CALCIUM EFFECT ON THE CONTENT OF DNA AND NYS-STAINED NUCLEAR, NUCLEOLAR AND CYTOPLASMIC PROTEINS IN CORTEX CELLS OF PEA (*PISUM SATIVUM* L.) ROOTS TREATED WITH HEAVY METALS

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ABSTRACT

Using cytophotometric procedures, following Feulgen-NYS staining, the measurements of DNA and nuclear, nucleolar and cytoplasmic protein contents in cortex cells of pea roots growing for 144 h in calcium and/or heavy metals (Cd^{2+} , Cr^{3+} , Pb^{2+}) presence were made. All tested metals treatment reduced the number of nuclei in 4C DNA class and induced appearance of nuclei with DNA amount below 2C, that was expressed in diminished DNA content. The level of NYS proteins in cells underwent also reduction. In lead presence protein content diminished in nucleus. On the other hand, increased amount of nuclear, nucleolar and cytoplasmic proteins was observed in material treated with cadmium while only of nucleolar protein content in chromium presence. In root cells treated with tested metals protein content in nucleus was related with ploidy level, disturbances in this relation appeared in nucleolus and mostly in cytoplasm.

Calcium added to chromium and mostly to lead solutions diminished the toxic effect of these metals that was demonstrated by an increase in DNA content, although calcium alone reduced DNA amount in nucleus due to lower number of 4C nuclei accompanied by appearance of 1C and 1-2C DNA classes. Calcium in different ways affected protein content changed by metal treatment. Present in cadmium solution it caused a further reduction in protein content in nucleus, nucleolus and cytoplasm but increased nuclear and cytoplasmic protein when added to lead, and nucleolar proteins – in chromium solution. Moreover, calcium ions presence in metal solutions did not restore the relationship between ploidy level and nucleolar and cytoplasmic NYS stained proteins and it did not disturb the relation existing in nucleus.

KEY WORDS: *Pisum sativum* L., root cortex cells, heavy metals, calcium effect, DNA content, NYS protein content.

INTRODUCTION

Numerous authors have described toxic effects of heavy metals on plant cells (Bonaly et al. 1980, Melnishuk et al. 1982, Romaniuk and Gabara 1988, Gabara and Romaniuk 1989, Wierzbicka 1989, Visviki and Rachlin 1994). However, the results are often different and sometimes contradictory. The mechanisms of toxic action at the cellular level are still not known well.

In one of earlier papers (Wojtyła-Kuchta and Gabara 1991) we described the effect of cadmium ions on DNA and NYS-stained proteins content in pea root cells. At that time we could not study the influence of other metals i.e. chromium and lead appearing in soil in a consequence of liming. Therefore, in the present research we not only repeated experiments with cadmium alone to check whether the obtained results are comparable with previous ones but also we wanted to establish effect of other heavy metals mentioned above. Moreover, we tried to find out whether calcium added to tested metal so-

lutions changed DNA and protein content, since calcium ions affected dry mass of nuclei and cytoplasm, their dimensions (Romaniuk and Gabara 1988, Gabara and Romaniuk 1989) as well as DNA (Gabara et al. 1992) and protein synthesis (unpubl) in pea root cells growing in the presence of cadmium, chromium and lead at the concentration of 10^{-4}M .

MATERIAL AND METHODS

Cortex cells of meristem of six-day-old primary roots of pea (*Pisum sativum* L.) were the object of the present studies. Seeds sterilized with 0.5% soap solution, washed in tap then distilled water were imbibed in solutions of: cadmium (CdCl_2), chromium [$\text{Cr}_2(\text{SO}_4)_3 \times 6\text{H}_2\text{O}$], lead (PbCl_2) at the concentration of 10^{-4}M each in the presence or absence of calcium (10^{-3}M). After 24 h seeds were treated with above solutions for 144 h as described earlier (Romaniuk and Gabara 1988).

The apical parts of roots (0.5 cm long) were fixed in MAF and double stained (DNA + NYS), according to procedure proposed by Gaub et al. (1975). After 1 h hydrolysis in 4N

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Fig. 1. Mean DNA content ($\bar{x} \pm SE$) in cortex cells of pea roots growing in the presence of heavy metals and after addition of calcium.

