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CADMIUM TOXICITY IN COINCUBATION SYSTEM *Pisum sativum/Plectonema boryanum*

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Abstract. Increased activities of antioxidative enzymes superoxide dismutase and peroxidase were established in accordance with growing cadmium concentration in the nutrition medium. It was shown that Cd caused two-fold increase of superoxide dismutase activity in experimental system I and 40% increase in experimental system II. There were almost no changes measured in experimental system III. On the contrary, peroxidase activity was inhibited and reached 60% of the control values in model system I. The rates of enzyme activity in experimental systems II and III remained close to the control. An increase of free proline content up to 500% towards the control was measured in experimental system I, at 30 mg/l $CdCl_2$. The enhancement was about 40% in experimental systems II and III. Cd was accumulated mainly in the algal cells – 1621 mg/kg at 10 mg/l $CdCl_2$ and 4450 mg/kg at 30 mg/l $CdCl_2$ in coincubation system III. The application of supernatant prevented Cd accumulation in pea roots up to 50%.

Keywords: coincubation system, *Pisum sativum*, *Plectonema boryanum*, cadmium, superoxide dismutase, peroxidase, proline.

AIMS AND BACKGROUND

Heavy metals, depending on their oxidation states, can be highly reactive and, as a consequence, toxic to most organisms. They are produced by an expanding variety of anthropogenic sources suggesting an increasingly important role for this form of pollution. The toxic effect of heavy metals appears to be related to production of reactive oxygen species and the resulting unbalanced cellular redox status.

Ameliorating environmental stresses typically involves the production of antioxidant enzymes that scavenge and detoxify the highly reactive oxygenated compounds. Hence, when heavy metal stress is present, concentrations and/or activities of antioxidants in plants are generally observed to be high[1].

Changes in antioxidant enzyme activities are often results of the contamination of the environment by prooxidants and, thus, these enzymes are good

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biochemical markers of stress and their increased activity may attest to a potential for remediation[2].

Cadmium, when not detoxified rapidly enough, may trigger, via the disturbance of the redox control of the cell, a sequence of reactions leading to growth inhibition, stimulation of secondary metabolism, lignification, and finally cell death. This view is in contrast to the idea that cadmium results in unspecific necrosis[3].

Free proline has been shown to play an important role in ameliorating environmental stress in plants and microorganisms, including heavy metal stress[4].

Significant accumulation of proline, usually thought to be an indicator of water stress, occurred at higher level of Cd. The excess levels of Cd also decreased the concentrations of soluble protein and chlorophylls and increased the ratio of chlorophyll *a*/*b* (Ref. 5).

One strategy to reduce heavy metal pollution is to use microalgae. These organisms, due to their ubiquitous occurrence in nature have been studied extensively in this regard. They can sequester heavy metal ions by absorption and adsorption as do by other microorganisms. This ability may be induced in response to stress by toxic heavy metal exposure. The use of microalgae for metal removal has the potential to achieve greater performance at a lower cost than the conventional technologies. This is consistent with the recent trend for growing interest in biosorbent technology for removal of trace amounts of metals from waste soils and waters[6].

A better understanding of the biological effects and response mechanisms of cyanobacteria to heavy metals exposure could be used to develop these bacteria for use in bioremediation. All organisms must possess mechanisms that regulate metal ion accumulation and, thus, avoid heavy metal toxicity. These mechanisms include resistance to metals that are always toxic to the cell and serve no beneficial role, such as cadmium and mercury, and also include resistance to metals such as copper, iron and zinc, which are toxic at high concentrations, but are absolutely essential in trace amounts. One of the most important mechanisms by which bacteria combat heavy metal exposure and subsequent accumulation is through internal metal sequestration. In the prokaryotic cyanobacteria metal ion sequestration within the cell is performed by the class II metallothioneins that sequester metal, thus preventing accumulation of potentially toxic forms of metal ions within the cell[7].

The aim of this study is to assess the role of co-cultivation of pea plants together with a cyanobacterium and to investigate the ability of the plant from the coincubation system to overcome cadmium toxicity.

