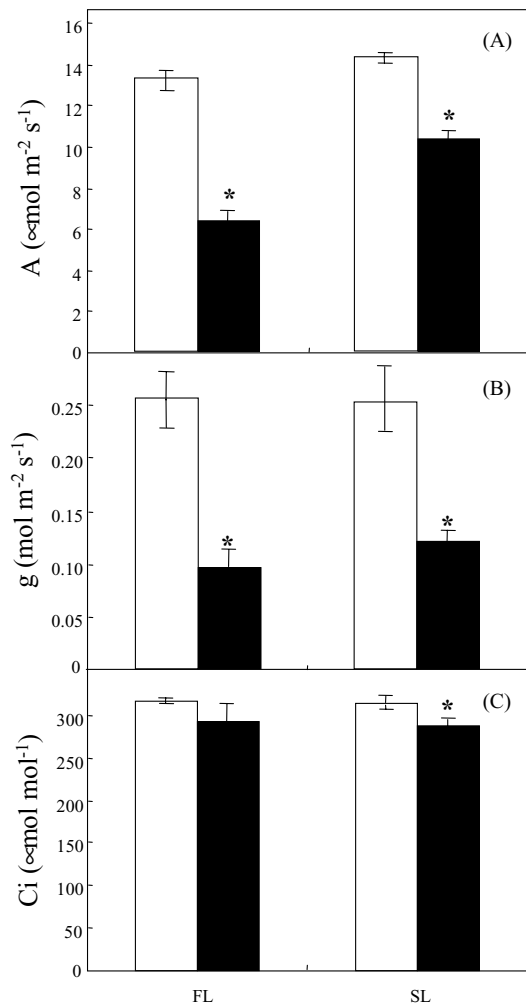

Fig. 2. Effect of copper stress on photosynthesis (A), stomatal conductance (B) and Ci (C) in first (FL) and second (SL) leaves. Data are mean ± S.E. of eight replicates per treatment. Significant levels between control (□) and stressed plants (■) are indicated by asterisks.

controls (Fig. 3A). This increase was more pronounced in the FL (155%) than in the SL (116%). In first and second leaves of the treated plants, sucrose content increased significantly compared to control plants (Fig. 3B). Unlike starch, sucrose content increased to the same extent in FL and SL of the stressed plants. In roots, starch was not detected in either stressed or control plants (Fig. 3A). Copper addition did not affect root sucrose content (Fig. 3B).

3.4. Ion analysis

In Cu-stressed plants, copper content increased significantly (71 and 133% in first and second leaves, respectively) compared to the leaves of control plants (Fig. 4A).

Potassium and magnesium contents decreased significantly both in first and second leaves of stressed plants (Fig. 4B and C) while calcium content only decreased in the first leaf of the stressed plants (Fig. 4D). Calcium content
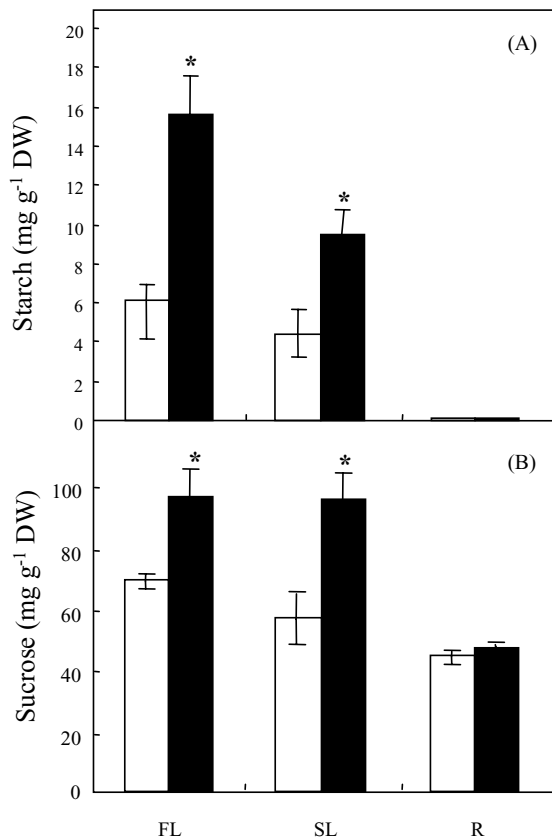
Fig. 3. Effect of copper stress on starch (A) and sucrose (B) contents in first (FL) and second (SL) leaves and in roots (R). Data are mean ± S.E. of eight replicates per treatment. Significant levels between control (□) and stressed plants (■) are indicated by asterisks.

in the second leaf was similar to the controls and was lower than that in the first leaf.

