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GROWTH, CELL WALL ELASTICITY AND PLASTICITY IN *ZEA MAYS* L. COLEOPTILES EXPOSED TO CADMIUM

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RESUMEN

CRECIMIENTO Y PROPIEDADES ELASTICAS Y PLASTICAS DE LAS PAREDES CELULARES DE COLEOPTILOS DE *ZEA MAYS* L. EXPUESTOS A CADMIO

Se ha estudiado la influencia de diferentes concentraciones de Cd (0, 0.05, 0.1, 1 y 10 mM) sobre el crecimiento en extensión y las propiedades físicas de las paredes celulares de coleoptilos de *Zea mays* L. El crecimiento en extensión fué seguido de forma continuada mediante un sistema de transductor de desplazamiento computerizado. El mismo sistema modificado permitió el análisis de las propiedades físicas de las paredes celulares, estimándose la extensibilidad total, la elasticidad y la plasticidad mediante la técnica Instron modificada. Todas las concentraciones de Cd suministradas inhibían de forma significativa el crecimiento. No se observó una correlación clara entre las propiedades de extensibilidad total, plasticidad y elasticidad de las paredes celulares con las concentraciones de Cd suministradas. De acuerdo con nuestros resultados la alteración de las propiedades físicas de las paredes celulares no parece ser la causa inicial de la disminución del crecimiento en extensión de coleoptilos sometidos a toxicidad por Cd.

Palabras clave: Cadmio. Inhibición crecimiento. Paredes celulares. Extensibilidad. Plasticidad. Sistema computerizado de desplazamiento lineal.

SUMMARY

The influence of different Cd concentrations (0, 0.05, 0.1, 1 and 10 mM) on extension growth and physical properties of cell walls was studied in *Zea mays* L. coleoptiles. Extension growth of the coleoptiles was continuously monitored with a computerized linear displacement transducer system. The same system, somewhat modified, was used for the analysis of the physical properties of the coleoptile cell walls. Total extensibility, cell wall plasticity and elasticity was measured by a modified Instron technique. All Cd concentrations supplied significantly inhibited extension growth of the coleoptiles. No clear correlation between the Cd concentrations supplied and cell wall extensibility, plasticity and elasticity was observed. We may conclude from our results that alteration of physical cell wall properties was not a primary cause for inhibition of extension growth by Cd in maize coleoptiles.

Key words: Cadmium. Growth inhibition. Cell wall. Extensibility. Plasticity. Elasticity. Computerized linear displacement transducer system.

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INTRODUCTION

Cell expansion growth generally is described by the modified Lockhart's equation (Dale and Sutcliffe, 1986):

$$\frac{dV}{dt} = \frac{L'_p \phi (\Delta\Psi + P - Y)}{\phi + L'_p}$$

where L'_p is the apparent hydraulic conductance, ϕ the extensibility of the wall, $\Delta\Psi$ the difference of water potential between the cell and its surroundings, Y the threshold turgor pressure, and P the actual turgor pressure. Any change of these parameters would alter the rate of cell expansion growth.

Cell expansion growth is generally severely inhibited by metal toxicity (Barceló and Poschenrieder, 1990; Breckle, 1991). It has been suggested that toxic levels of metals such as Al, Cd, Zn or Pb may bind to the pectin fraction of cell walls (Klimashevski and Dedov, 1975; Khan *et al.*, 1984; Peterson, 1969; Wierzbicka, 1987) and inhibit cell expansion growth by affecting cell wall extensibility. Cross-linking of the pectin carboxyl groups by metals has been suggested as a possible mechanism, but it seems more likely that metals affect physical properties of cell walls by influencing the synthesis and assambly of cell wall components. (Barceló and Poschenrieder, 1990; Barceló, 1991).

In the present short-term study we analysed the influence of different Cd concentrations on growth, cell wall extensibility, plasticity and elasticity, in order to establish the possible role of Cd-induced alterations of these parameters in growth inhibition by Cd.

MATERIALS AND METHODS

Two types of experiments were performed. *In vivo* measurement of extension growth (experiment I) and determination of *In-vitro* extensibilities of cell walls (experiment II).