EXPERIMENTAL

The coincubation system used in the present work consisted of 7-day old pea plants (*Pisum sativum* L.) and cyanobacterium *Plectonema boryanum* G o m., strain 594 (exponentially growing). Three different variants were elaborated to implement 10-day cultivation of pea: water + Cd (experimental system I), supernatant + Cd (experimental system II), algal suspension + Cd (experimental system III). Cadmium was added at concentrations 10 and 30 mg/l, as $CdCl_2$. Cultivating conditions are described in detail in our previous work[8]. The second leaf pair of pea plants was used for analysis.

Activity of superoxide dismutase (SOD, EC 1.15.1.1), (U/mg protein), was determined according to Beauchamp and Fridovich[9].

Peroxidase activity (PER, EC 1.11.7), (ΔE/min/mg protein), was measured after Hart et al.[10], and soluble protein content – after Bradford[11].

Proline content (μg proline/g fresh weight) was measured spectrophotometrically at 518 nm according to Bates[12].

The accumulation of cadmium in the stems and roots of pea plants, and in the cyanobacterial biomass was determined by means of atomic absorption spectrophotometer.

Results were presented as percent of control, which was accepted to be 100%. Data were averaged of triplicate measurements.

RESULTS AND DISCUSSION

Plant response to excess cadmium in the growth medium was assessed by a decrease in the leaf and root weight and length, a change in SOD and PER activities, and an accumulation of proline. Higher cadmium concentration (30 mg/l) inhibited growth of the stems about 50% (coincubation system I) and caused insignificant decrease in coincubation systems II and III. Fresh weight and length of pea plants were slightly affected in all model systems (data are not shown).

Generation of toxic oxygen species and consequently induced oxidative stress is considered as a possible mechanism of heavy metals action[13,14]. Our investigations of variations in the activities of SOD and PER sustained such an understanding. Activity of SOD in pea plants was significantly increased in experimental system I. It was increased with 87% towards the control at 10 mg/l $CdCl_2$ and reached the highest levels at 30 mg/l $CdCl_2$ – 196% above control. The same trend was observed when cultivating medium contained algal supernatant, even though it was not so obvious. SOD activity was increased with 20 and 40% at both Cd concentrations. Coincubation together with cyanobacterial suspension did not cause significant changes in the enzyme activity compared to the control variant (Fig. 1).

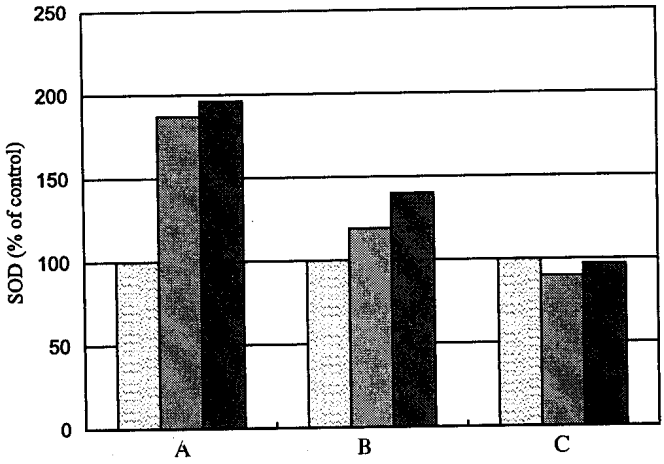

Fig. 1. Superoxide dismutase activity in pea leaves, treated with $CdCl_2$
Legend: A – experimental system I; B – experimental system II; C – experimental system III (all figures follow the same legend);
control ; 10 mg/l $CdCl_2$; 30 mg/l $CdCl_2$

PER activity of pea leaves in experimental system I was increased with 50 and 100% (at 10 and 30 mg/l $CdCl_2$). The rates of rising of enzyme activities were the same as these of SOD measured in coincubation system I. Cultivation in coincubation systems II and III led to a slightly increase of PER activity. When supernatant was added to the growth medium, 20% increase was registered at both cadmium concentrations. The activity of PER was 35% higher than the control in experimental system III, at 30 mg/l $CdCl_2$ (Fig. 2).