HCl and Feulgen reaction the roots were restained in 0.1% solution of NYS in 1% acetic acid for 30 min at room temperature.

DNA and protein content in nucleus, nucleolus and cytoplasm were determined cytophotometrically with Zeiss (Jena) histophotometer. Measurements of relative amount of DNA per nucleus were made at 500 nm and nuclear, nucleolar and cytoplasmic proteins at 480 nm. DNA values 2C and 4C were calculated from 20 telophase and 10 prophase nuclei, respectively. The content of DNA and protein was expressed in arbitrary units (AU). In each experimental series 100 cells from 10 roots were measured.

RESULTS

All tested metals but mostly lead treatment diminished DNA content in nuclei of pea root cortex cells (Fig. 1). Calcium ions presence alone as well as in cadmium solution caused a further reduction in DNA content but diminished toxic effect of lead (Fig. 1). Only in chromium treated material addition of calcium did not change visibly the pattern of DNA amount.

Mean DNA content results from the frequency of distribution of nuclei with different 2C DNA content (Fig. 2). While in control material three classes of nuclei were present (2C, 2-4C and 4C), in cadmium and lead treated cells four classes appeared, i.e. 1-2C, 2C, 2-4C, 4C and 1C, 1-2C, 2C, 2-4C, respectively. Moreover, in lead presence a 4C DNA class disappeared. Contrary to control material where 2C and 4C DNA nuclei dominated, in metal presence 2C nuclei were the most numerous.

In calcium treated root cells five classes of nuclei were observed (Fig. 2) while after this ion addition only to cadmium solution the number of classes increased to six (Fig. 2). Calcium added to metal solutions as well as this ion alone changed the pattern of particular C DNA nuclei distribution. Appearance of nuclei with low DNA content (1C, 1-2C) accompanied by smaller number of 2C, 2-4C, 4C nuclei along with the presence of 8C nuclei (Pb²⁺) explained the diminution in DNA content in studied material. On the other hand, an increase in the number of nuclei containing 4C DNA and appearance of 4-8C class was responsible for, similar as in

Fig. 2. Partition of the DNA content ($\bar{x} \pm SE$) among the cells of pea roots treated with calcium and/or heavy metals.

Fig. 3. NYS proteins content ($\bar{x} \pm \text{SE}$) in cortex cells of pea roots growing in the presence or absence of calcium.

control material (water), DNA content in pea roots treated with calcium and chromium.

Protein content

The content of NYS stained proteins in studied material was the highest in cytoplasm and the lowest in nucleolus (Fig. 3).

Only cadmium presence increased content of nuclear, nucleolar and cytoplasmic proteins. Enhanced level of NYS proteins in the other metals presence i.e. in chromium and lead was seen in nucleolus and in cytoplasm, respectively. Among tested metals only lead treatment diminished NYS proteins in nucleolus.

While calcium alone diminished the amount of nucleolar protein it did not affect the level of protein in nucleus and cytoplasm. Changes in protein content appeared also after this ion addition to metal solutions (Fig. 3). Lower protein content in calcium presence was noticed in nucleus, nucleolus and cytoplasm of cells treated with cadmium and higher protein content in nucleus and cytoplasm of cells treated with lead. Moreover, a marked increase in nucleolar protein content appeared in cells growing in chromium with calcium solution.

Nuclear protein content was related with ploidy level (Fig. 4). Such a relationship was observed in material growing both in water and heavy metal presence as well as in cells growing in calcium or calcium-cadmium solutions. Lack of this regularity was observed only in nuclei with very low DNA content (1C, 1-2C) in calcium alone or when this ion was added to cadmium (0-1C, 1C).

