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BRIEF COMMUNICATION

Effect of increasing concentrations of lead and cadmium on cucumber seedlings

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Abstract

The effect of lead and cadmium on biomass accumulation of cucumber seedlings (*Cucumis sativus* L.) as well as the contents of abscisic acid (ABA), free proline and soluble proteins in leaves were studied. Seedlings were subjected to lead nitrate or cadmium bromide in low concentrations (1 - 5 μM) for 1, 4 or 7 d, and then to the action of the same substances in high concentrations (500 - 1000 μM). The pretreatments of the seedlings with heavy metals in low concentrations enabled them to tolerate the subsequent high concentrations of cadmium and lead without injury. The plant responses to heavy metal treatment were accompanied by the accumulation of ABA, free proline and soluble proteins in leaf tissues.

Additional key words: abscisic acid, *Cucumis sativus*, growth, proline, soluble proteins.

Increased contamination of the environment with heavy metals has negative consequences for all kinds of organisms including higher plants. Heavy metals in high concentrations inhibit the growth and development of plants, and disturb many biochemical and physiological processes, for instance, injure cell membranes, reduce transpiration, cause breakdown of the protein synthesis, damage the photosynthetic apparatus and inhibit photosynthesis, affect the activity of several enzymes, raise lipid peroxidation, *etc.* (Foy *et al.* 1978, Sanità di Toppi and Gabrielli 1999).

Plant response to heavy metals is a complex phenomenon which includes mechanisms of avoidance, detoxification and repair (Sanità di Toppi and Gabrielli 1999). A first barrier against heavy metals stress, operating mainly at the root level, can be the immobilization of Cd by of means cell wall and extra-cellular sugars (Nishizono *et al.* 1989). Concentration of metals in the cytosol can be regulated by phytochelatins (Rauser 1990, Das *et al.* 1997) and cellular compartmentation (Rauser 1987). Heavy metal binding to phytochelatins is a widespread and decisive mechanism of metal detoxification in higher plants (Zenk 1996, Sanità di Toppi and Gabrielli 1999). A significant role in metal detoxification and tolerance is also played by vacuolar compartmentalization by organic acids, which prevents the free circulation of metal ions in the cytosol and forced them into a limited area (Rauser 1987). In animals, cyanobacteria, and fungi heavy metals can be complexed and detoxified by small cystein-rich proteins known as metallothioneins (Kägi 1991). Recently in *Arabidopsis thaliana* two Cu-induced metallothioneins called MT1 and MT2 were isolated (Murthy *et al.* 1997). However, there is no certain indication in the literature of the existence in higher plants of metallothioneins induced by cadmium (Sanità di Toppi and Gabrielli 1999) or lead. Nowadays, it is well known, that higher plants not only respond to heavy metal treatment by the synthesis of phytochelatins or related peptides, but also by the synthesis of stress proteins with a molecular mass ranging from 10 to 70 kDa (Didierjean *et al.* 1996, Bierkens *et al.* 1998). Stress proteins might limit and repair the damage

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to cell proteins caused by metals, and they could exert protective effects on cellular membranes (Lin *et al.* 1985, Didierjean *et al.* 1996).

However, acute effect of heavy metals in high doses and effects of gradually increasing concentrations may be different. While data concerning this phenomenon in the literature are relatively scarce (Brown and Martin 1981, Sobolev *et al.* 1982, Ali *et al.* 1998), the objective of this work was to study the plant response to the action of increasing concentrations of lead and cadmium.

The experiments were carried out on cucumber seedlings (*Cucumis sativus* L. cv. Alma-Atinskiĭ 1) grown for 3 d on 1/2 Knop solution (pH 6.2 - 6.4) at a temperature of 22 - 25 °C, air humidity 60 - 70 %, irradiance 220 μmol m^{-2} s^{-1} and photoperiod 14 h. The seedlings were placed in aerated solutions (volume of 250 cm^3) containing $Pb(NO_3)_2$ or $CdBr_2.4\,H_2O$ in low concentrations (1 - 5 μM) or in water (control) for 1, 4 or 7 d. Thereafter the plants were subjected to the action of the same metals but in higher concentrations (500 - 1000 μM), known to be damaging to the given plant (Titov *et al.* 1995) for 3 - 7 d. Other conditions of the experiment remained unchanged.

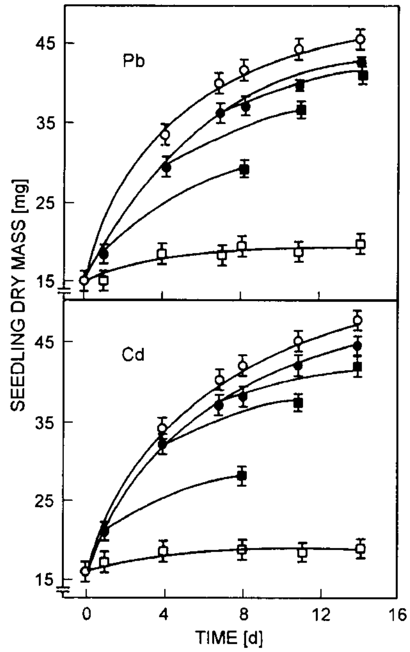

Fig. 1. Effect of lead and cadmium in increasing concentrations on dry biomass accumulation of cucumber seedlings (control - *open circles*, seedlings growing with 1 μM $Pb(NO_3)_2$ or 5 μM $CdBr_2$ - *closed circles*, seedlings growing with 1000 μM $Pb(NO_3)_2$ or 500 μM $CdBr_2$ - *open squares*, seedlings growing with 1 μM $Pb(NO_3)_2$ or 5 μM $CdBr_2$ for 1, 4, 7 d and exposed to 1000 μM $Pb(NO_3)_2$ or 500 μM $CdBr_2$ for 7 d - *closed squares*).