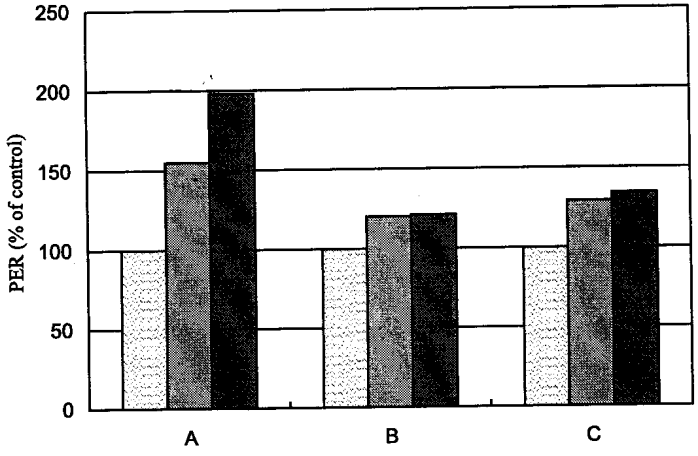

Fig. 2. Peroxidase activity in pea leaves, treated with $CdCl_2$

Maxima of activity of all three forms of peroxidase – soluble, ionically-bound, and covalently-bound peroxidases, in *M. crystallinum*, a halophyte, were found at the highest accumulation of cadmium[15].

It was established that in various species Cd-stressed plants the most important antioxidative role might be played by different enzyme – SOD, ascorbate- and guaiacol-peroxidase or catalase[16,17]. Probably it is due to the different sensitivity of SOD to H_2O_2 that is assumed to be the main product of reactive oxygen species during heavy metal stress. Another reason of the variation in level of antioxidative enzymes may be the binding of metal ions to the enzyme active center as suggested by Ref. 3. The results indicate that antioxidant enzymes are good markers of environmental stress, but they are only a component of a complex system responsible for plant tolerance to stress conditions.

Changes in the free proline as one of the major stress markers were investigated. The analysis of free proline content showed a drastic rise for pea plants grown in experimental system I – 400 and 500% towards the control at 10 and 30 mg/l $CdCl_2$. Proline content was slightly influenced in the medium containing supernatant or algal biomass. It was measured an increase about 20% at 10 mg/l $CdCl_2$ and 30-40% at higher cadmium concentration in coincubation systems II and III (Fig. 3).

Proline accumulation in the third leaf pair of *M. crystallinum* and in the roots occurred at a relatively low cadmium content[15]. It was reported that elevated proline reduced free radical levels in response to osmotic stress, as measured by malondialdehyde production and significantly improved the ability of plants to grow in conditions of high salinity[4]. These findings shed new light on the regulation of proline biosynthesis in plants and its role in reducing oxidative stress, in addition to its accepted role as an osmolyte.

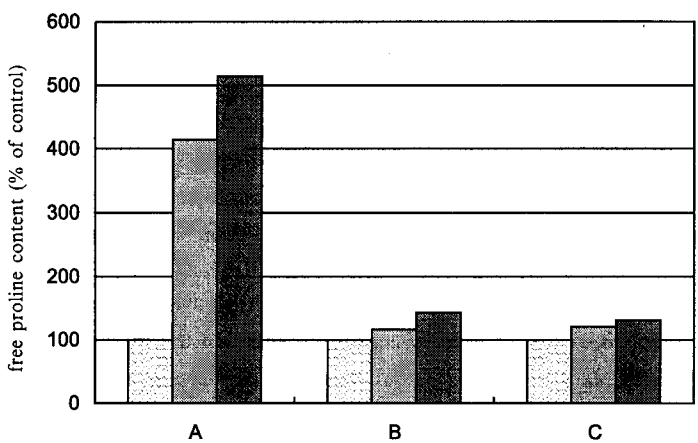

Fig. 3. Free proline content in the second leaf of *Pisum sativum*, treated with CdCl2

It was indicated[18] that Cd does not directly bind to free proline. It reveals a predominantly tetrahedral coordination of cadmium by sulphur or by 2 sulphur and 2 oxygen atoms in algal cells of different type. Analyses of the fatty acid products of free-radical damage, malondialdehyde, and determinations of glutathione reduced/glutathione oxidised ratios indicated that free proline reduces free radical damage and maintains a more reducing environment in the cells, particularly when they are exposed to toxic heavy metals (50 μM Cd). These results suggested that the free proline acts as an antioxidant in cadmium-stressed cells.

