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Effects of copper on root growth, cell division, and nucleolus of *Zea mays*

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Abstract

The effects of different concentrations (10^{-5} - 10^{-2} M) of copper sulfate on root growth, cell division and nucleoli in root tip cells of *Zea mays* L. were investigated. 10^{-5} M Cu stimulated root growth, but at higher concentrations (10^{-4} - 10^{-2} M) inhibited it. Cu had toxic effects on chromosomal morphology: c-mitosis, anaphase bridges, and chromosome stickiness were induced. Some nuclei had irregular shape and particles extruded from nucleoli to nuclei and finally from the nuclei into the cytoplasm.

Additional key words: chromosomal morphology, maize, mitosis, nucleus.

Introduction

The content of Cu in the environment is usually low, but it is considerably increased in mining areas and in the vicinity of smelters. Much of the Cu present in soils is in forms which are not readily available to plants because of the strong binding of Cu by organic matter and other soil colloids (Burton *et al.* 1983, Sanders *et al.* 1986). Copper solubility increased at lower pH, and Cu toxicity usually appears when the pH in the soil reaches 5 (Robson and Reuter 1981, Baker and Senft 1995). Copper is an essential nutrient for plants; it plays an irreplaceable role in the function of a large number of enzymes which catalyse oxidative reactions in a variety of metabolic pathways (Lolkema and Vooijs 1986, Marschner 1995). However, when absorbed in excess, copper can lead to inhibition of plant growth (Reboredo and Henriques 1991, Ouzounidou *et al.* 1992, Ouzounidou 1994a), inhibition of root elongation, disturbance of mitosis (Fiskesjö 1988), and damage to root epidermal cells and root cell membranes (Wainwright and Woolhouse 1977, Ouzounidou *et al.* 1995). Cu is also thought to inhibit several enzymes which play an important role in photosynthesis, such as ribulose-1,5-bisphosphate carboxylase (Stiborová *et al.* 1986a) and phosphoenolpyruvate carboxylase (Iglesias and Andreo 1984, Stiborová *et al.* 1986b).

Higher plant response to Cu differs in mechanisms of uptake and accumulation, and of avoiding damage and damage cause. The cytological effects of copper have been studied in *Allium cepa* by documentation c-mitosis (Levan 1945), quantification of chromosome aberrations (Fiskesjö 1988), and nucleolar behaviour (Liu *et al.* 1994). Maize (*Zea mays*) is one of the most important cereal crops and is very sensitive to Cu. The aim of this investigation was to increase our the understanding of the effects of different concentrations of Cu^{2+} on the root growth, cell division and nucleolus of *Zea mays.*

Materials and methods

The seeds of *Z. mays* were kindly provided by Institute of Food Crops, Tianjin Academy of Agricultural Sciences, Tianjin, P.R. China, and were used in the present investigation. The seeds were soaked for 24 h in tap water (pH 6.5), and then were treated with different copper sulfate ($CuSO_4 . 5H_2O$) concentrations ranging from 10^{-2} to 10^{-5} M. Tap water was used for the control experiment. The seeds were allowed to germinate in Petri

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dishes at temperature 26°C for 48 h. The seeds were protected from direct sunlight. Twenty root tips in each treatment group were cut and fixed in 95 % ethanol : 99 % acetic acid (3:2; v/v) for 4 to 5 h and hydrolyzed in 1 M hydrochloric acid : 95 % ethanol : 99 % acetic acid (5:3:2) for 4 - 5 min at 60°C. For the observation of chromosomal morphology, 10 root tips were squashed in Carbol Fuchsin solution (Li 1982) and for the observation of nucleolus changes, the others were squashed in 45 % acetic acid, dried, and on day 2 stained with silver nitrate (Li *et al.* 1990, Liu and Jiang 1991).

Results

The effects of Cu^{2+} on root growth of *Z. mays* varied with the concentrations used (Fig. 1). The root growth was stimulated at 10^{-5} M Cu, but inhibited at 10^{-4}-10^{-2} M Cu.

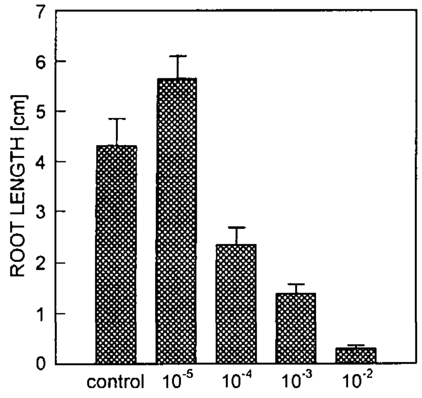

Fig. 1. Effect of different concentrations (10^{-5} - 10^{-2} M) of copper sulphate on root growth of *Zea mays*.

The effects of Cu on the morphology of the roots also varied with the concentrations. At 10^{-5} M, the morphology of the roots was normal during the whole treatment (48 h). At 10^{-4} M, the roots appeared slightly yellow, and at 10^{-3} M, the roots showed a yellow and twisted appearance after 48 h treatment. At 10^{-2} M, the roots were yellow-green and in appearance and rotten.

