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Effects of Pb^{2+} on Root Growth, Cell Division, and Nucleolus of *Zea mays* L.

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Lead (Pb) exists in many forms in natural sources throughout the world. It has been demonstrated that its main sources in the atmosphere come from smelting and refinery operations from Pb mining, metallurgical industries, gas and industrial activities (Alloway and Ayres 1994). Pb toxicity in plants does not appear when organic matter and other mineral nutrients are in abundant supply (Baumhardt and Welch 1972), and its toxicity occurs most commonly on waste heaps from mining operations where the organic matter and nutrient content of the soil are low (Woolhouse 1983). The problems of Pb pollution have increased due to the widespread use of metals for industrial and agricultural purposes. Lead toxicity in many nontolerant plants was reported to be associated with the disturbance of mitosis (Levan 1945; Ahlberg et al. 1972; Ramel 1973; Wierzbicka 1989; Liu et al. 1994), toxicity to nucleoli (Liu et al. 1994), induction of binuclear cells (Swieboda 1976) and inhibition of root elongation (Lane and Martin 1980). There are reports on Pb poisoning of leaves, leading to chlorosis (Johnson et al. 1977; Johnson and Proctor 1977) and decrease in photosystem II (Branquinho et al. 1997). Pb is also thought to inhibit activity of enzymes (Hampp et al. 1973).

Maize (*Zea mays* L.) is one of the most important cereal crops. However, few investigations of Pb on cell division in root tip cells of *Z. mays*, especially on nucleoli, are reported. The aim of this investigation was to increase our understanding of the effects of different concentrations of Pb^{2+} on the root growth, cell division and nucleolus of *Z. Mays*, and to know what is the difference with other plants treated with Pb such as, *Allium cepa* (Liu et al. 1994) and *Brassica juncea* (Jiang and Liu 1999).

MATERIALS AND METHODS

The seeds of *Z. mays* were kindly provided by the Institute of Food Crops, Tianjin Academy of Agricultural Sciences, Tianjin, P.R. China, and were used in the present investigation. The test Pb ion concentrations were made up from lead nitrate ($Pb(NO_3)_2$), ranging from $10^{-2} \sim 10^{-5}$ M. The solutions were prepared in tap water (pH = 6.5). Tap water was used for the control experiment. The seeds were soaked for 24 h in tap water before starting the experiments, and then, were treated with different concentrations of Pb solutions. They were allowed to germinate producing roots in Petri dishes at temperature 26 °C for 24 and 48 h. They were protected from direct sunlight. The test liquids were changed regularly every 24 h. Twenty root tips in each treatment group were cut and fixed in 3 parts 95% ethanol : 2 parts acetic acid for 4 to 5 h and hydrolyzed in 5 parts 1 M hydrochloric acid : 3 parts 95% ethanol : 2 parts acetic acid for 4-5 min at 60 °C. For the observation of chromosomal morphology, 10 of the 20 root tips were squashed in Carbol Fuchsin solution (Li 1982) and for the observation of nucleolus changes, the others were squashed in 45% acetic acid, dried, and on day 2 stained

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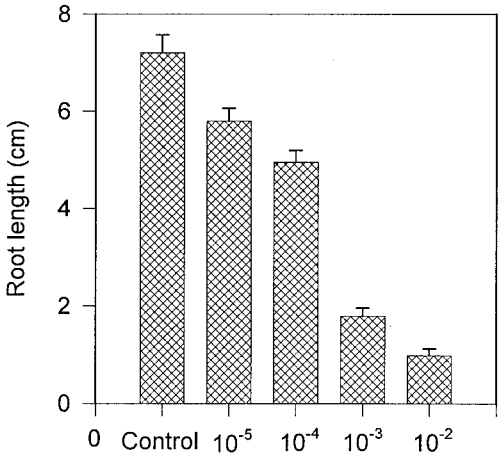

Figure 1. Effects of Pb^{2+} on root growth of *Zea mays* (48h)

with silver nitrate (Li et al. 1990; Liu and Jiang 1991).

