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EFFECT OF CADMIUM ON ION UPTAKE, TRANSPIRATION AND CYTOKININ CONTENT IN WHEAT SEEDLINGS

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Sumarry. In wheat seedlings (*Triticum durum)*, uptake of potassium and nitrate, transpiration and shoot growth were inhibited by adding cadmium to the nutrient medium. A sharp reduction in cytokinin content was observed within two hours of supplying cadmium. The reduction in cytokinin content was probably a result of hormone breakdown. In support of this, Cd-treatment elevated cytokinin oxidase activity. Similarities between the effects of cadmium on both cytokinin content and transpiration are discussed in terms of an involvement of the hormone in protecting plants from the toxic action of this heavy metal.

Key words: *Triticum durum*, transpiration, growth, cytokinin, cadmium

Introduction

Pollution of the environment by heavy metals underpins much interest in their actions on plants. Consequently, there is considerable published information about the action of Cd^{2+} on plant growth and on physiological and biochemical processes. Harmful effects produced by Cd might be explained by its ability to inactivate enzymes possibly through reaction with the SH-groups of proteins (Fuhrer, 1982). Detrimental effects are manifested in inhibition of photosynthesis and in oxidative stress leading to membrane damage (Prasad, 1995). Plants possess many substances capable of binding and inactivating Cd: phytochelatins, metallothioneins, organic acids (etc.) (Sanita' di Toppi and Gabbrielli, 1999), which allow them to accumulate the element in significant quantities (Costa and Morel, 1993). However, induction of synthesis of substances capable of binding heavy metals demands some time while plants still experience the toxic

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effects of the active agent. Consequently plant tolerance to toxic metals cannot be attributed only to the formation of their complexes with some chelating substances. Hormones are assumed to play an important role in the adaptation of plants to stressful environments (Salisbury and Marinos, 1985). Accumulation of ABA and decrease in cytokinin content have been reported in the case of plants treated with toxic metals (Pochenrieder et al., 1989; Prasad, 1995). However the effects were observed a long time after the start of the exposure. Fast changes in hormone content have been shown in response to the action of unfavourable factors other than toxic metals (Kudoyarova et al, 1998), and the results suggest a possible implication of short-term hormonal effects in response to Cd. However no data are available in the literature. To rectify this deficiency we have studied the rapid effects of cadmium ions on cytokinin content, transpiration and ion uptake in wheat seedlings. Cytokinins were chosen for study in the light of reports about the increase in sensitivity of heavy metal-treated plants to the effect of synthetic cytokinins (Sayed, 1999), which might be indirect evidence of a decreased content of endogenous cytokinins.

Materials and Methods

Plant material and growth conditions. Seedlings of durum wheat (*Triticum durum* Desf. cv. Bezenchukskaya 139) were grown in darkness at 24ºC between empty glass tubes sealed at the ends, tied together and floated in containers filled with 0.1 strength Hoagland-Arnon nutrient solution. Three days later the containers were transferred into the light, and grown-on under illumination of 90 W/m^2 PAR from ZN and DNAT-400 fluorescent lamps, with a 14-h photoperiod and a temperature of 21ºC. Nine-day-old wheat plants were used to study the effects of Cd^{2+}. Sufficient cadmium acetate was added to the nutrient medium to make a concentration of 0.04 mM. In preliminary experiments this concentration was shown to provide fast inhibition of shoot growth. In some cases zeatin was added to the nutrient medium three hours prior to cadmium to make a concentration of 1 mg/l.

Physiological measurements. One, two and three hours after addition of cadmium to the nutrient medium decreases in nitrate and potassium content in the nutrient medium were measured with ion-selective electrodes and the data used to calculate ion uptake by the plants. At the same time, transpiration was measured gravimetrically. An analogue inductive electromechanical position sensor was used to monitor extension growth of the second leaf. The output signal from the sensor was tracked continuously using a chart recorder.

Purification and immunoassay of cytokinins was carried out as described earlier (Kudoyarova et al, 1998).

Activity of cytokinin oxidase was measured as described earlier (Vysotskaya et al., 2001).

Results

During the first 30 min after adding cadmium to the nutrient medium, extension growth of the second leaf of wheat seedlings decreased from 31±5 µm/min to 14±2 µm/min and remained at this level for next 100 min. Pre-treatment of wheat seedlings with zeatin reduced Cd-induced inhibition of leaf growth (25±3 µm/min).

