BioMetals 1993, **6**, 245–249

Responses of elongation growth rate, turgor pressure and cell wall extensibility of stem cells of *Impatiens balsamina* to lead, cadmium and zinc

Sharifah Barlian Aidid* & Hishashi Okamoto†

Laboratory of Plant Physiology, Biological Institute, Nagoya University, Nagoya, Japan;
**Present address: Physics Department, Universiti Kebangsaan Malaysia, Selangor, Malaysia;*
†Present address: Graduate School of Integrated Science, Yokohama University, Yokohama, Japan

Received 11 February 1993; accepted for publication 15 March 1993

Elongation growth rate of stem cells of *Impatiens balsamina* was inhibited by the heavy metals Pb^{2+}, Cd^{2+} and Zn^{2+} due to their suppression on cell wall extensibility. Effective turgor was also inhibited by Pb^{2+} and Cd^{2+} but it played a secondary role in reducing the stem cell elongation growth rate. The major rate-limiting factor for cell elongation growth was the cell wall extensibility. Furthermore, Cd^{2+} was found to be more toxic than Pb^{2+}, while Pb^{2+} was more toxic than Zn^{2+}.

Keywords: cell wall extensibility, heavy metals, *Impatiens balsamina*, stem cell elongation, turgor pressure

Introduction

Heavy metals such as lead, cadmium and zinc have received considerable attention during recent years as a result of increased environmental burdens from industrial, agricultural, energy and municipal sources. Although some metals, e.g. copper, zinc and magnesium, are essential for plant growth, they are generally toxic and able to interfere and inhibit plant development. They can ultimately cause the death of some plants when present at elevated levels in soils (Kabata-Pendias & Pendias 1984).

Inhibitory effects of heavy metals on plant growth have been demonstrated by many investigators through the measurement of growth parameters like root elongation (Trivedi & Erdei 1992, Symeonidis & Karataglis 1992), protein concentration (Sharma & Bisen 1992) and fresh weight change (Greger *et al.* 1991).

Plant cell enlargement is due to the irreversible expansion of the plant cell which results from two interdependent physical processes: water absorption and cell wall yielding. As first formulated by Lockhart (1965), plant cell enlargement, elongation or relative growth rate is a linear function of turgor pressure P in excess of a critical turgor Y (the yield threshold) and cell wall extensibility. In view of this, inhibitory effects on plant growth by heavy metals can be demonstrated by studying the responses of the cell elongation, the effective turgor pressure $(P - Y)$ and the cell wall extensibility of the stem cells of *Impatiens balsamina* when subjected to lead, cadmium or zinc treatments.

Address for correspondence: S. B. Aidid, Physics Department, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia. Fax: (+60) 3 8256086.

Materials and methods

Growth conditions

Seeds of *I. balsamina* (balsam) were soaked for about 8 h in a medium containing 0.5 mM $CaSO_4$, washed thoroughly with tap water and sown in wet vermiculate in porcelain pot. After 30 days of growth in 12 h dark and 12 h light at 28 °C in a growth chamber, segments of 20 mm long were cut from the stems of the balsam seedlings and used as experimental materials. The upper cut end of the segment was always 5 mm below the cotyledonary node of the seedling.

Measurement of growth parameters

The segment was mounted in a perfusion chamber and filtered standard experimental solution that contained

10 mM KCl, 0.1 mM $CaCl_2$, 30 mM sucrose and 0.01 mM indole acetic acid (IAA), pH 5.7, was forced into the xylem of the stem segment at room conditions by the application of a constant pressure. The details of the perfusion chamber and the perfusion technique have been described by Okamoto *et al.* (1978). The elongation growth of the segment was monitored with a displacement transducer and the growth rate would normally attain a steady value after 4 h of perfusion with the standard experimental medium at constant pressure. The cell wall extensibility was measured by the pressure-jump method which had been reported previously by Okamoto *et al.* (1989). The values of the wall extensibility were measured after the elongation growth rate of the segment had reached a steady level. Any segment with a steady elongation growth rate lower than 15 $\mu m\,h^{-1}$ after perfusion for 4 h with the standard experimental medium was normally discarded and replaced with a new segment.

