Effect of uranium and cadmium uptake on oxidative stress reactions for *Phaseolus vulgaris*

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Abstract. Bean seedlings were grown under controlled conditions on a Hoagland solution. Ten-day-old seedlings were exposed to 0, 0.1, 1, 10, 100 and 1000 μM U or 0.5 and 1 μM Cd. Following 7 days' exposure, plants were sampled for determination of contaminant uptake, biometric parameters (shoot and root length, area of primary leaves, weight of shoot, root and primary leaves) and activity of enzymes involved in the plant's anti-oxidative defense mechanisms. Generally we did not observe a significant difference in plant development between control and treated plants based on biometric parameters. Enzyme activities in roots were stimulated with increasing contaminant concentrations (though generally not significantly). However, for roots exposed to 1000 μM U, enzyme activity was generally significantly reduced. In shoots no significant difference in the defense mechanism between the treatments was observed.

Introduction

Large areas of land have been contaminated by radionuclides and fission-by products including uranium from nuclear weapons facilities, above ground nuclear testing, nuclear reactor operations, improper waste storage practices and nuclear accidents. Radioactive contamination of the environment surrounding facilities where uranium or uranium bearing minerals have been mined and processed has occurred in many countries. Uranium is reported to be the most frequent radionuclide contaminant in ground and surface water soils of the United States Department of Energy facilities (Entry et al. 1996). Uranium is both radiotoxic and chemotoxic, yet the mechanisms of toxicity has been predominantly studied for man and for some animal species (Ribera et al. 1996). On the other hand, little information on uranium toxicity at the cellular level is available for plants. Chemical

toxicity would be predominantly caused by the aqueous hexavalent ion UO_2^{+2}. General physiological phenomena occurring at the cellular level following radiation are direct effects on molecules or indirectly through a water radiolytic reaction resulting in the production of highly reactive oxygen species (ROS).

In plants, environmental adversity often leads to the increase in formation of ROS. Under natural (non-stress) conditions ROS occur in the plant cell and therefore plants possess several anti-oxidative defense mechanisms to control the redox state of the cell which is essential for normal physiological and biochemical functioning. The defense systems comprise anti-oxidative enzymes (superoxide dismutases, peroxidases, catalases, glutathione reductase) and antioxidants (e.g. glutathione, ascorbate,…). Heavy metal toxicity results in an enhancement of the anti-oxidative defense system (Clijsters et al. 1999, Cuypers et al. 2002). Resistance to such conditions may be correlated with enzymes in oxygen detoxification (Bowler et al. 1991).

The present study aims to analyze the biological effects induced by bioaccumulation of uranium by *Phaseolus vulgaris.* Following a 1 week exposure, plant development and the capacity of enzymes involved in the anti-oxidative defense mechanism of the plant were analyzed. The enzymes studied cover enzymes for the acorbate-gluthatione cycle (Gluthatione reductase), some enzymes capable of quenching reactive oxygen species (Guaiacol and syringaldazine peroxidase, superoxide dismutase), and enzymes which catalyze reactions leading to the reduction of $NAD(P)^+$.

Materials and methods

Bean seedlings were grown in plastic bowls containing 3 L of a modified Hoagland nutrient solution (fourth strength for macronutrients and full strength of micronutrients) which was adjusted to pH 5.0 by addition of KOH. Dwarf beans, *Phaseolus vulgaris* L. cv. Limburgse vroege, were grown under controlled conditions. Bean seeds received a cold treatment (+4°C) for 3 days to break dormancy and to synchronize germination. They were transferred to the growth chamber to germinate for 4 days between water-soaked rock wool. Subsequently the seed coats were removed and the seeds were placed on perforated polystyrene floats in an aerated modified Hoagland's nutrient solution, which was renewed every other day. The conditions in the growth chamber were a 10 h photoperiod at 65 % relative humidity and day/night temperatures of 22°C. Light was supplied by cool white fluorescent lamps at a photosynthetic photon flux density of 165 $\mu molm^{-2}s^{-1}$ at the leaf level. Ten days old bean seedlings were treated with different concentrations of ^{238}U, supplied as uranylnitrate (0, 0.1, 1, 10, 100 and 1000 µM), or Cd, supplied as 0.5 or 1 µM $CdSO_4$. Per treatment, 2 containers were prepared containing each 10 plants. After application of the metals to the nutrient solution, plants were harvested after 1, 2, 4 and 7 days, processed and stored prior to the measurements. The solution was changed after every sampling. Only the results after 7 days exposure will be presented here. Plants were analyzed for shoot and

