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Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic

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Abstract

The effects of different concentrations (10^{-7} to 10^{-2} M) of cadmium chloride on root growth, cell division and nucleoli in root tip cells of *Allium sativum* L. were investigated. At lower concentrations of Cd^{2+} (10^{-7} to 10^{-6} M), Cd^{2+} did not influence the root growth, even had a stimulation effects during a short treatment. The results showed that the rate of root growth per day at the treatment groups (10^{-4} to 10^{-2} M Cd^{2+}) decreased with increasing duration of the treatment and increasing Cd^{2+} concentration. Cd^{2+} induced c-mitosis, anaphase bridges, chromosome stickiness and on nucleoli, causing some particles of similar silver-stained material scattered in the nuclei and making the silver staining reaction at the periphery of the nucleolus weaker.

Additional key words: *Allium sativum*, anaphase bridges, chromosome stickiness, mitosis.

Introduction

It has been demonstrated that Cd is a substantial pollutant due to its high toxicity and great solubility in water (Lockwood 1976). Cd inhibits root growth and cell division in some plants sensitive to Cd^{2+}, such as onion (Fiskesjö 1988, Liu *et al.* 1992) and bean (Oehlker 1953), induces leaf chlorosis accompanied by a lowering of photosynthetic rate (Bazzaz *et al.* 1974, Hampp *et al.* 1976, Bazynski *et al.* 1980, Salt *et al.* 1995, Das *et al.* 1997), disturbs cell proliferation (Rosas *et al.* 1984), impedes respiration (Lee *et al.* 1976), mitochondrial electron transport (Miller *et al.* 1973), enzyme activities (Weigel and Jäger 1980) and inhibits uptake of other elements, such as Zn (Root *et al.* 1975, Christensen 1984a) and Ca (Christensen 1984b). Plants nonsensitive to Cd^{2+} can accumulate Cd^{2+} to a relatively high level without adverse effects on growth (Bingham 1979, Kuboi *et al.* 1986). It was reported that that garlic (*A. sativum*) has considerable ability to accumulate substantial amounts of cadmium (Jiang *et al.* 2001). However, few cytological researches on the toxic effects of Cd^{2+} on nucleolus in root tip cells of *A. sativum*, using a silver staining technique, have been reported. This paper reports the effects of Cd^{2+} on root growth, cell division and nucleoli of *A. sativum*.

Materials and methods

Healthy and equal-sized garlic (*Allium sativum* L.) cloves were chosen from the bulbs that had not started the formation of green leaves or root growth. Before commencing the experiment, the dry scales of the bulbs were removed. With the same test set-up as for *A. cepa* in the *Allium* test (Fiskesjö 1993), twelve garlic cloves were the starting material in each series, and the best 10 garlic cloves were selected for testing. Cd was provided as cadmium chloride ($CdCl_2 . 2.5\ H_2O$), ranging from 10^{-2} to 10^{-7} M. The healthy and equal-sized garlic cloves were soaked for 24 h before starting the experiments. They are allowed to sprout out and produce roots, and then they

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were treated with different concentrations of Cd solutions in Petri dishes at temperature 20 °C in the dark for 24, 48, and 72 h. The test liquids were changed regularly every 24 h. In each treatment ten treated roots were examined for the morphological observation. Twenty root tips in each treatment group were cut and fixed in ethanol + acetic acid (3:2) for 4 to 5 h and hydrolyzed in 1 M hydrochloric acid + 95 % ethanol + 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 changes in the nucleoli, the last ten were squashed in 45 % acetic acid, then drying and 2 staining with silver nitrate (Li *et al.* 1990, Liu and Jiang 1991). Data for root length were analyzed with standard statistical software (*SigmaPlot*).

Results and discussion

The effects of Cd^{2+} on root growth of *A. sativum* varied with the different concentrations of $CdCl_2$ solutions used (10^{-2} to 10^{-7} M). The higher concentrations of Cd^{2+} (10^{-4} to 10^{-2} M) obviously decreased the root growth with increasing duration of the treatment and increasing Cd^{2+} concentration and the root growth was completely inhibited after 24 h treatment. The lower concentrations of Cd^{2+} (10^{-7} to 10^{-6} M) did not obviously influence the root growth, and had even a stimulatory effect during a short treatment period (Fig. 1, Table 1).