Fiskesjö (1985) introduced the standard types of aberrant chromosome behavior referred to in the modified *Allium* test. It is very important to monitor the chromosomal changes in root tip cells of plants caused by metal ions. This method was used in the present investigation. Also, the root length was measured and the morphology of the roots was observed in order to understand the effects of Pb on root growth.

RESULTS AND DISCUSSION

The effects of Pb^{2+} on root growth of *Z. mays* varied with the different concentrations of lead nitrate solutions used (Fig. 1). Pb had an inhibitory effect on the root growth at all concentrations (10^{-5}~10^{-2} M) used during the entire treatment (48 h). At 10^{-3} M Pb, the root length was strongly inhibited, and at above 10^{-2} M Pb, there was slight root growth after 24 h of treatment.

The effects of Pb on the morphology of the roots also varied with the concentrations of lead nitrate. At 10^{-5} M, the morphology of the roots was normal during the whole treatment (48 h). At 10^{-4} M, the roots showed a twisted appearance after 48 h of treatment. The roots became slightly brownish or blackish. At 10^{-3} and 10^{-2} M, the most roots appeared brownish and blackish and rotten.

The mitotic index reflects the frequency of cell division and it is regarded as an important parameter when determining the rate of root growth. As can be seen from Table 1, the mitotic index decreased progressively with increased Pb concentration. This fits well with the above mentioned effects of lead nitrate on root growth. The mitotic index can be correlated with the rate of root growth, suggesting that the inhibition of root growth resulted from inhibition of the cell division.

Chromosome aberrations have been used as a measure of reproductive success and as a method for the detection of possible genetic damage by environmental agents (such as herbicides, insecticides, fungicides and heavy metals) in plants for many years, and can provide both qualitative and quantitative data on the effects (Grant 1978). Figure 2A-I in the present investigation shows the effects of Pb^{2+} on root tip cells of *Z. mays*.

Table 1. Effects of lead nitrate ($Pb(NO_3)_2$) on cell division in root tips of *Zea mays*

Time (H)	Treatment (M)	Mitotic index (‰)	Number of cells	Normal dividing cells in %		Anomalous dividing cells in %			Anomalous mitoses in %
				Metaphases	Anaphases	c-mitosis	Chromosome bridges	Chromosome stickiness	
24	Control	38	500	55.6	43.6	0.2	0.6		0.8
	10^{-5}	35	500	58.6	39.6	0.7	0.4	0.7	1.8
	10^{-4}	32	500	50.2	41.2	4.4	0.4	3.8	8.6
	10^{-3}	29	500	38.8	39.9	12.5	1.9	6.9	21.3
	10^{-2}	23	310	7.7	13.5	66.3		12.5	78.8
48	Control	39	500	51.2	47.9	0.2	0.7		0.9
	10^{-5}	33	500	53.4	42.6	1.1	0.8	2.1	4.0
	10^{-4}	30	500	46.6	40.3	7.4	0.5	5.2	13.1
	10^{-3}	27	390	27.9	7.2	49.7		15.2	64.9
	10^{-2}	15	200			72.1		27.9	100.0

