The changes in the proteins profile induced by cadmium stress in flax (*Linum usitatissimum* L.)

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Summary

Influence of $Cd(NO_3)_2$ concentration on the process of flax (*Linum usitatissimum* L.) germination and changes occurring on the level of synthesized proteins or peptides were evaluated. The studies did not reveal differences in seed germination in control conditions and in presence of cadmium solution. Extracts obtained from two-week-old seedlings were exposed to ammonium sulphate saturation (0–30 and 50–80%). Ion exchange chromatography on DEAE Cellulose revealed appearance of proteins or peptides rich in the cysteine residues which were not present in control group (absorbance 280 and 254 nm). Particular proteins were observed in three extract fractions eluted by NaCl gradient (0.3–0.5 M NaCl) what could suggests formation of (Cys-rich) protein or peptides in presence of the cadmium solution during detoxification process. The largest absorbance indicated a high concentration of (Cys-rich) proteins or peptides related probably to incubation with cadmium solution observed at extract fraction eluted with 0.4 M NaCl.

Key words: *Flax, heavy metals, soil contamination, phytoremediation*

INTRODUCTION

In many regions of the world, content of metals have exceeded permissible limits which excludes the usage of crop in food production. There are two ways of heavy metals contamination management: by obtaining plants resistant to high contamination for phytoremediation purposes or resistant genotypes that have no or very low ability to accumulate heavy metals from the soil [1]. The new concept of the existence of resistant genotypes cultivated on contaminated soil but with acceptable level of heavy metals in their edible parts was investigated in the Chinese cabbage and other crop genotypes [2]. Certain plant species could take part in soil cleaning due to ability for accumulation of heavy metals in phytoremediation process. The pot experiments conducted on common flax revealed suitability of flax usage for phytoremediation of the soil contaminated by cadmium [3]. Application of plants that not only accumulate cadmium in harvestable parts but also are useful for non-food purposes could be the best solution. In the future usage of plant, cell culture could be a very valuable tool in the development of variable plants for phytoremediation processes, also for flax [4]. Studies on potential energy of crops like flax, hemp and peanut have revealed that these plants have high tolerance to cadmium contamination and can accumulate high amounts of heavy metals in their shoots [5]. The presence of cadmium in environment influences organisms' metabolism and plants exposed to this kind of stress produce protective phytochelatins [6-8] or methallothioneins [9]. Phytochelatins are oligomers of glutathione produced by the enzyme phytochelatin synthase (Cys-rich peptides). They act as chelators, while metallothioneins are a family of cysteine-rich, low molecular weight proteins and have the capacity to bind heavy metals through the thiol group of its cysteine residues. Both protein and peptides play a role in detoxification process. The aim of the preliminary study was determination of the changes of the proteins profile induced by cadmium stress in flax (*Linum usitatissimum* L.) and its potentially usage in phytoremediation process. The influence of applied $Cd(NO_3)_2$ concentration on process of germination and changes on the level of synthesized protein were evaluated. The continuation of the initial investigations on the characteristic of the (Cys-rich) proteins or peptides could allow to increase the heavy metals accumulation in flax, a very important attribute for phytoremediation process.

MATERIAL AND METHODS

Flax (*Linum usitatissimum* L.) seeds of Cristal cultivar were germinated (50 seeds per Petri dish of 11 cm in diameter) on filter papers moistened with distilled water (control) or in the test solution (0.5 mM $Cd(NO_3)_2$) at 21°C in darkness. The two-week-old seedlings were homogenized with 20 mM Tris/HCl buffer (pH 8.8) and the homogenate was centrifuged at 10,000x g for 60 minutes [9]. The extracts

obtained from seedlings were saturated using ammonium sulphate precipitation (0–30% and 50–80%). After centrifugation, supernatant was dialyzed against 20 mM Tris-HCl buffer (pH 8.8). Anion-exchange chromatography on DEAE Cellulose equilibrated with 20 mM Tris/HCl buffer (pH 8.8) was performed. Proteins were eluted with a step gradient of NaCl (0.2, 0.3, 0.4, 0.5, 0.6 and 0.8 M NaCl, respectively) and 1.5 ml fractions were collected. The protein content was examined in all fractions by measurements of absorbance at 280 nm (protein concentration) and 254 nm (Cys-rich proteins and peptides concentration), respectively. Proteins containing cysteine residues could potentially bind cadmium ions.