To measure the extensibility of cell wall based on the pressure-jump method, a small increase in pressure was applied to the xylem vessel via the perfusion device. A new steady rate of elongation V_2 was obtained when this increased pressure was introduced for a period of time (3 min). This increase in pressure was known as pressure jump $\Delta P'$. The cell wall extensibility ϕ was calculated from V_2, $\Delta P'$ and the elongation rate V_1 just prior to the application of the pressure jump, as follows: $\phi = (V_2 - V_1)/\Delta P'$. Then the effective turgor $(P - Y)$ was calculated from V_1/ϕ based on Lockhart growth equations (1965). The values of ϕ were examined at 30 min intervals.

The effects of Pb^{2+}, Cd^{2+} and Zn^{2+} on these growth parameters were studied by perfusing the system with experimental solutions containing the composition of the standard solution with added appropriate concentration of the metal under study.

Results

The elongation growth rate of the stem segment perfused with the standard experimental solution, i.e. the control, reached a steady value after 4 h of perfusion and this value remained constant throughout the course of the experiment (Figure 1A). The measured values of cell wall extensibility ϕ for the control estimated by the pressure-jump method reached a steady value of $(3.8 \pm 0.4) \times 10^{-5}\ kPa^{-1}\,h^{-1}$, 5 h after excision. This value is the mean ± standard error from five measurements (Figure 1B). As seen from Figure 1(B) the values of ϕ decreased slowly with the passage of time. The values of the effective turgor $(P - Y)$ for the control were calculated from the data in Figure 1 (A and B) for the control, and are shown in Figure 1 (C). The value of $(P - Y)$ was 33.3 ± 5.0 kPa for the control, 5 h after excision. The change in the effective turgor of the control was the opposite of that of the cell wall

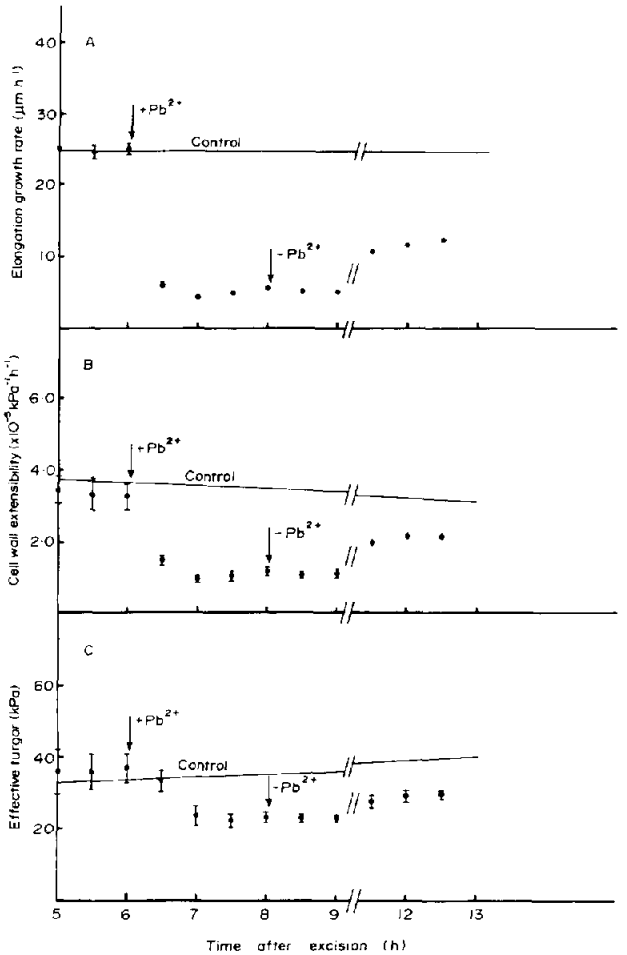

Figure 1. Control (——) and effect of 1.0 mM Pb^{2+} (●) on (**A**) the cell elongation growth rate, (**B**) the cell wall extensibility and (**C**) the effective turgor. Arrows with positive signs indicate lead addition to standard experimental medium and arrows with negative signs indicate lead removal from the standard experimental medium. For simplicity the mean data points ± standard errors bars for cell elongation, cell wall extensibility and effective turgor for all the control data are not shown. Each control data presented is averaged from five control experiments and each data point presented for each treatment is also the average of five similarly treated conditions.