Cadmium in the nutrient medium inhibited ion uptake by 55–60%. This was observed within 1 h of the start of treatment (Table 1).

Table 1. Ion uptake by control and Cd-treated wheat seedlings during the first hour after the start of treatment. Values represent means +SE, n=5.

Concentration of Cd in nutrient medium, mM	Ion uptake, µmol seedling^{-1}.h^{-1}	
	NO_3^-	K^+
0	65+7	43+3
0,04	26+4	19+2

The treatment affected transpiration rate with the same rapidity (Fig. 1). During the first hour it was almost halved compared with controls and remained much lower than in controls for a further hour.

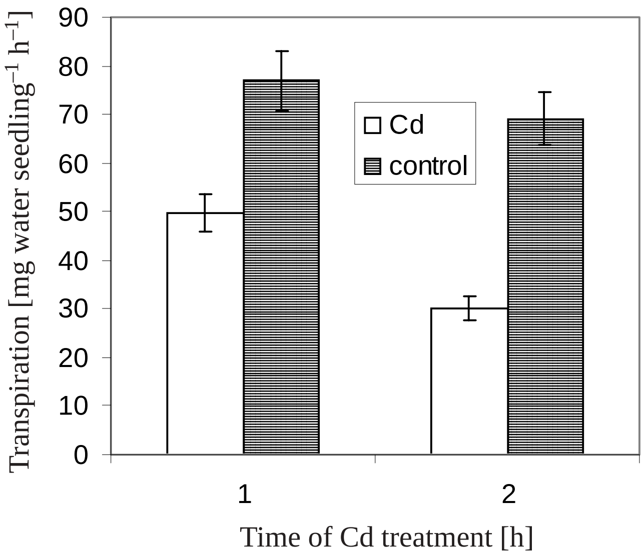

Fig. 1. Effect of the presence of 0.04 mM cadmium acetate in the nutrient medium on transpiration of nine-day-old wheat seedlings. Vertical bars represent SE.

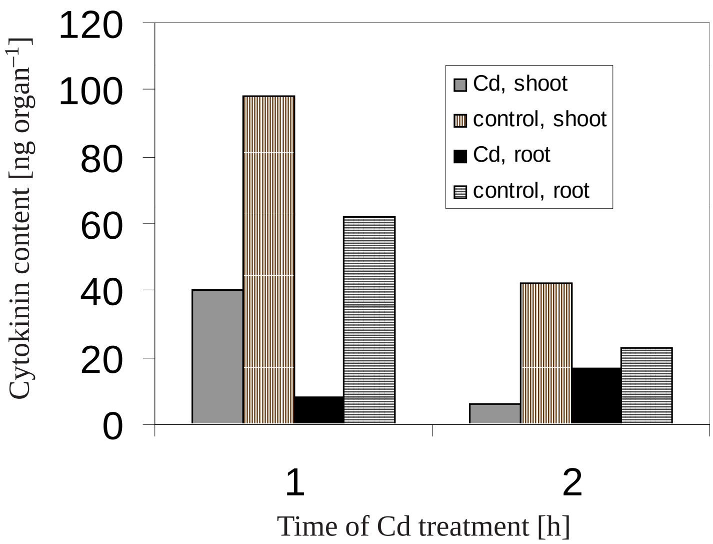

Fig. 2. Effect of the presence of 0.04 mM cadmium acetate in the nutrient medium on total cytokinin content in shoots and roots of nine-day-old wheat seedlings. Vertical bars represent SE.

The amount of cytokinins in the shoots and roots of Cd plants was less than half that in shoots of control plants after only 1 h of treatment, and after 2 h was still substantially below control values (Fig. 2).

Addition of 0,04 mM cadmium acetate to the incubation medium increased the activity of cytokinin oxidase. The quantity of degraded isopentenyleadenine was 485 pmol/mg protein with cadmium acetate and 305 pmol/mg protein without.