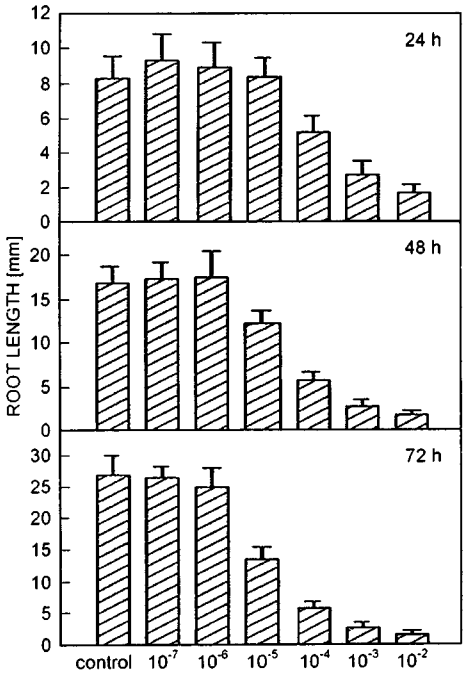

Fig. 1. Effects of different concentrations of Cd^{2+} on root growth of *A. sativum*. *Vertical bars* denote SE (n = 10).

After treatment with 10^{-7} to 10^{-6} M Cd^{2+}, the morphology of the roots was more or less normal during the whole treatment. At 10^{-5} to 10^{-4} M Cd^{2+}, the root tips showed a slightly twisted appearance after 24 h of treatment. At 10^{-3} to 10^{-2} M Cd^{2+}, the root tips were abnormally stubby and stiff.

The mitotic index decreased progressively with increased Cd concentration and duration of time, except for the seedlings exposed to 10^{-7} M Cd^{2+} (Table 1). This fits well with the above mentioned effects of $CdCl_2$ on root growth.

C-mitosis was observed in the root tip cells of all treated groups after treatment with Cd^{2+}. The frequency of cells with c-mitosis increased with increasing Cd^{2+} concentration and duration of treatment (Table 1). The severely condensed chromosomes are randomly scattered in the cell (Fig. 2*A*).

Chromosome bridges are due either to breaks in chromosomes or chromatids (often resulting in

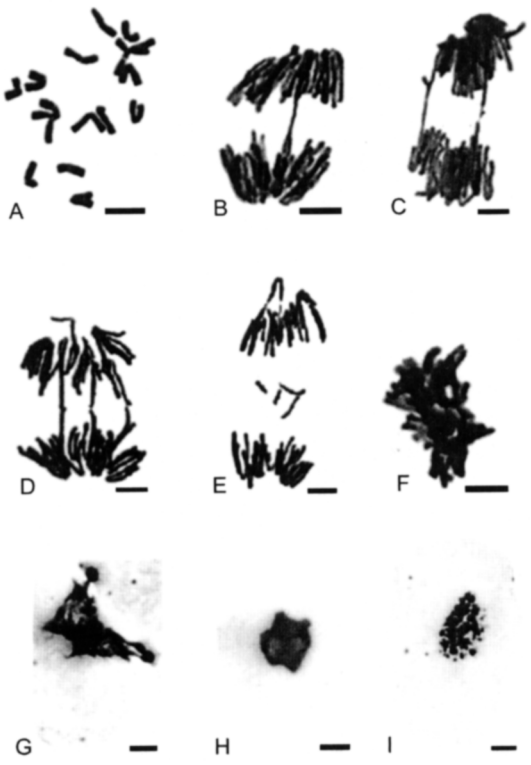

Fig. 2. The effects of Cd^{2+} on root tip cell division of *Allium sativum*. *A*: c-metaphase (10^{-5} M Cd^{2+}, 24 h); *B-D*: chromosome bridges (*B* - 10^{-5} M Cd^{2+}, 24 h, *C* - 10^{-6} M Cd^{2+}, 48 h, *D* - 10^{-3} M Cd^{2+}, 24 h); *E*: chromosome fragments (10^{-3} M Cd^{2+}, 24 h); *F*: chromosome stickiness (10^{-2} M Cd^{2+}, 48 h); *G*: micronuclei (10^{-7} M Cd^{2+}, 48 h); *H*: irregular nuclei in shape (10^{-2} M Cd^{2+}, 24 h); *I*: nucleus disintegration (10^{-2} M Cd^{2+}, 72 h). *Scale* = 5 μm.

Table 1. Effects of $CdCl_2$ on root growth (increments per 24 h) and cell division in the root tip cells of *A. sativum* (NDC - no dividing cells).