extensibility, it became gradually large with the passage of time. For simplicity the mean data points ± standard error bars of cell elongation growth rate, cell wall extensibility and effective turgor, averaged from five control experiments, are not shown in all the figures presented as control.

When Pb^{2+} of 1.0 mM was added to the perfusion medium the elongation growth rate, cell wall extensibility and effective turgor were greatly reduced (Figure 1) but the inhibitory effect on these growth parameters by 1.0 mM Pb^{2+} was partially abolished

after 5 h of perfusion without Pb^{2+}. Pb^{2+} at concentrations lower than 0.2 mM had no significant effect on the stem cell elongation growth rate or the cell wall extensibility nor the effective turgor when the balsam stem segment was perfused for more than 4 h with perfusion medium containing such low concentration of Pb^{2+} (data not shown).

Cd^{2+}, at a concentration of 0.1 mM, exhibited an inhibitory effect on the elongation growth rate and the cell wall extensibility after a time lag of about 1 h. The inhibition of the effective turgor was less pronounced (Figure 2). When 1.0 mM Cd^{2+} was introduced into the standard perfusion solution an inhibitory effect occured immediately and the elongation growth rate together with the cell wall extensibility were reduced nearly to zero. At such a concentration of Cd^{2+}, significant inhibition of the effective turgor was noted. No recovery in the elongation growth rate and cell wall extensibility was noticed when Cd^{2+} was removed from the system (Figure 3).

Low concentrations of Zn^{2+} did not show any inhibitory effect on the growth parameters of the balsam stem (results not shown). However, when the Zn^{2+} concentration was raised to as high as 1.0 mM the inhibitory effect on the cell elongation growth rate and cell wall extensibility became apparent. The effective turgor was not significantly affected. The inhibitory effect was reversible when Zn^{2+} was removed from the system but it could not be abolished completely during the course of the experiment (Figure 4).