RESULTS AND DISCUSSION

The conducted studies did not reveal any difference in seed germination in control environment and in presence of cadmium, what confirmed the results obtained in another experiment carried out on *Phragmites australis* [10]. The extract was purified using ion exchange chromatography on DEAE Cellulose to separate proteins. The analysis of control extract and extract with 0.5 mM $Cd(NO_3)_2$ after ammonium sulphate saturation to 30% and dialysis revealed appearance of the proteins rich in cysteine residues (presence of peaks at 254 nm absorbance) in extract fraction no. 12, 18 and 24 (gradient 0.3, 0.4 and 0.5 M NaCl, respectively) from seedlings incubated with cadmium solution (fig. 1.). These peaks were not observed in the extract from control seedlings incubated with water. In the control extract the fraction No. 17, containing proteins interconnected with cadmium were observed, what suggested that material used in the studies contained Cysrich proteins or peptides. The research carried out on more purified extracts after 80% ammonium sulphate saturation (50–80%) and dialysis showed presence of the proteins with the cysteine residues at extract fraction No. 13, 18 and 24 (gradient 0.3, 0.4 and 0.5 M NaCl) in the sample cultivated in cadmium presence (fig. 2.).

Separation using ion exchange chromatography on DEAE Cellulose revealed the appearance of proteins which were not present in control group (absorbance 280 and 254 nm). Proteins synthesized in flax, probably in response to cadmium presence, were eluted by 0.3, 0.4 and 0.5 M NaCl gradient what can suggest the occurrence of proteins. The largest absorbance value, indicating the highest concentration of Cys-rich proteins probably induced by incubation with cadmium solution, was observed at extract fraction eluted with 0.4 M NaCl after saturation both 0–30% and 50–80% of $(NH_4)_2SO_4$. Other studies, focused on flaxseed protein extract, showed a presence of phytochelatin-like or methallothionein-like components in extract fractions eluted at 0.45 and 0.5 M NaCl gradient [7].

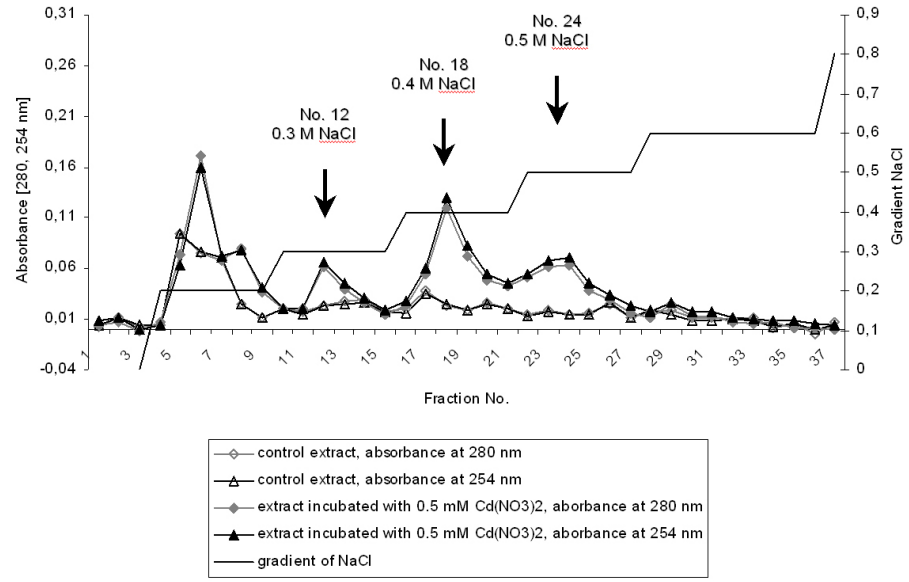

Figure 1. Elution profile (DEAE Cellulose) of protein extracts from flax seedlings cultured in 0.5 mM $Cd(NO_3)_2$ solution in comparison with control incubated in water after 30% ammonium sulphate saturation and dialysis