Experiment I: For investigating the influence of Cd on the extension growth of maize coleoptiles, *Zea mays* L. cv Honeycomb (Semillas Batlle SA, Barcelona) seeds were germinated for seven days on sterilized Petri dishes in darkness (25 °C). Only uniform seedlings with stright coleoptiles of an initial length of approximately 15 mm were used.

For extension growth monitoring, a linear displacement transducer device (LVDT) similar to that described by Penny *et al.* (1973) and modified by Cramer *et al.* (1988) was used. The transducer (Sangamo, U.K., type DF-1 with a DCU1B conditioner) was connected to the coleoptile with a nylon thread. Data adquisition was made with a personal computer (PC/XT, Elbe Microsystems, PCX, Barcelona). Seedlings were grown in continuously aerated, flowing solutions, containing 0.4 mM Ca (controls) or 0.05, 0.1, 1 or 10 mM Cd as $CdCl_2 . 2.5\ H_2O$). A scheme of our device is shown in figure 1.

Growth of the coleoptiles was monitored for 4 h. After this, roots

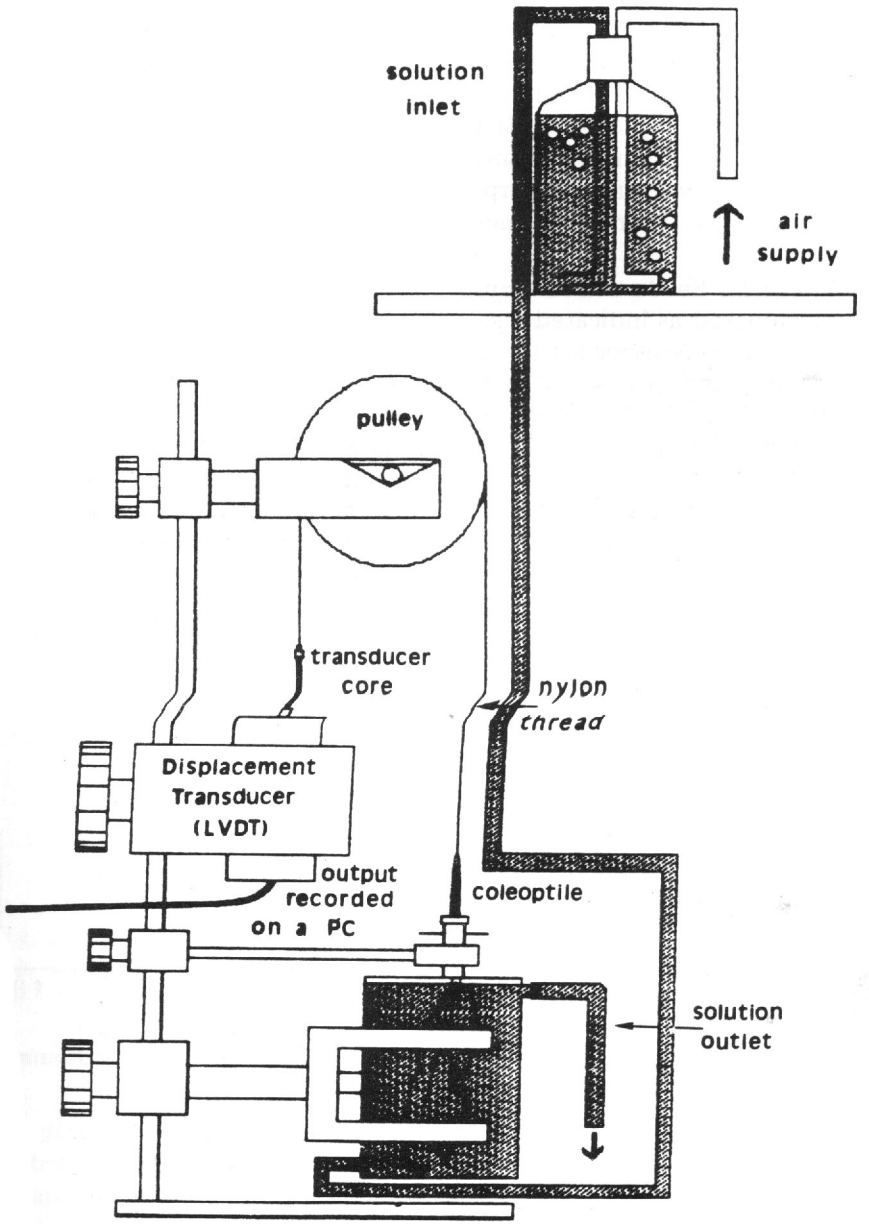

FIG. 1.—*Device for extension growth monitoring (modified after Penny et al., 1973).*