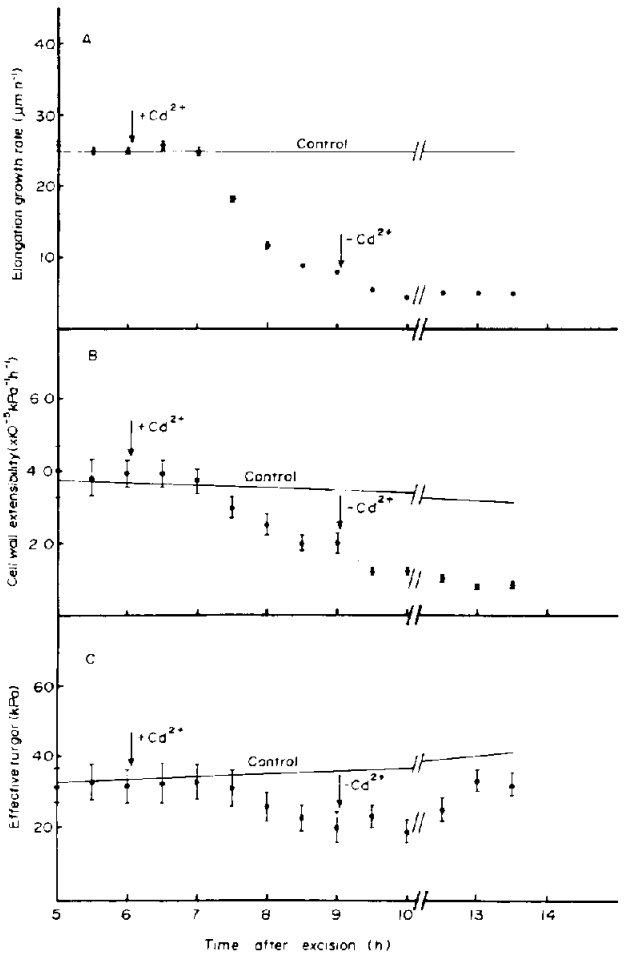

Figure 2. Effect of 0.1 mM Cd^{2+} on (**A**) the cell elongation growth rate, (**B**) the cell wall extensibility and (**C**) the effective turgor. The data for the control are similar to those in Figure 1. All symbols convey similar meanings as in Figure 1.

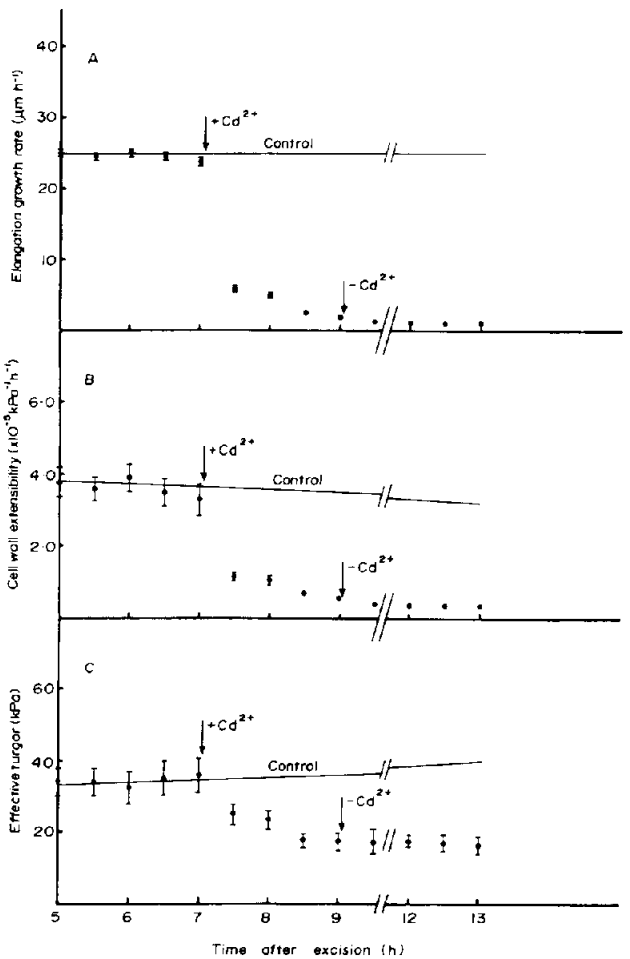

Figure 3. Effect of 1.0 mM Cd^{2+} on (**A**) the cell elongation growth rate, (**B**) the cell wall extensibility and (**C**) the effective turgor. The data for the control are similar to those in Figure 1. All symbols convey similar meanings as in Figure 1.