The mitotic index reflects the frequency of cell division and it is regarded as an important parameter when determining the rate of root growth. The mitotic index decreased progressively with increased Cu concentration (from 10^{-4} to 10^{-2} M) (Table 1). This fits well with the above mentioned effects of copper sulfate on root growth. The mitotic index can be correlated with rate of growth, suggesting that the inhibition of root growth resulted from inhibition of cell division.

The standard types of aberrant chromosomes (modified *Allium* test introduced by Fiskesjö 1985), were also observed in the root tip cells of *Z. mays* after treatment with copper sulfate.

Table 1. Effects of copper sulfate at different concentrations on cell division in root tips of *Zea mays* determined after 48 h treatment. Cell division was completely stopped at 10^{-2} M Cu.

$CuSO_4$ [M]	Mitotic index [‰]	Number of cells	Normal dividing cells [%] metaphases	anaphases	Anomalous dividing cells [%] c-mitosis	chromosome bridges	chromosome stickiness	Anomalous mitoses [%]
Control	40	1000	64.7	34.4	0.6	0.3	-	0.9
10^{-5}	43	1000	56.2	43.2	0.4	0.2	-	0.6
10^{-4}	33	1000	55.4	35.1	5.3	0.8	3.4	9.5
10^{-3}	27	520	3.0	12.1	7.8	3.0	74.1	84.9

C-mitosis was observed in the root tip cells of all Cu-treated groups (Fig. 2*A*), except at 10^{-2} M Cu. The frequency of cells with c-mitosis increased with increasing Cu^{2+} concentration (Table 1).

Anaphase bridges involving one or more chromosomes (Fig. 2*B,C*) were found after the Cu treatment. The frequency of cells with chromosome bridges also increased with increasing Cu^{2+} concentration (Table 1). Anaphase configurations with chromosome bridges exhibiting stickiness were observed only in the treatment with 10^{-3} M Cu (Fig. 2*D*). This type of toxic effect is most likely irreversible.

Klášterská *et al.* (1976) and McGill *et al.* (1974) indicated that chromosome stickiness arises from improper folding of the chromosome fiber into single chromatids and that chromosomes become attached to each other by subchromatid bridges. The chromosome pattern reflects highly toxic effects, usually of an irreversible type, and probably leads to cell death (Fig. 2*E*). The frequency of cells with chromosome stickiness also progressively increases with increasing Cu concentration.

Some nuclei in the root tip cells treated with Cu^{2+} (above 10^{-3} M) became irregular in shape (Fig. 2*F,G*). In addition, broken nuclei were also found at the highest concentration (10^{-2} M) (Fig. 2*H,I*).

Normally, the diploid nucleus of *Z. mays* contains one or two nucleoli (Fig. 3*A*). The effects of Cu on nucleoli varied with the different concentrations of copper sulfate solutions used. Three phenomena were observed after these treatments. Firstly, after 48 h treatment with 10^{-4} M Cu, a few particles of silver-stained nucleolar material (Fig. 3*B*) were on the way from the nucleolus to the nucleus (Fig. 3*C*). More and more particle material were gradually accumulated around the nucleus (Fig. 3*D*), after 48 h treatment with 10^{-3} M Cu. The frequency of cells with this type progressively increases with increasing Cu concentration. Secondly, at higher concentrations of Cu (10^{-2} and 10^{-3} M), the silver staining reaction at the periphery of the nucleolus became weaker (Fig. 3*E*). Thirdly, nucleolar material was extruded from the nucleus into the cytoplasm (Fig. 3*F*).

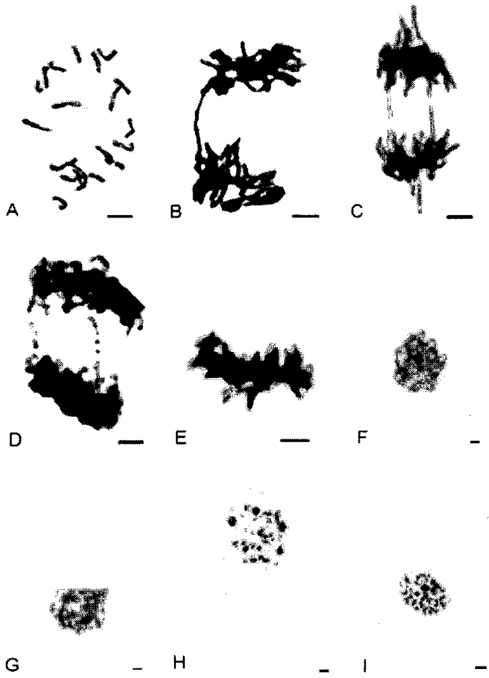

Fig. 2. The effects of Cu^{2+} on root tip cell division of *Zea mays* (48-h treatment): *A* - c-metaphase (10^{-4} M Cu^{2+}), *B,C* - chromosome bridges (10^{-3} M Cu^{2+}), *D* - anaphase configu-rations with chromosome bridges exhibiting stickiness (10^{-3} M Cu^{2+}), *E* - chromosome stickiness (10^{-3} M Cu^{2+}), *F,G* - irregularly shaped nuclei (10^{-3} M Cu^{2+}), *H,I* - broken nuclei (10^{-2} M Cu^{2+}). Scale = 5 μm.