As it was shown in our experiments, the proline content was slightly increased in coincubation systems II and III. It allowed considering that adding of supernatant and algal biomass into the growth medium prevented to some extent the development of oxidative stress symptoms in pea, treated with $CdCl_2$.

Our results confirmed that plants accumulate cadmium mainly in the root system. According to Ref. 15, resistance to excess cadmium in the medium was achieved by its predominant accumulation in roots, where excess cadmium is compartmentalised in the apoplast and seems to be subjected to detoxification through pectate formation.

We found that Cd accumulation in pea plants followed the changes of concentration. The major quantity of toxic metal (87 mg/kg dry weight) in the stems and leaves was accumulated in the plants growing on the water + 30 mg/l $CdCl_2$ (experimental system I). Cadmium content decreased nearly three-fold in the stems and leaves of plants coincubated with supernatant and cyanobacterial suspension (experimental systems II and III). There was only a weak correlation between cadmium accumulation and the extent of a biomass decrease in the leaves, when cadmium concentration in the medium was toxic (data are not shown).

Strongly increased levels of Cd accumulation were observed in the pea roots. Its content was highest in the water medium containing 30 mg/l $CdCl_2$ – 386 mg/kg dry weight. In the coincubation systems II and III cadmium content decreased nearly three times, and the lowest values were registered at concentration 10 mg/l (Table 1).

Algae and cyanobacteria respond to heavy metals by induction of several antioxidants, including diverse enzymes and synthesis of low molecular weight compounds. At high, or acute, levels of metal pollutants, damage to algal cells occurs because reactive oxygen species levels exceed the capacity of the cell to cope. At lower or chronic levels algae accumulate heavy metals[19].

Analysing the content of Cd accumulated in the dry cyanobacterial biomass from the coincubation system III it was found that it contained significantly higher content of accumulated cadmium ions in comparison with pea plants from the same model system. It was measured Cd content of 1621 and

Table 1. Accumulation of Cd (mg/kg dry weight) in the shoots and roots of pea (*Pisum sativim* L.) and *Plectonema boryanum* biomass after cadmium treatment

Variants	*Pisum sativum* Cd (mg/kg dry weight)		*Plectonema boryanum* Cd (mg/kg dry weight)
	stems	roots	
Water + 10 mg/l $CdCl_2$	70 ± 3.8	351 ± 16.8	–
Water + 30 mg/l $CdCl_2$	87 ± 6.2	386 ± 19.9	–
Supernatant + 10 mg/l $CdCl_2$	42 ± 2.0	133 ± 9.47	–
Supernatant + 30 mg/l $CdCl_2$	26 ± 1.8	139 ± 6.88	–
Algal suspension + 10 mg/l $CdCl_2$	37 ± 2.5	145 ± 10.4	1621 ± 105
Algal suspension + 30 mg/l $CdCl_2$	24 ± 1.7	162 ± 9.92	4450 ± 286

4450 mg/kg dry weight, respectively at 10 and 30 mg/l $CdCl_2$ (Table 1). That confirmed expected high levels of accumulation of Cd from the cyanobacterium *Plectonema boryanum*, thus providing better conditions for growing and development of higher plant in the coincubation system.

CONCLUSIONS

It was clearly indicated that toxic effect of Cd was due to the enhanced generation of reactive oxygen species. Pea leaves could be characterised by a high activity of the antioxidant enzymes superoxide dismutase and peroxidase, and an increase of free proline at high cadmium concentrations.

It is not known whether algal cells or supernatant protect plants from Cd-induced injury by preventing access of cadmium to sensitive extra- or intracellular sites, or by excreted or intrinsic metal-chelators, or by other defense systems.

Our results are a good reason to presume that algal suspension and products released in the supernatant as a result of metabolic processes of *Plectonema boryanum* accumulate and detoxify cadmium from the growing medium. Hence, the development of stress-tolerant plant-cyanobacterial associations may be a promising new strategy for phytoremediation and soil amelioration measures.

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