Nucleolar protein content in water or calcium treated material was related to level of ploidy (Fig. 5). Heavy metals alone or in calcium solution disturbed the pattern of this relation in different ways. Lack of relationship was noticed in chromium treated material and after calcium addition. On the other hand, in cadmium and lead treated pea root cells in the presence or absence of calcium such relation existed between groups of nuclei i.e. 1-2C and 2C, 2-4C, 4C DNA (Cd^{2+}); 1C, 1-2C and 2C (Pb^{2+}); 0-1C, 1C, 1-2C, 2C and 2-4C, 4C DNA ($\text{Cd}^{2+} + \text{Ca}^{2+}$), or 1-2, 2C and 2-4, 4C DNA ($\text{Pb}^{2+} + \text{Ca}^{2+}$).

Generally cytoplasmic proteins amount was not related to the level of ploidy (Fig. 6). The disturbances in this relation were noticed in cadmium or chromium treated material with or without calcium ions. Symptoms of relationship between nuclear DNA and cytoplasmic NYS protein amount were seen in groups of nuclei with low amount of DNA and 2-4, 4C in both controls (H_2O , Ca^{2+}) and in lead in the presence or absence of calcium ions.

DISCUSSION

Results of the present experiments clearly demonstrate negative effect of heavy metals on nuclear DNA content that is in agreement with the data obtained by other authors (Falchuk et al. 1975, Bonaly et al. 1980) as well as our earlier investigations on cadmium (Wojtyła-Kuchta and Gabara 1991). Moreover, lead presence was more toxic than chromium or cadmium. Heavy metals interacted directly with DNA molecules and in consequence blocked DNA replication (Berger and Skinner 1974, Bianchi et al. 1977, Wallace and Anderson 1984), although, in Bonaly et al. (1980) opinion DNA synthesis continued slowly in cadmium presence. The lower intensity of radioactive thymidine incorporation to pea root in tested metals presence (Gabara et al. 1992) seemed to indicate that slower DNA synthesis could be responsible for reduction in DNA amount. Lower number of 4C nuclei also confirmed the above suggestion. On the other hand, the highest reduction in amount of DNA in lead presence could not be attributed to lower DNA synthesis since the intensity of ^3H thymidine uptake to these cells was on the similar level as in control material (Gabara et al. 1992).

Fig. 4. Relationship between nucleolar protein content ($\bar{x} \pm \text{SE}$) and ploidy level in pea root cells treated with calcium and/or heavy metals.

Fig. 5. Relationship between nuclear protein content ($\bar{x} \pm \text{SE}$) and ploidy level in pea root cells treated with calcium and/or heavy metals.

Contrary to our earlier results indicating the highest protein amount in nucleolus and the lowest in nucleus (Wojtyła-Kuchta and Gabara 1991) in the present studies in cadmium treated cells the most abundant in NYS protein was cytoplasm and the less – nucleolus. Although in the present experiment cytophotometric analysis of protein content was carried out with another model of cytophotometer the reason of differen-

ces in the level of NYS protein is difficult to explain. The second intriguing problem was the contradictory cadmium effect on nuclear protein content observed earlier (Wojtyła-Kuchta and Gabara 1991) in comparison with the present studies. It cannot be excluded that differences mentioned above might indicate from different species specificity of pea used in previous and present experiments.

Fig. 6. Relationship between cytoplasmic protein content ($\bar{x} \pm SE$) and ploidy level in pea root cells treated with calcium and/or heavy metals.

Our present investigations indicated that only lead treatment diminished protein amount in nucleus. The other metals did not change protein content or slightly increased it in nucleus, nucleolus and cytoplasm. An enhancement in the apoplasmic protein content and its polypeptide composition was described and well documented also in barley leaves (Brune et al. 1994). In the presence of cadmium excess in roots of maize seedlings Tukendorf and Rauser (1990) discovered that during synthesis of phytochelutins a diminution in the level of total glutathione and in free cysteine content occurred. It cannot be excluded that high protein content in cells treated with cadmium indicated synthesis of phytochelutine. On the other hand, lower NYS protein content in lead presence might be explained as reduction in phytochelutine level. Why cadmium and lead affected protein synthesis in different ways remains obscure.