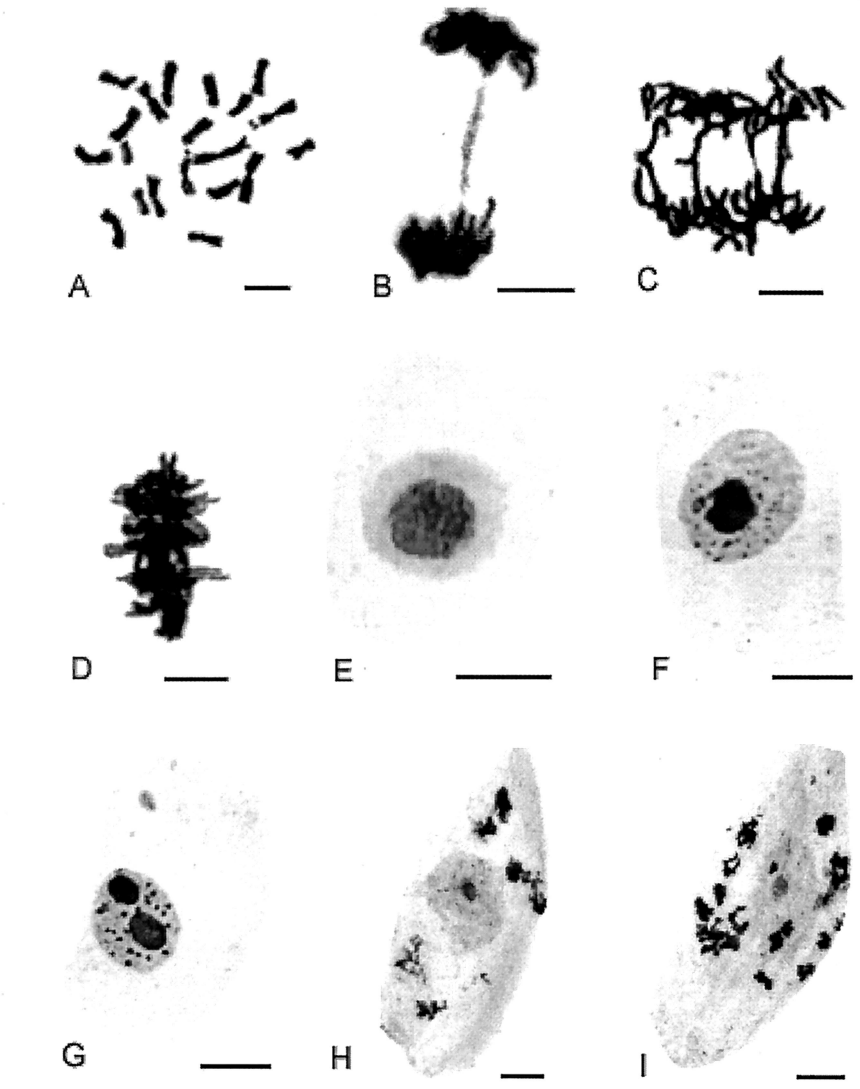

Figure 2A-I. The effects of Pb^{2+} on root tip cells of *Z. mays*. (A) c-metaphase (10^{-4} M Pb^{2+}, 48h,); (B-C) Chromosome bridges (10^{-3} M Pb^{2+}, 24h,); (D) Chromosome stickiness (10-2 M Pb^{2+}, 48 h); (E) Control cell (tap water, 48 h); (F) Some silver-stained particulate material scattered in the nucleus and nucleolar material is on the way from the nucleolus to the nucleus (10^{-3} M Pb^{2+}, 24 h); (G) A few of particulate silver-stained material released from the nucleus into cytoplasm (10^{-3} M Pb^{2+}, 48 h); (H-I) more and more silver-stained materials of particulate nature in the cytoplasm. Nucleolar remnants in the nucleus becoming smaller in size and their silver staining reaction weaker (10^{-2} M Pb^{2+}, 48h). Scale = 5 μm.

C-mitosis was observed in the root tip cells of all the treated groups after treatment with Pb. As it shows from Table 1, the frequency of cells with c-mitosis progressively and obviously increased with increasing Pb^{2+} concentration and time of expose. The severely condensed chromosomes are randomly scattered in the cell (Fig. 2A). Pb has c-mitotic effects as its main effects after 24 h of treatment at concentrations of 10^{-2} to 10^{-3} M Pb^{2+}. This type of abnormality was produced as a result of inhibition of spindle fiber formation. Pb^{2+} is thought to be extremely c-mitotically active.