In roots, copper content was far higher in stressed plants than in control plants (Fig. 4A). In contrast to leaves, potassium, magnesium and calcium contents in the roots remained unaffected in spite of addition of the copper in the nutrient solution (Fig. 4B–D).

4. Discussion

In the present work, addition of copper affected only leaf expansion while the dry weight accumulation in leaves, stem and roots remained unaffected. The first target of copper inhibition was thus leaf expansion rather than dry weight accumulation. This is consistent with previous results showing a preferential inhibition of the elongation process by copper excess in runner bean plants [5,22]. In the same way, Cook et al. [23] showed that leaf area decreased more markedly than the leaf dry weight when leaf copper concentration increased in *Phaseolus* plants.

Photosynthesis in both first and second leaves decreased in copper stressed plants. After copper exposure, stomatal closure occurred in both first and second leaves. However,

C_i was only slightly decreased in spite of a pronounced inhibition of photosynthesis. This indicates that photosynthesis inhibition was not a consequence of stomatal closure and this is consistent with previous work on rice [6]. Despite a higher copper accumulation in the leaves, C_i was only slightly decreased. These results suggested an indirect effect of copper inhibition on photosynthesis processes. Indeed, carbohydrate analysis showed a significant increase both in starch and sucrose contents in the two kinds of leaves. Accumulation of assimilate did not occur in roots and was limited to the foliar compartment. This starch accumulation especially in the source leaves may result from a decrease in phloem loading and capacity of assimilate transport or from a fall in the rate of utilisation of the assimilates in the sink organs. Root growth expressed as biomass accumulation was unaffected by copper excess even if copper was sequestered primarily in roots. Because only the expanding leaves exhibited a reduced growth, it could be hypothesised that sink activity (i.e. leaf expansion) is affected by copper rather than phloem loading and assimilate transport. Leaves became a weaker sink for the photosynthetic requirements as soon as their expansion declined under copper effect, and this could account for the observed accumulation of non-structural carbohydrates in source leaves. This carbohydrate accumulation in leaves of stressed plants could in turn induce a feedback inhibition of photosynthesis. Thus, the relationships between photosynthate accumulation and feedback inhibition of photosynthesis is well documented [24]. The inhibition of photosynthesis in Cu-stressed cucumber leaves is more likely a consequence of an altered source–sink relationship, rather than due to toxic effects of copper on the photosynthetic apparatus [14]. Similarly, 6-day-old rice seedlings submitted to cadmium or nickel stress showed an increase in their carbohydrate contents which was accompanied by a decrease in net photosynthesis [25]. Therefore, a feedback inhibition of photosynthesis via growth reduction and assimilate accumulation might be a common response to moderate stress.

In the presence of copper excess, ion partitioning was markedly affected. Copper was sequestered primarily in the root compartment. This is consistent with previous reports showing that copper is located primarily in roots of *Phaseolus* plants submitted to copper stress [23]. In Cu-stressed cucumber plants, root potassium, magnesium and calcium contents were unaffected by copper. However, a highly significant decrease in calcium content was observed only in the first leaf. Calcium translocation from roots to leaves was disrupted in the presence of copper, as already reported in beans plants [12]. The second leaves showed a decline in area (36–37%) similar to the first leaves in spite of the unaffected calcium content and higher copper content. These results contradict the hypothesis that copper binding by the cell wall could alter cell wall elasticity either directly or by displacing one part of the calcium content in the wall [26,27]. In rice seedlings, Chen et al. [28] have shown that copper stress-induced H_2O_2-dependent peroxydase could be
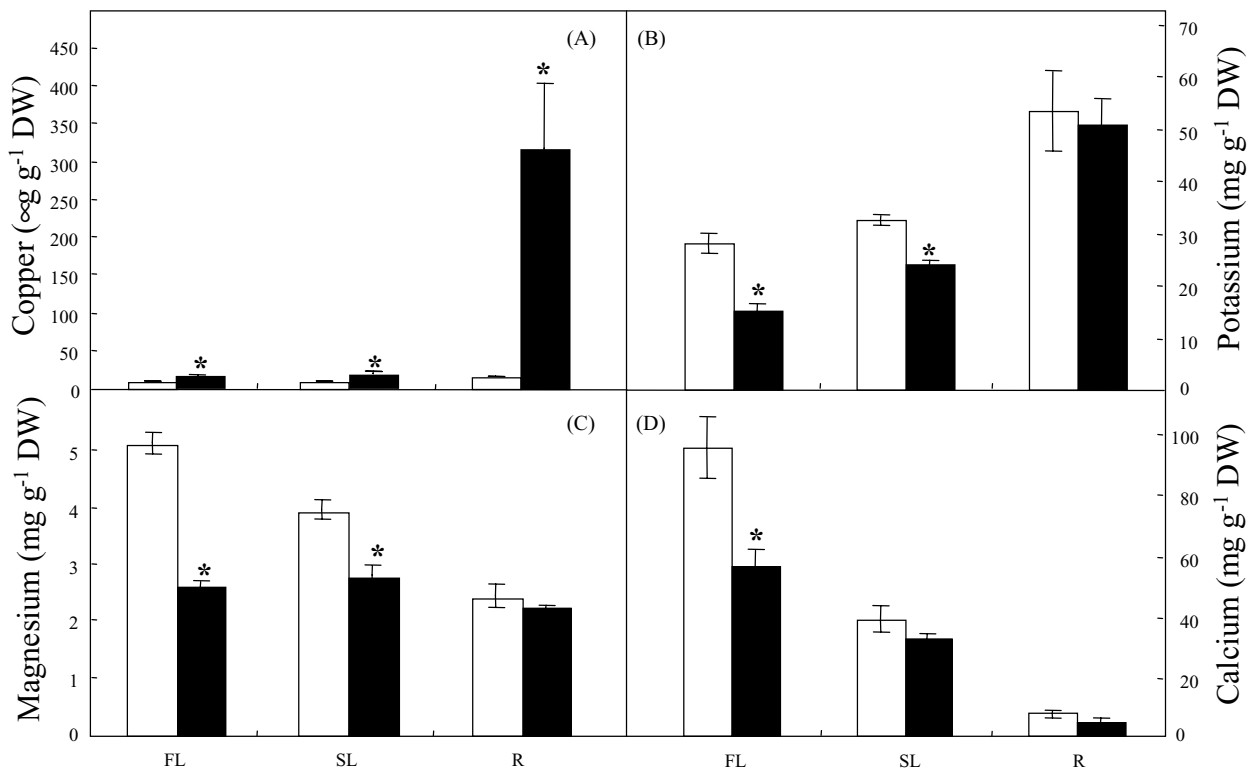
Fig. 4. Effect of copper stress on copper (A), potassium (B), magnesium (C) and calcium (D) contents in first (FL) and second (SL) leaves and in roots (R). Data are mean ± S.E. of eight replicates per treatment. Significant levels between control (□) and stressed plants (■) are indicated by asterisks.

