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 (CdCl₂, 2.5 H₂O), ranging from 10^{-3} to 10^{-1} 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

Received 3 June 2002, accepted 15 November 2002.
Acknowledgements: This project was supported by the National Natural Science Foundation of China.
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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 on root growth of *A. sativum* varied with the different concentrations of CdCl₂ solutions used (10⁻² to 10⁻⁷ M). The higher concentrations of Cd (10⁻⁴ to 10⁻³ M) obviously decreased the root growth with increasing duration of the treatment and increasing Cd concentration and the root growth was completely inhibited after 24 h treatment. The lower concentrations of Cd (10⁻⁷ to 10⁻⁵ 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 on root growth of *A. sativum*. Vertical bars denote SE (n = 10).](image)

After treatment with 10⁻⁷ to 10⁻⁶ M Cd, the morphology of the roots was more or less normal during the whole treatment. At 10⁻⁵ to 10⁻⁴ M Cd, the root tips showed a slightly twisted appearance after 24 h of treatment. At 10⁻³ to 10⁻² M Cd, 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 (Table 1). This fits well with the above mentioned effects of CdCl₂ on root growth.

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

![Fig. 2. The effects of Cd on root tip cell division of *Allium sativum*. A: c-metaphase (10⁻³ M Cd; 24 h); B-D: chromosome bridges (B - 10⁻⁴ M Cd; 24 h, C - 10⁻⁵ M Cd; 48 h, D - 10⁻⁶ M Cd; 24 h); E: chromosome fragments (10⁻⁵ M Cd; 24 h); F: chromosome stickiness (10⁻² M Cd; 48 h); G: micronuclei (10⁻⁷ M Cd; 48 h); H: irregular nuclei in shape (10⁻² M Cd; 24 h); I: nucleus disintegration (10⁻² M Cd; 72 h). Scale = 5 μm.](image)