Calcium added to heavy metal solutions as well as this ion alone changed DNA and NYS protein content in pea root cells. Our results partly confirm the data obtained by other authors demonstrating that calcium diminished toxic effects of heavy metals (Garland and Wilkins 1981, Gabbrilli and Pandolfini 1984, Gabara and Romaniuk 1989, Gabara et al. 1992).

The mechanism of calcium action in diminution of metals toxicity is far to knowledge although these ions presence in tested metal solutions increased DNA synthesis and labelling index in pea roots (Gabara et al. 1992), affected RNA synthesis in cytoplasm and nucleolus (Gabara et al., unpublished work) and diminished radioactive lysine incorporation to pea root cells (Gabara in prep.). In Kawasaki and Moritsugu (1987) opinion calcium reduced heavy metals absorption that was expressed in their lower toxicity. Another explanation of diminished negative effects of heavy metals was based on calmoduline action. This calcium binding protein by interaction with metals reduced their concentration in cells (Means et al. 1982, Roberts et al. 1986) and in consequence their toxicity.

It is difficult to say at the moment whether diminished metals toxicity was caused by their reduced absorption or concentration in cells. We hope that determination of heavy metals concentration after calcium addition will help us to resolve this problem.

On the other hand, calcium action seems to be complex since added to cadmium solution it caused a further reduction in DNA and NYS-protein content while in lead solution increased or even stimulated DNA as well as nuclear and cytoplasmic protein amount. In Matsumoto and Takyu (1986) opinion calcium ion regulates template activity of chromatin, the enhanced as well as diminished level of NYS-proteins might result from disturbances in template activity of chromatin.

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WPŁYW WAPNIA NA ZAWARTOŚĆ DNA I BARWIĄCYCH SIĘ NYS BIAŁEK JĄDROWYCH, JĄDERKOWYCH I CYTOPLAZMATYCZNYCH W KOMÓRKACH KORY PIERWOTNEJ KORZENI GROCHU (*PISUM SATIVUM* L.) TRAKTOWANYCH METALAMI CIĘŻKIMI

STRESZCZENIE

Stosując metody cytofotometryczne po barwieniu Feulgen-NYS, zmierzono zawartość DNA oraz białek jądrowych, jąderekowych i cytoplazmatycznych w komórkach kory pierwotnej korzeni grochu rosnących 144 godz. w roztworze wapnia i/lub metali ciężkich (Cd^{2+} , Cr^{3+} , Pb^{2+}). Obecność testowanych metali powodowała redukcję liczby jąder w klasie 4 C oraz pojawienie się jąder o zawartości poniżej 2C, co uwidoczniło się w spadku zawartości DNA. Zmianom uległ poziom białek barwiących się NYS w komórkach. W obecności ołowiu zmalała zawartość białek w jądrze. Z drugiej strony, w materiale traktowanym kadmem wzrosła zawartość białek jądrowych, jąderekowych i cytoplazmatycznych a traktowanym chromem – tylko białek jąderekowych. W komórkach korzeni rosnących w obecności testowanych metali obserwowano relacje między zawartością białek w jądrze a stopniem ploidalności, zakłócenia tej relacji uwidoczniły się w jąderku a zwłaszcza w cytoplazmie.

Mimo, iż sam wapń obniżał poziom DNA w jądrze, dodany jedynie do roztworu ołowiu zmniejszał toksyczne skutki działania tego metalu, co ujawniło się we wzroście zawartości DNA. Wapń w różny sposób wpływał na zmiany w zawartości białek spowodowane działaniem metali. Dodany do roztworu kadmu powodował dalszą redukcję zawartości białek w jądrze i jąderku. Natomiast wapń w obecności ołowiu – zwiększał poziom białek jądrowych i cytoplazmatycznych a dodany do roztworu chromu – białek jąderekowych. Ponadto, jony wapnia w roztworach metali nie powodowały odtworzenia relacji między stopniem ploidalności a poziomem białek barwiących się NYS w jąderku i cytoplazmie, nie zakłócały równocześnie istniejących relacji w jądrze.

SŁOWA KLUCZOWE: *Pisum sativum* L., komórki kory pierwotnej korzeni, metale ciężkie, rola wapnia, zawartość DNA, zawartość białek NYS