The response of seedlings to the effect of lead and cadmium ions was evaluated by measuring the accumulation of biomass. The content of soluble proteins in the seedling leaves was analyzed following Bradford method (1976). Amount of free proline in the leaves was estimated by Bates *et al.* (1973). The concentration of free ABA in the leaves was analyzed by enzyme-linked immunosorbent assay using the modification of Kudoyarova *et al.* (1986). The experiments were performed in triplicate.

The action of lead in a concentration of 1000 μM on cucumber seedlings caused a sharp retardation of biomass accumulation, whereas its treatment in a concentration of 1 μM just slightly decelerated this process (Fig. 1). Pretreatment of the seedlings with lead in a low concentration (1 μM) for 1, 4 or 7 d resulted in markedly less suppression of biomass accumulation at subsequent exposure to a high Pb concentration (1000 μM) (Fig. 1). Therefore, pretreatment of cucumber seedlings with lead in a low concentration caused an increase in their resistance to this metal.

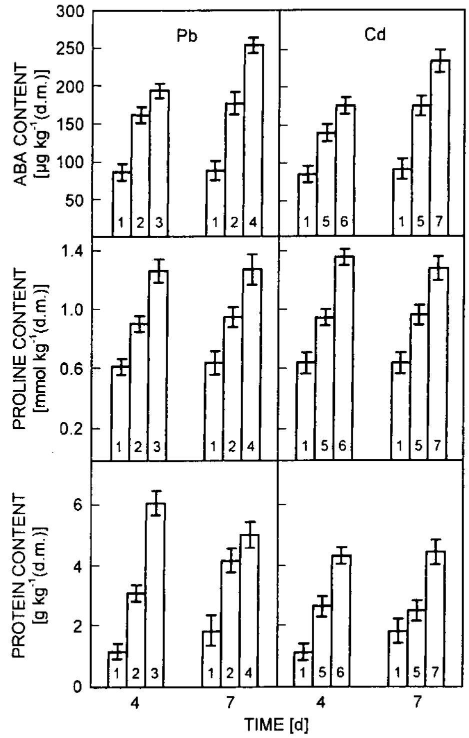

Fig. 2. Effect of lead and cadmium in increasing concentrations on the content of ABA, free proline and soluble proteins in leaves of cucumber seedlings: *1* - 0 μM (control), *2* - 1 μM $Pb(NO_3)_2$, *3* - 1 μM (1 d) + 1000 μM $Pb(NO_3)_2$, *4* - 1 μM (4 d) + 1000 μM $Pb(NO_3)_2$, *5* - 5 μM $CdBr_2$, *6* - 5 μM (1 d) + 500 μM $CdBr_2$, *7* - 5 μM (4 d) + 500 μM $CdBr_2$.

Similarly, the cadmium stress in acute dose (500 μM) resulted in significant reduction in the cucumber growth. The pretreatment of seedlings with a low cadmium concentration (5 μM) for 1, 4 and 7 d prior to exposure to a high concentration (500 μM) caused them to produce greater biomass than those grown on 500 μM without pretreatment (Fig. 1). These results are in agreement with data on *Holcus lanatum* L.: roots were pretreated with a low cadmium concentration (0.2 μg cm^{-3}) at subsequent treatment with a high concentration (1 μg cm^{-3}) grew better than roots given no cadmium pretreatment (Brown and Martin 1981).

The response of cucumber seedlings to lead or cadmium stress was accompanied by the accumulation of free ABA in leaf tissues (Fig. 2). Even the treatment with low metal concentrations for 4 or 7 d caused a significant increase in the content of this hormone. Further exposure of the seedlings to high Pb or Cd concentrations for 3 d resulted in additional enhancement of ABA content in the leaves. In addition, the accumulation of free proline and water soluble proteins in the leaf tissues was observed at the Pb and Cd in a concentration of 1 or 5 μM (Fig. 2). The following increase in the concentration of Pb and Cd to 1000 and 500 μM, respectively, resulted in greater enhancement of free proline and protein contents.

ABA plays an essential role in plant tolerance to heavy metals as it promotes stomatal closure and/or modify gene expression (Leung and Giraudat 1998). For instance, the increase in concentration of ABA linked the Cd stress with the changes in wax coverage via stimulated expression of lipid transfer protein genes in barley (Hollenbach *et al.* 1997). Besides, the enhancement of ABA content in plant promotes the accumulation of proline (Younis *et al.* 1994). Recently, Schat *et al.* (1997) demonstrated that proline accumulation under Cd treatment in leaves of *Silene vulgaris* is a consequence of a Cd-induced water deficit, rather than a consequence of direct effect of this metal. All the same time, proline is believed to protect plant tissues against heavy metal stress by acting as N-storage compound, osmotically active solute and protectant for enzymes, other macromolecules and cellular structures (Shah and Dubey 1997, Ali *et al.* 1998, Muñoz *et al.* 1998).

The data presented demonstrate that pretreatment of plants with a low concentrations of Cd or Pb may have significant effect upon their tolerance to subsequent high concentrations. The enhancement of metal resistance of cucumber plants to increasing (high) concentrations of Pb and Cd may be connected with the accumulation of free proline, ABA and soluble proteins in tissues.

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