Time [h]	Conc. [M]	Growth [cm]	Mitotic index	Number of cells	Normal dividing cells [%] metaphases	anaphases	Anomalous dividing cell [%] c-mitosis	chromosome bridge	chromosome stickiness
24	Control	0.8	23	1000	58.6	40.3	0.5	0.6	0.0
	10^{-7}	0.9	28	1000	57.4	41.6	0.5	0.5	0.0
	10^{-6}	0.9	22	1000	62.7	35.6	0.8	0.7	0.2
	10^{-5}	0.8	19	1000	60.8	37.4	0.9	0.6	0.3
	10^{-4}	0.5	16	870	56.2	34.1	1.9	2.1	5.7
	10^{-3}	0.3	11	460	48.0	32.8	3.2	4.8	11.2
	10^{-2}	0.2	9	270	28.2	18.4	7.1	10.5	35.8
48	Control	0.9	24	1000	56.7	41.7	0.8	0.7	0.1
	10^{-7}	0.8	29	1000	57.6	40.9	0.8	0.6	0.1
	10^{-6}	0.9	23	1000	60.5	37.2	1.0	0.8	0.5
	10^{-5}	0.4	20	1000	59.3	34.9	2.0	1.5	2.3
	10^{-4}	0.1	8	210	50.1	29.4	3.4	2.2	14.9
	10^{-3}	0.0	NDC	NDC					
	10^{-2}	0.0	NDC	NDC					
72	Control	1.0	24	1000	52.9	43.5	1.6	1.1	0.9
	10^{-7}	0.9	27	1000	55.5	41.6	1.5	0.9	0.5
	10^{-6}	0.8	21	1000	56.7	38.4	2.1	1.1	1.7
	10^{-5}	0.1	16	740	51.4	34.9	3.2	2.9	7.6
	10^{-4}	0.0	NDC	NDC					
	10^{-3}	0.0	NDC	NDC					
	10^{-2}	0.0	NDC	NDC					

fragments) or to chromosome stickiness. Anaphase bridges involving one or more chromosomes (Fig. 2*B-D*) were found after the Cd^{2+} treatment. The frequency of cells with chromosome bridges increased with increasing Cd^{2+} concentration and duration of treatment (Table 1). Chromosome fragments (Fig. 2*E*) were also observed.

The chromosome pattern reflects highly toxic effects, usually of an irreversible type, and probably leads to cell death (Fig. 2*F*). The frequency of cells with chromosome stickiness also progressively increases with increasing Cd^{2+} concentration and duration of treatment.

In addition to the aberrations mentioned above, interphase cells with micronuclei were observed after 48 h treatments with above 10^{-7} M Cd^{2+} (Fig. 2*G*). Also, nuclei irregular, in shape, were found in the root tip cells after 24 h treatment with 10^{-2} M Cd^{2+} (Fig. 2*H*) and nucleus disintegration occurred with increasing duration of treatment (Fig. 2*I*), which was one of severely toxic phenomena, usually leading to death of the cells.

Normally, the diploid nucleus of *A. sativum* contains one or two nucleoli (Fig. 3*A*). The effects of Cd^{2+} on nucleoli varied with the different concentrations of $CdCl_2$ solutions used. The two phenomena resulting from effect of Cd^{2+} on the nucleoli in root tip cells were observed. First, the cells revealed that a few particles of nucleolar silver-stained material were observed together with main nucleolus (Fig. 3*B*) after the 24 h treatment with lower concentration (10^{-5} M Cd^{2+}) and 72 h treatments with 10^{-6} to 10^{-7} M Cd^{2+}. With increasing concentration of Cd^{2+} and duration of treatment, more and more particle material gradually accumulated in the nucleus (Fig. 3*C-F*) and occupied the whole nucleus (Fig. 3*E*), and aggregated into irregular shapes (Fig. 3*D,F*). The frequency of cells with this type of particles progressively increases with increasing Cd^{2+} concentration. Second, the silver staining reaction at the periphery of the nucleolus became weaker (Fig. 3*G-I*). This phenomenon usually occurred in the cells exposed to (10^{-4} to 10^{-2} M Cd^{2+}) after 72 h treatment. Once the phenomenon occurred, the root growth was almost or completely stopped.

It is common knowledge that silver impregnation is regarded as a specific stain of the nucleolus and the nucleolar organizing region (NOR). The silver staining technique has been widely applied in cytological studies to try to understand the nucleolar cycle and the nucleolar organization in both animals and plants. The results of the present investigation indicated that Cd^{2+} can induce some similar silver-stained material scattered in the nuclei and make the silver staining reaction at the periphery of the nucleolus weaker. Besides, it has toxic effects on chromosomal morphology including c-mitosis, anaphase bridges and chromosome stickiness.

The low Cd^{2+} concentrations of 10^{-5} to 10^{-7} M have a stimulatory effect on root growth of *A. sativum*. Such low Cd^{2+} concentrations, however, affect the nucleoli of some root tip cells so that some silver-stained particulates appear in the nucleus. But once the nucleolus was affected, many silver-stained particles were scattered in

the nucleus and the nucleus broken, the root growth of *A. sativum* was clearly inhibited. At 10^{-4} to 10^{-2} M, Cd^{2+} has inhibitory and toxic effects on both root growth and nucleoli. At the same time, the silver staining reaction at the periphery of the nucleolus became weaker and the root growth was almost or completely stopped. These effects of Cd^{2+} on nucleoli are, to some degree, similar to those observed after lead treatment and copper treatment (Liu *et al.* 1994a,b).