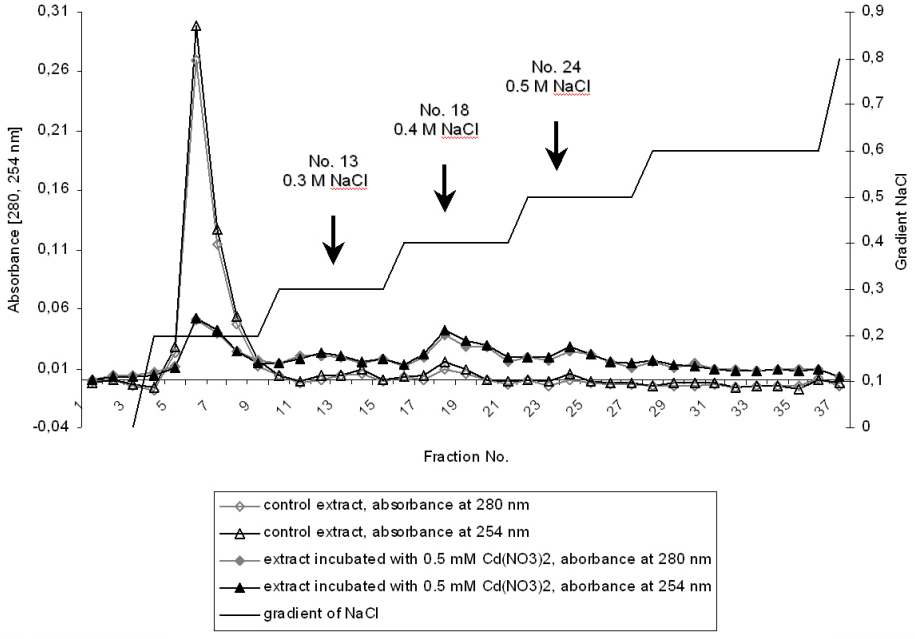

Figure 2. Elution profile (DEAE Cellulose) of protein extracts from flax seedlings cultured in 0.5 mM $Cd(NO_3)_2$ solution in comparison with control incubated in water after 80% ammonium sulphate saturation and dialysis

CONCLUSIONS

The initial studies did not reveal difference in seed germination in control conditions and in the presence of cadmium. Particular proteins were observed in three fractions, eluted by NaCl gradient (0.3–0.5 M), what suggests formation of different Cys-rich proteins or peptides in presence of cadmium solution, which could be used for the detoxification process in these plants. The highest absorbance value, indicating the high concentration of proteins or peptides rich in the cysteine residues, probably induced by incubation with cadmium, was observed in extract fraction eluted with 0.4 M NaCl at 0–30% and 50–80% $(NH_4)_2SO_4$ saturation. Continuation of our studies enables characterization of Cys-rich proteins or peptides produced in response to the presence of the cadmium solution. The knowledge concerning peptides or proteins (Cys-rich) in the future could be helpful to increase heavy metals accumulation in plants and their usage in the process of phytoremediation.

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ZMIANY PROFILU BIAŁEK INDUKOWANE PRZEZ STRES KADMOWY W LNIE (*LINUM USITATISSIMUM* L.)

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S t r e s z c z e n i e

Oceniano wpływ obecności $Cd(NO_3)_2$ na kiełkowanie lnu (*Linum usitatissimum* L.), a także zmiany zachodzące na poziomie syntetyzowanych białek lub peptydów. Przeprowadzone badania nie wykazały różnic w kiełkowaniu nasion tak w warunkach kontrolnych, jak i w obecności roztworu kadmu. Ekstrakty uzyskane z dwutygodniowych siewek poddano wysalaniu siarczanem amonu (0–30 i 50–80% wysycenia). Rozdział na kolumnie jonowymiennej DEAE Cellulose ujawnił pojawienie się białek lub peptydów bogatych w reszty cysteinowe, które nie były obecne w grupie kontrolnej (absorbancja 280 i 254 nm). Poszczególne białka lub peptydy obserwowano w trzech frakcjach ekstraktu, przy wymywaniu gradientem NaCl (0,3–0,5 M NaCl), co sugeruje powstanie białek lub peptydów bogatych w cysteinę w środowisku zawierającym kadm. Największą wartość absorpcji wskazującej na wysokie stężenie białka lub peptydu (bogate w cysteinę) związanego prawdopodobnie z inkubacją z roztworem kadmu wykazano we frakcji ekstraktu wymywanej 0,4 M NaCl.

Słowa kluczowe: *len, metale ciężkie, zanieczyszczenie gleby, fitoremediacja*