root length, leaf area, weight of roots, primary leaves and the rest of the plant. The roots from the control and metal treated plants were rinsed in 10 mM $CaCl_2$ to remove the adhering uranium or cadmium. For the determination of the tissue metal content, roots and primary leaves were dried at 100°C. The metal content was determined by ICP-MS after wet digestion of the dried material in concentrated HCl. Prior to the measurements of enzyme activities, samples of ~0.5 g were frozen in liquid nitrogen and stored at -70°C.

Plant tissue was homogenised (Smeets et al. 2005) and enzymes were measured spectrophotometrically. Guaiacol or syringaldazine peroxidase capacities (GPOD, SPOD, EC.11.1.7.) was measured according to Bergmeyer (1974) and Imberty et al. (1984), respectively. Superoxide dismutase (SOD, EC 1.15.1.1) catalyses the conversion of superoxide radicals into dioxygen and hydrogen peroxide and is measured according to McCord and Fridovich (1969). Analysis of the glutathione reductase (GR, EC 1.6.4.2) capacity was based on the reduction of GSSG, using NADPH (Bergmeyer 1974). Measurement of the capacities of glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) and isocitrate dehydrogenase (ICDH; EC 1.1.1.42) was performed as described by Bergmeyer (1974).

Statistical analysis of data was performed with the statistical software *Statistica for Windows* (Statsoft, 2003). Significant differences were considered at $P= 0.05$, and mean values were ranked by Tukey's multiple range tests when more than two groups were compared with ANOVA. All data are represented as mean±stdev, unless explicitly mentioned.

Results

For all growth parameters studied the same tendency with treatment is observed (Fig. 1): the values reached a peak at a uranium concentration in the nutrient solution of 1 or 10 µM and then steadily declined with a steep decrease between 100 and 1000 µM uranium in the solution. However, there was no significant difference in plant development observed between treatments and control. The hormesis-like response up to a 1-10 µM uranium concentration, could perhaps be explained by the use of uranylnitrate and the response of the plant to nitrate. The presence of Cd in the nutrient solution at concentrations of 0.5 and 1 µM tended to promote plant growth though not significantly.

The bottom pictures of Fig. 1 represent the uranium concentrations in the roots and primary leaves. The uranium concentration (FW) in roots ranged from 31 ± 4 ppm to 14916 ± 2208 ppm at a 0.1 µM and 1000 µM solution concentration, respectively. A significant difference is only observed between the root uranium concentration at 1000 µM and the other treatments. Concentrations in primary leaves range from 0.7±0.5 ppm to 16.5±6.3 ppm at a 0.1 µM and 1000 µM solution concentration, respectively, or a factor 40 to 900 lower than the concentrations observed in the roots. The observed root/shoot ratios agree with those reported in literature (Dushenkov et al. 1997; Shahandeh and Hossner 2002). The Cd concentrations (FW) were 27.2±7.1 ppm and 19.6±7.2 ppm for the roots and

3.5±2.6 ppm and 2.0±1.4 ppm for the leaves, for an external Cd concentration of 0.5 and 1 ppm Cd, respectively. The 10-fold higher Cd content in the roots as compared to the leaves has been extensively reported and is in accordance to the literature (Nan et al. 2002).