were submerged for 30 min in 0.4 mM $CaCl_2$ solution, followed by distilled water. The plant material was dried at 60 °C and, after digestion (0.1 g dry matter in 0.2 mL $HClO_4$ and 1.4 mL H_2O_2 in closed vials), Ca and Cd concentrations were determined by atomic absorption spectrometry (Perkin Elmer 703).

Experiment II: Seeds of *Zea mays* L. were germinated as indicated above. Ten uniform coleoptiles per treatment were cut and placed in Petri dishes with 15 mL of control or Cd-containing solution (0.05, 0.1 1 or 10 mM as $CdCl_2.2H_2O$). The dishes were gently shaken for 2, 4 or 24 h. After washing with a 0.4 mM Ca solution, the coleoptiles were frozen at −30 °C. Before measuring cell wall extensibility, coleoptiles were allowed to thaw.

Cell wall extensibility, elasticity and plasticity were determined with the Instron technique according to Kutschera y Schopfer (1986). The same linear displacement transducer from experiment I was used. The weight for cell wall extension was 20 g. The displacement caused by the cell wall extension was monitored by a personal computer as indicated above. Total extensibility (Et), elastic extensibility (Ee) and plastic extensibility (Ep) were determined on the graphics as shown in figure 2.

Cadmium and Ca concentrations of coleoptiles were determined as in experiment I.

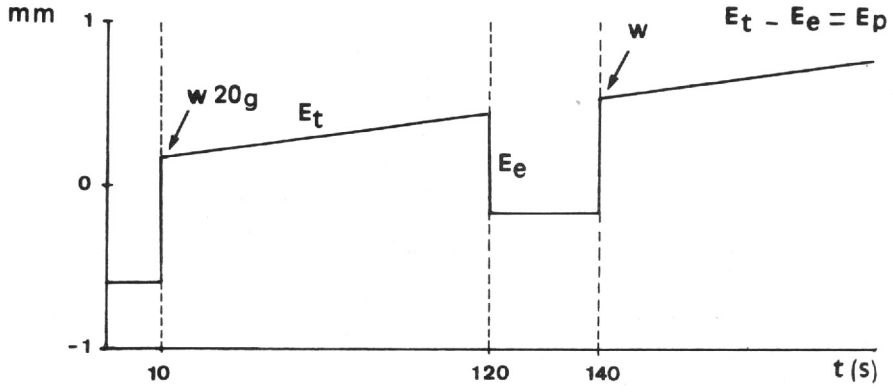

FIG. 2.—*Schematic representation of an* in vitro *extensibility curve, indicating total extensibility, plastic and elastic extensibility (Kutschera and Schopfer, 1985).*

RESULTS

In vivo growth measurement showed that all Cd concentrations assayed had a significant inhibitory effect on coleoptile growth (Fig. 3). The growth rate of control coleoptiles generally increased during the whole experiment (4 h) (Fig. 4). The growth rates of coleoptiles exposed

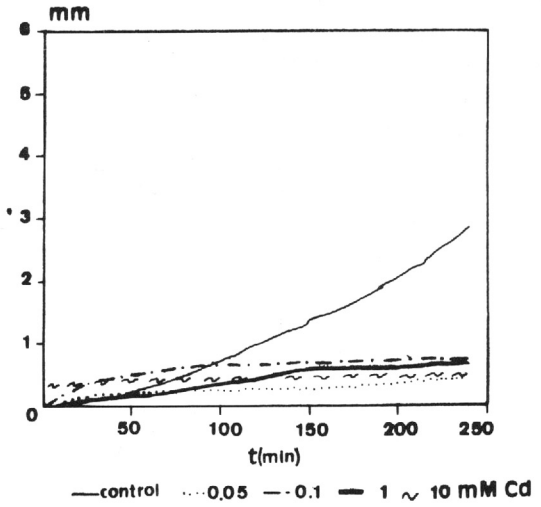

FIG. 3.—*Growth (mm) of* Zea mays *L. coleoptiles grown in control or Cd-containing nutrient solution.*