implied in growth inhibition by catalysing the formation of cross-linking among cell wall polymers.

Potassium and magnesium contents decreased significantly both in first and second leaves, and this decrease was more pronounced in the first leaf. The observed ion distribution between roots and leaves suggested that the uptake and the upward translocation of these ions could be disturbed by copper excess. In spinach plants, Ouzounidou et al. [13] reported that iron, sodium, potassium, calcium and magnesium content declined under Cu treatment. It has also been observed that heavy metals such Cu and Cd interfere with the root uptake of mineral nutrients [29,30]. In magnesium deficient plants, assimilate accumulation in the leaves has been demonstrated for a number of plant species [31]. These authors suggested that accumulation of assimilates in the source leaves of Mg deficient plants results from a lack of utilisation of these assimilates in the sink leaves [32] leading to a decrease in photosynthesis [31]. In the stressed cucumber plants, copper induced a sharp decline in leaf magnesium content (40% in FL + SL expressed as mg Mg per plant) and this might account for the observed assimilate accumulation and associated feedback inhibition of photosynthesis. Magnesium plays a key role in the modulation of RuBP carboxylase in the stroma of the chloroplasts [33]. Thus, after copper stress, the magnesium content decrease in cucumber leaves could reinforce the reduction of net assimilation rate.

Potassium is the most abundant cation in plant tissues. It plays an important role in the vacuole where it contributes largely to the osmotic pressure and thus to the turgor pressure, a neccessary process for leaf expansion [17,34]. Cells extension in leaves is closely related to their potassium content [17]. In cucumber cotyledons, potassium supply enhances extension by a factor of 4 in response to the application of cytokinin [35]. Similarly, Mengel and Arneke [36] have shown that in expanding leaves of bean plants submitted to potassium deficiency, turgor, cell size and leaf area were significantly lower that in expanding leaves of the control plants. The potassium content of Cu-treated cucumber plants decreased significantly both in first and second leaves. This decline in potassium content could induce a drop in osmotic potential of leaf cells leading to a decrease in leaf cell extension.

In conclusion, copper excess leads to unbalanced nutrient uptake and decreased upward translocation in cucumber plants. Thus, copper could inhibit potassium uptake—an important ion for turgor and leaf expansion—and magnesium uptake, an essential mineral nutrient for photosynthesis metabolism and for phloem loading. We suggest that the decrease in leaf potassium content plays a crucial role in leaf expansion inhibition which may account for the feedback inhibition of photosynthesis via carbohydrate accumulation in leaves. In addition, magnesium content decline may reinforce this photosynthesis inhibition.

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