Chromosome bridges at anaphase are due either to breaks in chromosomes or chromatids (often resulting in fragments) or to chromosome stickiness (disturbing the normal cell division). Anaphase bridges involving one or more chromosomes (Fig. 2B, C) were observed in all Pb treatments.

Klásterá et al. (1976) and McGill et al. (1974) indicated that chromosome stickiness arises from improper folding of the chromosome fiber into single chromatids and that chromosomes become attached to each other by subchromatid bridges. The chromosome pattern reflects highly toxic effects, usually of an irreversible type, and probably leads to cell death.The frequency of cells with chromosome stickiness (Fig. 2D) also progressively increases with increasing Pb concentration and duration of treatment. Almost all of the sticky chromosomes exhibit c-mitosis in the treated roots with Pb from 10^{-4} to 10^{-2} M.

Normally, the diploid nucleus of *Z. mays* contains one or three nucleoli (Fig. 2E). After 24 h of treatment with 10^{-3} M Pb, nucleolar material is on the way from the nucleolus to the nucleus and some small particulate silver-stained material was observed together with nucleolus/nucleoli in the nucleus of some root tip cells (Fig. 2F). The frequency of cells with this type progressively increases with increasing Pb concentration. The amount of this particulate material increased progressively and nearly occupied the whole nucleus, and some particulate silver-stained material was released from the nucleus into cytoplasm (Fig. 2G). The more and more nucleolar material was extruded from the nucleus into the cytoplasm and the nucleolar material in the cytoplasm is disintegrated, forming the silver-stained particulate material, which increases progressively and aggregates into irregular shapes, as duration of the treatment increases (Fig. 2H, I). The nucleolar remnants in nucleus become smaller in size and their silver staining reaction becomes weaker (Fig. 2H, I). Irregularly shaped nucleoli were also found in most of the cells with higher concentration Pb (10^{-2} M).

The results in the present investigation indicated that lead inhibits the root growth of *Z. mays* at all the concentrations of Pb^{2+} (10^{-5} ~10^{-2} M) and has obviously a inhibitory effect on it at concentrations (above 10^{-3} M Pb^{2+}); the Pb effects on chromosomal morphology include c-mitosis and anaphase bridges and chromosome stickiness. Pb, at higher concentrations (10^{-3} ~ 10^{-2} M Pb), can induce toxic effects on nucleoli, causing some particulate silver-stained material scattered in the nuclei and inducing the nucleolar material released from the nucleus into the cytoplasm. Once the nucleolus was severely affected, the root growth of *Z. mays* almost or completely stopped.

Pb is not generally considered as an essential element for the growth of plants, but appears to stimulate plant growth in some plants in small amounts (Dou 1988; Jiang and Liu 1999). The results of the present experiment are more or less the same as those described by Liu et al. (1994) and Jiang and Liu (1999) for the effect of Pb^{2+} on the root growth and root tip cells of *Allium cepa* and *Brassica juncea*, but with a few differences. 1) Pb has a stimulatory effect on root growth of *Brassica juncea* at concentrations of 10^{-4} ~ 10^{-5} M and obviously inhibits root growth only at above 10^{-3} M Pb^{2+} (Jiang and Liu 1999), while Pb inhibits the root growth of *Allium cepa* (Liu et al. 1994) and *Z. mays* in the present study at 10^{-4} M Pb^{2+}. Pb has no beneficial effects on root growth of *Allium cepa* and *Z. mays*. 2) Pb^{2+} is thought to be extremely c-mitotically active. Pb has c-mitotic effects as its main effects in this investigation. The frequency of cells with c-mitosis increases as Pb concentration and duration of treatment increase which is in agreement with the observations by Levan (1945), Wierzbjcka (1988) and Liu et al. (1994), while at the same concentration the frequency of cells with c-mitosis

increases sharply when compared with the findings noticed by Jiang and Liu (1999). 3) Pb, at higher concentrations of 10^{-3} to 10^{-2} M, can induce the nucleolar material released from the nucleus into the cytoplasm in the root tip cells of *Z. mays*. This phenomenon has not been observed in the treatments in which the authors studied the effects of Pb on the nucleoli in the root tip cells of *Allium cepa* (Liu et al. 1994) and *Brassica juncea* (Jiang and Liu 1999). Apparently, *Z. mays* is more sensitive to Pb on an equimolar basis, compared with *Allium cepa* and *B. juncea,* as for the toxic effects on the nucleoli.