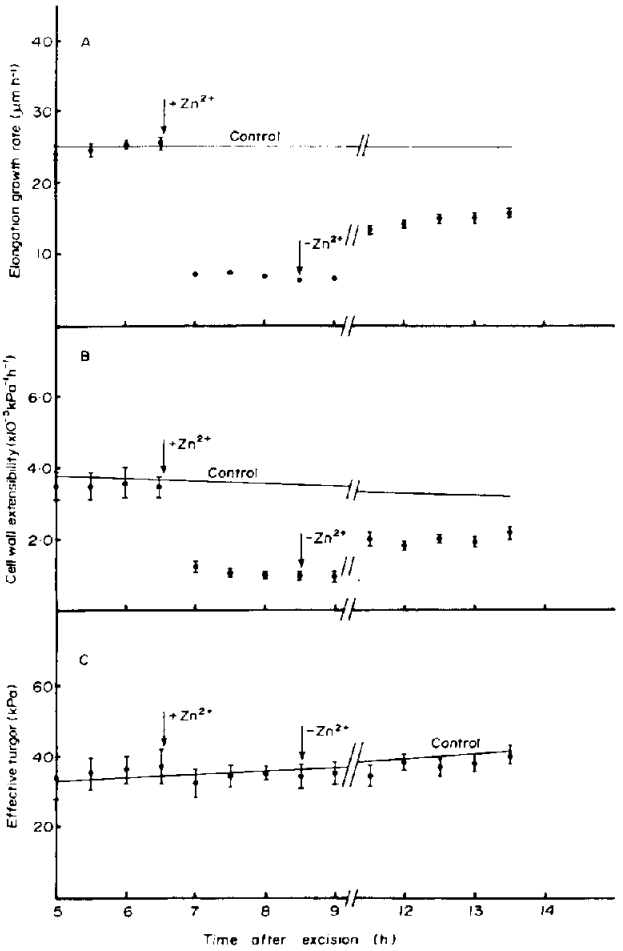

Figure 4. Effect of 1.0 mM Zn^{2+} on (**A**) the cell elongation growth rate, (**B**) the cell wall extensibility and (**C**) the effective turgor. The data for the control are similar to those in Figure 1. All symbols convey similar meanings as in Figure 1.

Discussion

The enlargement of plant cells is considered to be due to the irreversible yielding of the cell wall to the stress produced by cell turgor. As such, two simultaneous processes are required for cell enlargement: (i) water uptake and (ii) irreversible expansion of the cell wall as predicted by Lockhart growth equations (Lockhart 1965). Okamoto *et al.* (1978) have designed a pressurized intra-organ perfusion system where the two proton pumps (an outer electrogenic ion pump located on the cell membrane of the organ surface and an inner electrogenic ion pump located on the boundary membrane between the parenchyma symplast and the xylem vessel) could be measured and studied separately. From their studies water and solute uptake for the generation of turgor or osmotic pressure have been found to correlate with the activity of the xylem proton pump operating at the xylem/symplast interface (De Boer *et al.* 1985, Okamoto & Katou 1988) and membrane permeability; whereas the proton pump operating at the organ surface was found to be responsible for the loosening of the cell wall (Mizuno *et al.* 1980).

This study showed that the inhibitory effect on elongation growth rate of balsam stem cells by 1.0 mM Pb^{2+} was due to the decrease in cell wall extensibility and the effective turgor (Figure 1). Aidid & Okamoto (1992) found that low concentrations of Pb^{2+} (0.2 mM) depolarized the respiration-dependent component of the xylem/symplast membrane potential or the xylem proton pump without any significant inhibition of the elongation growth rate and inhibition on the elongation growth rate became significant when 0.5 mM Pb^{2+} was used. This could mean that the inhibitory effects of Pb^{2+} on the elongation growth rate could be due to the damage at the sites responsible for the active component of the surface membrane potential which plays an important role in the operation of the surface proton pump, which is responsible for the loosening of the cell wall for cell wall extensibility. However, the change in membrane permeability and the suppression on the electrogenic component of the xylem/symplast membrane potential or in other words the xylem proton pump which are responsible for the uptake of water and nutrients for the generation of turgor pressure, might play secondary roles in cell elongation.

Cd^{2+} inhibited growth at much lower concentrations than Pb^{2+} and Zn^{2+} (Figures 1, 2 and 4). The inhibition was irreversible even at low concentrations of Cd^{2+}. Although at such low concentrations of Cd^{2+} the effective turgor was not significantly inhibited, the elongation growth showed significant inhibition (Figure 2). This indicated that cell wall extensibility might be the growth parameter that had more control on the elongation growth rate of the stem cells of balsam plants.

In this study the cell wall extensibility was greatly reduced by 1.0 mM Zn^{2+} without showing any significant reduction in the effective turgor and yet the elongation growth rate was greatly reduced (Figure 4). Similar high concentrations of Zn^{2+} (1.5 mM) were found to decrease the elongation growth rate of stem cells of the balsam plant without depolarizing the xylem/symplast membrane potential, i.e. without inhibiting the proton pump operating at the xylem/symplast membrane responsible for the turgor or osmotic pressure, as demonstrated by Aidid & Okamoto (1992). Hence the results of this

study are consistent with the findings of Aidid & Okamoto (1992).