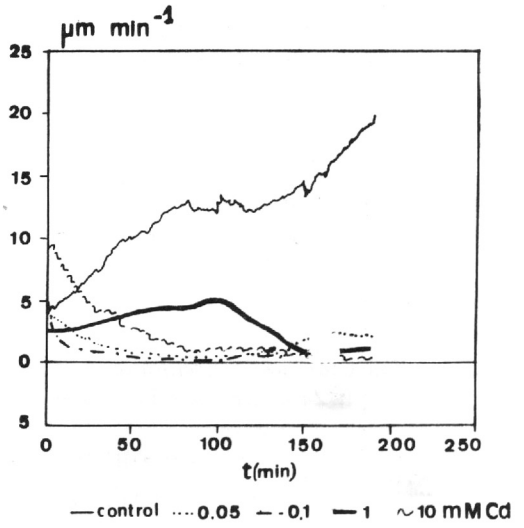

FIG. 4.—*Growth rates (μm min^{-1})* Zea mays *L. coleoptiles grown in control or Cd-containing nutrient solution.*

to Cd generally decreased inmediately after Cd supply and felt to zero after approximately 90 min exposure to Cd. Coleoptiles exposed to 1 mM Cd showed a different behaviour, reaching a zero growth rate after approximately 150 min exposure to Cd. Coleoptiles exposed to 0.1 mM Cd or higher concentrations did not recover a significant growth rate within the experimental time, while coleoptiles treated with 0.05 mM exhibited a small increase of growth rate after approximately 145 min.

Table 1 shows the concentrations of Ca and Cd in roots and coleoptiles. All plants treated with Cd showed higher Ca concentrations in roots and lower Ca concentrations in shoots than controls. The lowest Ca

TABLE 1

Concentration of Ca and Cd ($\mu g\ g^{-1}$) in roots and coleoptiles exposed for 4 h to control or Cd-Containing solutions.

Cd conc. in solution	Roots		Coleoptiles	
	Calcium	Cadmium	Calcium	Cadmium
Control	10.68	n. d.*	15.24	n. d.
0.05 mM	18.95	166.67	8.94	n. d.
0.1 mM	17.26	283.43	4.99	n. d.
1 mM	15.34	1324.17	10.40	7.97
10 mM	Missing	Missing	12.31	307.12

* n. d., not detectable.

concentrations were found in shoots of the 0.1 mM Cd treatment. The Cd concentrations within both roots and shoots significantly increased with Cd supply. In control plants and in shoots of plants exposed to 0.05 or 0.1 mM Cd, the Cd concentrations were below the detection limit.

Table 2 shows the Ca and Cd concentrations of coleoptiles floated for different times (2 h, 4 h or 24 h) on control (0.4 mM Ca) or Cd-containing

TABLE 2

Calcium and Cadmium concentrations of coleoptiles floated for different times (2 h, 4 h, 24 h) on control (0.4 mM Ca) or Cd-containing solutions.

EXPOSURE TIME	CADMIUM CONCENTRATION IN SOLUTION				
	Control	0.05 mM	0.1 mM	1.0 mM	10 mM
	Calcium concentration $\mu g\ g^{-1}$ dry weight				
2 h	8.11	12.53	17.60	39.10	1.04
4 h	23.83	3.74	16.64	12.49	122.48
24 h	18.73	31.75	225.55	17.08	20.12
	Cadmium concentration $\mu g\ g^{-1}$ dry weight				
2 h	n. d.*	n. d.	107.40	625.61	1009.52
4 h	n. d.	147.87	Missing	893.37	638.21
24 h	n. d.	286.71	743.49	3159.29	9498.58

* n. d., not detectable.

solutions. Generally both Cd treatment and exposure time significantly influenced Ca concentrations of coleoptiles, but no correlation between Cd supply and Ca concentration could be established. The cadmium concentration in the coleoptiles significantly increased with the Cd concentration supplied and with the exposure time.