Inhibition of cell division in root apical meristems is a rapid response to lead treatment as indicated in the present investigation. Lane et al. (1978) indicated that the inhibiting effects of Pb on growth may arise from the interference of Pb with auxin-regulated cell elongation. The cell membrane is an obvious initial site for toxic metal action. Brown and Wells (1988) indicated that cations bound to the cell wall can be replaced efficiently by other cations with a stronger affinity for those binding sites. It should be noted that Ca^{2+} can be replaced by Pb^{2+}, because they have similar radii (Ca^{2+} 0.99 Å; Pb^{2+} 1.25 Å) (Chai and Zhu 1983). The results from Gabara and Golaszewska (1992) confirm not only the role of calcium in maintaining the normal structure of mitotic spindle but of chromatin and chromosomes as well (Clarkson and Hanson 1980; Marme 1986). Calcium ions added to lead solution can increase the mitotic index and decrease the number of cells with chromosome fragments and chromatid bridges in the cortex cells of *Pisum sativum* (Gabara and Golaszewska 1992). Calcium alone and calcium added to Pb solutions enhanced the size of a single nucleolus as well as the total nucleolar dimension in cortex cells of pea (Gabara et al. 1995).

From what indicated, we suggest that the toxic phenomena of lead on root growth, cell division and nucleoli may result from uptake and accumulation of lead in the root tip cells and inhibition of calcium uptake. This inhibition is mainly the result of cation competition for, or blocking of, binding sites, leading to the restricted transport of Ca^{2+} across the plasma membrane into the cytoplasm to varying degrees. Calmodulin (CaM) is a small Ca^{2+}-binding protein that acts to transduce second messenger signals into a wide array of cellular responses (Zielinski 1998). Because of the result of cation competition, the level of free Ca^{2+} in the cell is very low and CaM does not activate Ca-ATPase (Xu 1985), leading to failure in regulation of calcium concentration and to disturbance of the physiological activities of CaM. Means and Dedman (1980) showed that CaM was located specifically in the mitotic spindle and involve in the processes of chromosome movement through regulation and control of depolymerization and polymerization of the microtubules (Li and Sun, 1991). If the regulation and control functions of CaM is affected, CaM does not regulate and control microtubules, or tubulin synthesis may be inhibited. Our data showed that there are a large number of cells with c-mitosis at higher concentrations ($10^{-3} \sim 10^{-2}$ M Pb), suggesting that lead mainly disturbs and destroys the proceeding of cell division, showing a large amount of the cells with c-mitosis caused by destroying the formation of the spindle fibers.

As is well known, the nucleolus is the metabolic centre of RNA. The integrity of the nucleolus depends on the existence of Ca^{2+} (Wang 1988). Li and Sun (1991) studied the CaM distribution in the nucleoli of root tip cells of maize, using immunoelectron microscopic technique, and found that CaM might play a role in regulating and controlling RNA synthesis and that the nucleolar behaviour might be regulated and controlled during interphase. Using enzymelabelled immunohistochemistry, Cheng et al. (1991) found that there are more CaM in cytoplasm, but the nuclei, especially nucleoli also contained CaM, after studied the CaM distribution in the cultured tabacco cells. This finding may support the idea that the phenomenon of nucleolar material extrusion from the nuclei into the cytoplasm and the disintegration of this material may result from calcium deficiency in the cells and CaM failing to activate its functions. However, the mechanism behind the expression of this phenomenon needs to be further investigated.

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