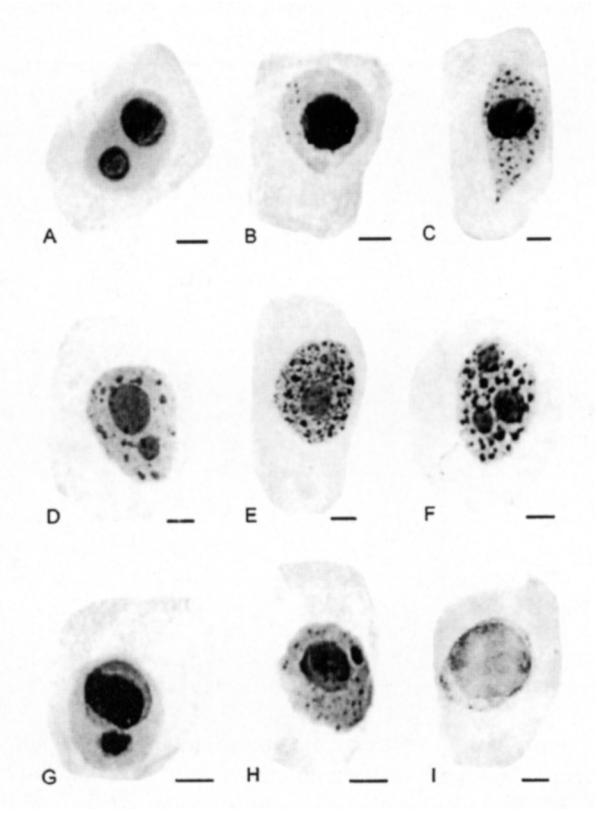

Fig. 3. Effects of Cd^{2+} on nucleoli in root tip cells of *Allium sativum*. *A*: control cells (72 h); *B*: a few of silver-stained particles scattered in the nucleus (10^{-4} M Cd^{2+}, 24 h); *C-F*: increasing number of silver-stained particles scattered in the nucleus (*C* - 10^{-4} M Cd^{2+}, 48 h, *D* - 10^{-3} M Cd^{2+}, 24 h, *E-F* - 10^{-3} M Cd^{2+}, 72 h); *G-I*: The silver staining reaction at the periphery of the nucleolus becomes weaker (10^{-3} M Cd^{2+}, 48 h; 10^{-2} M Cd^{2+}, 48 h; 10^{-2} M Cd^{2+}, 72 h). *Scale* = 5 μm.

At higher concentrations, Cd^{2+} inhibits root growth, resulting from disturbance of the cell division, and inhibition of cell expansion growth by direct or indirect effects of Cd^{2+} on auxin metabolism or auxin carriers (Prasad 1995). Rivetta *et al.* (1997) indicated, after studying the effects of Cd^{2+} on radish seed germination, that Cd^{2+} enters the cells through Ca^{2+} channels in the plasma membrane. Ca^{2+} could be replaced by Cd^{2+} (Clarkson and Lüttge 1989, Rivetta *et al.* 1997), because they have similar radii (Ca^{2+}, 0.099 nm; Cd^{2+}, 0.097 nm) (Wen *et al.* 1989). Means and Dedman (1980) indicated that calmodulin (CaM) was located specifically in the mitotic spindle and is involved in the processes of chromosome movement through regulation and control of depoly-merization and polymerization of microtubules (Chueng 1980, 1983). As noted by Rivetta *et al.* (1997), Cd^{2+} binds to CaM and competes with Ca^{2+} in this binding. This may explain the mitotic abnormalities caused by Cd^{2+} such as chromosome bridges, c-mitosis, chromosome stickiness and chromosome fragments.

It is well known that the nucleolus is the metabolic center of RNA. The integrity of the nucleolus depends on the existence of Ca^{2+} (Wang 1988). The effects of Cd^{2+} on the nucleolus in *A. sativum* root tip cells may result from the excessive uptake and accumulation of Cd^{2+} as well as from the reduced Ca^{2+} uptake (Smeyers-Verbeke *et al.* 1978). If the cell concentration of free Ca^{2+} is low, CaM does not activate Ca-ATPase (Xu 1985), leading to failure in regulation of calcium concentration, which, in turn disturbs and destroys the physiological activities and regulation of CaM. The mechanism behind the expression of this phenomenon (*e.g.* the presence of Cd^{2+} in the nucleolus) remains to be explained.

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