Total extensibility, elastic and plastic extensibility of cell walls are shown in Table 3. In controls, a significant decrease with time of total extensibility and plastic and elastic extensibility was observed. In coleoptiles exposed to Cd the response of physical cell wall properties was more complex. Generally an increase of total extensibility was observed after 2 or 4 h exposure. After 24 h exposure all Cd-treated coleoptiles showed a higher total extensibility than controls. A similar behaviour was found for plastic extensibility. In control plants, the elastic extensibility significantly decreased with time. After 2 h exposure, plants treated with Cd showed significantly lower cell wall elasticity than controls, but differences were not significant after 4 h exposure. After 24 h, coleoptiles treated with 0.05 mM Cd showed significantly higher, and

TABLE 3

Total extensibility, plastic extensibility and elastic extensibility (μm) of cell walls of maize coleoptiles exposed for different times to control or Cd-containing solutions.

EXPOSURE TIME	TOTAL EXTENSIBILITY				
	Control	0.05 mM	0.1 mM	1.0 mM	10 mM
2 hours	608.89ae*	423.75bc	640.17ace	489.82ce	862.70e
4 hours	401.17b	689.25bc	612.91c	632.34c	642.68ce
24 hours	327.76b	458.15cdf	401.38bdf	417.02bcf	545.60ef
	PLASTIC EXTENSIBILITY				
	Control	0.05 mM	0.1 mM	1.0 mM	10 mM
2 hours	301.51a	244.43bd	393.76abc	331.65abc	626.27ce
4 hours	290.69ad	529.64de	678.86be	464.85cde	467.92de
24 hours	183.11b	246.16bd	349.41bf	306.40cf	386.60ce
	ELASTIC EXTENSIBILITY				
	Control	0.05 mM	0.1 mM	1.0 mM	10 mM
2 hours	307.38a	179.32be	246.41ab	158.17b	236.43b
4 hours	110.48bd	168.62bde	134.04d	167.49bd	174.76bd
24 hours	144.65bc	211.99e	51.97f	110.61bfg	158.93ceg

* Values followed by the same letter are not significantly different ($p < 0.05$).

those treated with 0.1 mM Cd significantly lower cell wall eslasticity than controls. No significant correlation between Cd concentration and cell wall extensibility could de established.

DISCUSSION

Our results confirm earlier findings, that Cd causes severe decrease of extension growth in plants (Poschenrieder *et al.*, 1989). Our observation that extension growth of coleoptiles decreased almost immediately after supply to roots of Cd concentrations which were not translocated in detectable amounts to the coleoptiles (0.05 mM and 0.1 mM Cd treatments), suggests that inhibition of extension growth in the above-ground parts was not due to an *in situ* effect of Cd, but a consequence of the translocation of a root-induced signal. In plants exposed for 4 h to 1 mM Cd a significant amount of Cd was detectable in the coleoptile, but growth rate was less affected than that of plants receiving lower Cd supply.

Our results on *in vitro* extensibility of coleoptiles floated on control solutions are in line with the hypothesis that cell wall extensibility and elasticity decrease with plant age and increase of cell size (Tyree and Jarvis, 1982). The tissue Cd concentrations achieved by floating sectioned coleoptiles on Cd solutions were much higher than toxic tissue concentrations reported for maize plants (Bingham *et al.*, 1975). Nevertheless, Cd did not decrease but increase total extensibility of cell walls, which may be related to the smaller cell size in Cd-treated plants. In coleoptiles exposed to Cd, we only observed a transient decrease of elastic extensibility after 2 h exposure. Excepting coleoptiles from the 0.1 mM Cd treatment, values similar or even higher than that of controls were achieved after 24 h. Thus the short-term effect of Cd on cell wall elasticity in maize was clearly distinct from the long-term Cd effect described in *Phaseolus vulgaris* L. leaves (Barceló *et al.*, 1986).

CONCLUSIONS

We may conclude from our results, that the Cd-induced inhibition of extension growth in coleoptiles of *Zea mays* L. is initially not caused by a decrease of cell wall extensibility. Toxic effects in roots seem to be responsible for the initial growth inhibitory response in upper plant parts.

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