Stunted growth in plants caused by heavy metals could be due to inhibition of cell elongation growth. Cell elongation growth could be controlled mainly by cell wall extensibility, although cell turgor plays an important role. All of the three heavy metals studied here showed an inhibitory effect on cell elongation by suppressing cell wall extensibility. In other words, heavy metals might be extremely detrimental to the proton pump operating at the surface responsible for cell wall extensibility. Sharma & Bisen (1992) also found that heavy metals like Hg^{2+} and Cd^{2+} induced inhibition of light-induced proton efflux in *Anabaena flos-aquae*.

Many heavy metals are toxic to plant growth; however, the degree of toxicity of each metal to a particular species of plant is quite different. In the present study it was demonstrated that the degree of toxicity by the three metals towards the stem cell elongation growth of *I. balsamina* was $Cd^{2+} > Pb^{2+} > Zn^{2+}$.

Acknowledgments

The support of Universiti Kebangsaan Malaysia and Nagoya University for S.B.A. during her periods of sabbatical leave is gratefully acknowledged. The authors wish to thank the Iwatani Naoji Foundation of Japan for the financial support. The support of the Malaysian government in providing the Research and Development grant (IRPA 4–07–03–02) to S.B.A. is also greatly appreciated.

References

Aidid SB, Okamoto H. 1992 Effects of lead, cadmium and zinc on the electric membrane potential at the xylem/symplast interface and cell elongation on *Impatiens balsamina*. *Environ Exp Bot* **32**, 439–448.

De Boer AH, Katou K, Mizuno A, *et al.* 1985. The role of electrogenic xylem pumps in K^+ absorption from the xylem of *Vigna unguiculate*: the effects of auxin and fusicoccin. *Plant, Cell Environ* **8**, 579–586.

Greger M, Brammer E, Lindberg S, *et al.* 1991 Uptake and physiological effects of cadmium in sugar beet (*Beta vulgaris*) related to mineral provision. *J Exp Bot* **42**, 729–737.

Kabatan-Pendias A, Pendias H. 1984 *Trace Elements in Soils and Plants*. Boca Raton, FL: CRC Press.

Lockhart JA. 1965 An analysis of irreversible plant cell elongation. *J Theoret Biol* **8**, 264–275.

Mizuno A, Katou K, Okamoto H. 1980 Structure and function of the elongation sink in the stems of higher plants I. Effects of anoxia and IAA on the growth rate and the spatially separate electrogenic ion pumps. *Plant Cell Physiol* **21**, 395–403.

Okamoto H, Ichino K, Katou K. 1978 Radial electrogenic activity in the stem of *Vigna sesquipedalis*: involvement of spatially separate pumps. *Plant, Cell Environ* **1**, 179–284.

Okamoto H, Katou K. 1988 Demonstration of respiration-dependent water uptake and turgor generation in *Vigna hypotyl*. *Plant Cell Physiol* **29**, 509–515.

Okamoto H, Liu Q, Nakahori K, *et al.* 1989. A pressure-jump method as a new tool in growth physiology for monitoring physiological wall extensibility and effective turgor. *Plant Cell Physiol* **30**, 979–985.

Sharma SK, Bisen PS. 1992 Hg^{2+} and Cd^{2+} induced inhibition of light induced proton efflux in the cyanobacterium *Anabaena flos-aquae*. *BioMetals* **5**, 163–167.

Symeonidis L, Karataglis S. 1992 Interactive effects of cadmium, lead and zinc on root growth of two metal tolerant genotypes of *Holcus lanatus* L. *BioMetals* **5**, 173–178.

Trivedi S, Erdei L. 1992 Effects of cadmium and lead on the accumulation of Ca^{2+} and K^+ and on the influx and translation of K^+ in wheat of low and high K^+ status. *Physiologia Plantarium* **84**, 94–100.