Discussion

Cadmium treatment led to an inhibition of growth rate, transpiration and ion uptake by wheat seedlings. Rapid inhibition of root function was evident in terms of reductions in both ion and water uptake. This may not be altogether surprising since roots are the first to come in contact with the injurious cadmium. These results are in accordance with reports indicating inhibition of water conductance in roots by toxic metals (Barcelo and Poschenreider, 1990). The decrease in transpiration of Cd-treated plants is likely to be due to stomatal closure. Cd-induced reduction in stomatal conductivity is in accordance with the literature (Pearson and Kirkham, 1981). Its physiological significance might be in limiting water losses when water uptake by roots is reduced by Cd. A beneficial effect of decreased stomatal conductivity was assumed to be in limiting Cd transported with the transpiration flow (Salt et al., 1995). However the mechanism of Cd-induced stomatal closure remains unclear (Sanita' di Toppi and Gabbrielli, 1999). The rapid decline in cytokinin content observed in Cd-treated plants might be important for the control of transpiration since this hormone is known to maintain stomata in the open state (Bengston et al., 1979).

The observed decline in cytokinin content might be due to an accelerated degradation of this hormone. Oxidation of cytokinins is an important pathway for their inactivation (Kaminek et al., 1997). Since Cd-treatment is known to produce oxidative stress (Somashekaraiah et al., 1992) increased oxidation of hormones might be due to this effect. However the results of *in vitro* measurement of the effect of Cd on the activity of cytokinin oxidase suggests a more direct effect of this element on the process of hormone metabolism. In accordance with this, we found that 0,04 mM cadmium acetate increased activity of cytokinin oxidase. The ability of Cd to influence the activity of different enzymes is known from the literature (Van Assche and Clijsters, 1990). The decreased production of hormones by injured roots is also not excluded as contributing to the decrease observed in the shoots.

Inhibition of leaf growth also contributes to a decline in water losses. Thus a decrease in leaf surface is a well-known non-specific response to water stress, which is induced by many unfavourable factors. Decreased cytokinin content in Cd-treated plants might also be responsible for their growth response.The ability of this hormone to influence cell division and expansion is well-known (Mok, M., 1994). Thus cytokinins have recently been shown to influence the activity of expansins (proteins in-

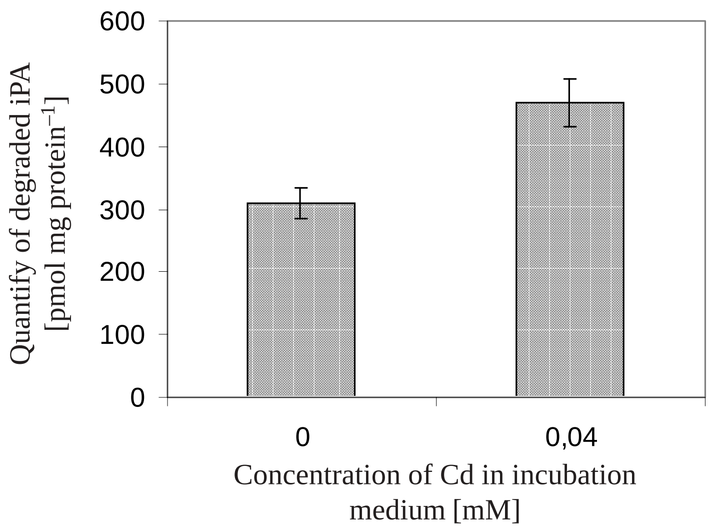

Fig. 3. Effect of the presence of different concentrations of Cd in the incubation medium on the activity of cytokinin oxidase measured *in vitro*. Vertical bars represent SE.

volved in the control of cell expansion) (Downes and Crowell, 1998). Consequently inhibition of leaf expansion of wheat seedlings treated with Cd might be due to a Cd-induced decline in cytokinin content. This assumption is supported by the fact that in this study the inhibitory effect of cadmium on leaf growth was lower in plants which were pre-treated with zeatin. The decrease in the growth inhibitory effect of Cd in plants treated with exogenous cytokinin is in accordance with the literature (Gadallah and El-Enany, 1999).

Thus, the decrease in cytokinin content in plants treated with Cd, which might be a result of acceleration of cytokinin metabolism caused by Cd-induced activation of cytokinin oxidase, is likely to be responsible for decreased transpiration and leaf growth. These effects might lead to limited Cd flow drawn by transpiration flow, which might protect plants from its injurious effects until induced syntheses of Cd-binding substances makes them resistant to the action of this heavy metal.

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