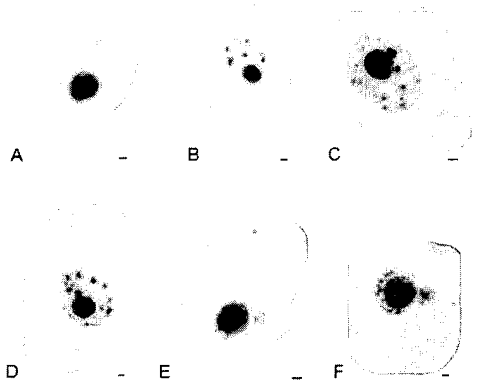

Fig. 3. The effects of Cu^{2+} on nucleoli of root tip cells of *Zea mays*. *A* - control cells, *B* - a few of silver-stained particles scattered in the nucleus (10^{-4} M Cu^{2+}), *C* - nucleolar material is released on the way from the nucleolus to the nucleus (10^{-3} M Cu^{2+}), *D* - more silver-stained particles scattered in the nucleus (10^{-3} M Cu^{2+}), *E* - the silver staining reaction at the periphery of the nucleolus becomes weaker (10^{-4} M Cu^{2+}), *F* - nucleolar material is released from the nucleus to the cytoplasm. Scale = 5 μm.

Discussion

The results in the present investigation indicated that copper affects *Z. mays* at concentrations from 10^{-4} to 10^{-2} M. These are in agreement with the findings of Liu *et al.* (1994) for the effect of Cu^{2+} on the root growth and tip cells of *Allium cepa*, but with a few differences. For instance, *1*) there is not so many nucleolar particles scattered in the nuclei and so much nucleoar material released from nuclei into cytoplasm in *Z. mays*, when compared with *A. cepa*; *2*) Cu toxicity on the nucleoli in root tip cells of *Z. mays* is not stronger than those of *A. cepa,* which shows that *A. cepa* is more sensitive to Cu compared with *Z. mays.* The results in the present investigation also indicated that once the nucleolus was poisoned, showing some silver-stained particles scattered in the nucleus, and the nucleus was broken, the root growth of *Z. mays* was obviously inhibited. And once the silver-stained nucleolar material was extruded from the nucleus into the cytoplasm, the root growth was almost or completely stopped.

Several authors reported that the inhibition of root elongation caused by copper may be due to metal interference with cell division, including inducement of chromosomal aberrations and abnormal mitosis (Agarwal *et al.* 1987, Eleftheriou and Karataglis 1989, Punz and Sieghardt 1993, Kahle 1993), which can explain the inhibition of root growth in this investigation.

A large proportion of the Cu absorbed by the plants is retained in the roots (Tukendorf and Baszynski 1985). The results Lastra *et al.* (1987) demonstrated that active uptake of Cu occurs by the binding of Cu^{2+} to a specific carrier (probably a polypeptide) located on the outer surface of root cell plasmalemma, and that the Cu^{2+}-

ionophore complex is transported across the plasma membrane into the cytoplasm, where it changes configuration and releases Cu^{2+} into the cytoplasm. According to the investigation (Clijsters and Van Assche 1985), Cu toxicity can induce deficiency of other essential elements, suggesting that this deficiency result in inhibition of metal-ion dependent reactions. Fernandes and Henriques (1991) stated that at high metal concentrations, Cu can damage cell walls and plasma membrane integrity, and the plants lose their uptake selectivity. Ouzounidou (1994b) thought that copper accumulation influences the tissue distribution of Ca, Mg, Fe and K. It was demonstrated that copper ions tend to displace Ca^{2+} ions from exchange sites and are strongly bound in root-free space (Jensén and Adalsteinsson 1989).

From what is indicated above, we infer that the inhibitory and toxic effects of Cu on root growth, cell division and nucleoli of root tip cells of *Z. mays* may result from excessive uptake and accumulation of Cu and interference of normal cell division, damage to cell walls and plasma membrane integrity (De Vos *et al.* 1993, Strange and Macnair 1991), loss of uptake selectivity, and inhibition of Ca uptake. The integrity of the nucleolus depends on the existence of Ca^{2+} (Wang 1988). Because of the low level of free Ca^{2+} in the cells, calmodulin (CaM) does not activate Ca-ATPase (Xu 1985), which leads to failure in regulation of calcium concentration and disturbance or inhibition of various cellular metabolic processes. However, the mechanisms remain to be further studied.

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