The capacity of some enzymes involved in the antioxidative defense mechanism in bean roots and primary leaves were analyzed. Between treatments, there were no significant differences or consistent patterns in enzyme capacity observed for the leaves. Therefore, enzyme capacity is only discussed at the root level (Fig. 2). In the roots, uranium seems to have an effect on the biochemical parameters studied: up to an ambient concentration of 1 or 10 µM uranium, the capacities of

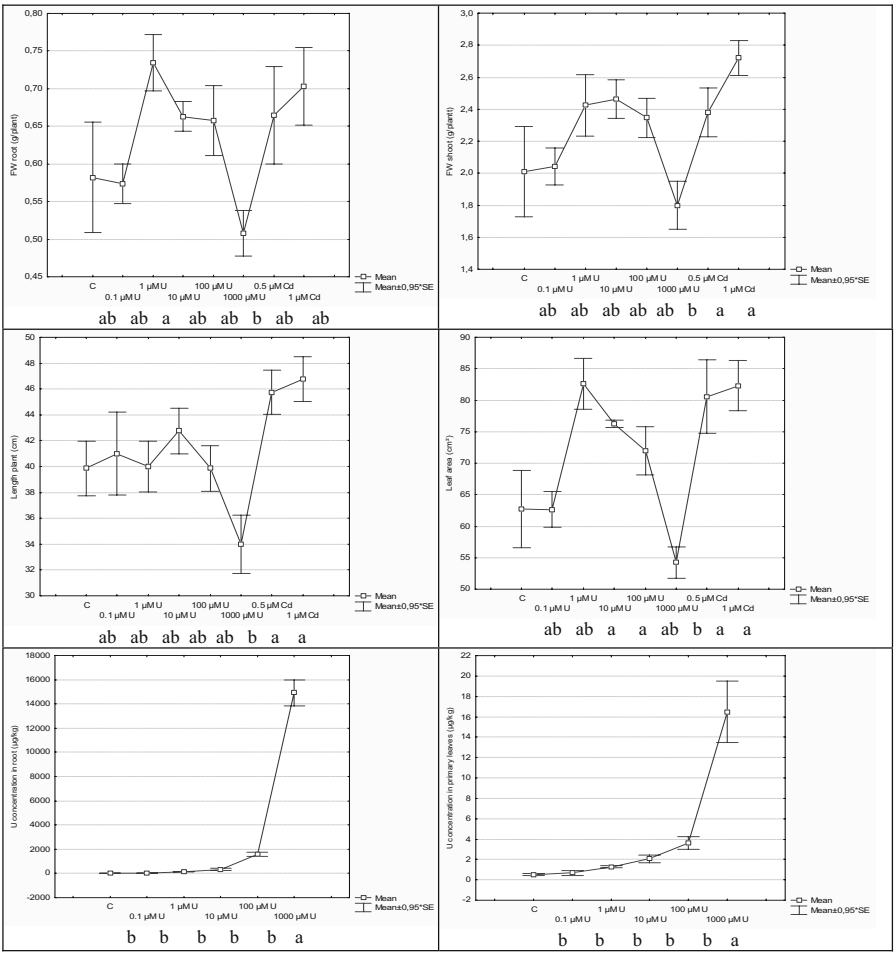

Fig. 1. Effect of exposure of beans (*Phaseolus vulgaris)* to different uranium and cadmium concentrations on shoot and root fresh weight, plant length and surface area of primary leaves and uranium concentrations in roots and primary leaves. Treatments with different letter annotations are significantly different.

guaiacol and syringaldazine peroxidase (GPOD, SPOD), gluthatione reductase (GLUR) and the $NADP^+$-reducing enzymes isocitrate dehydrogenase (ICDH) and glucose-6P-dehydrogenase (G-6P-DH) increase. This tendency is, however not significant. The capacity of superoxide dismutase (SOD) showed an irregular pattern with treatment and no significant differences were observed. For all enzymes but SOD, the capacity almost ceased at 1000 µM uranium.

Exposing the roots to 0.5 or 1 µM Cd resulted in a non-significant increase in enzyme capacity compared to the control.

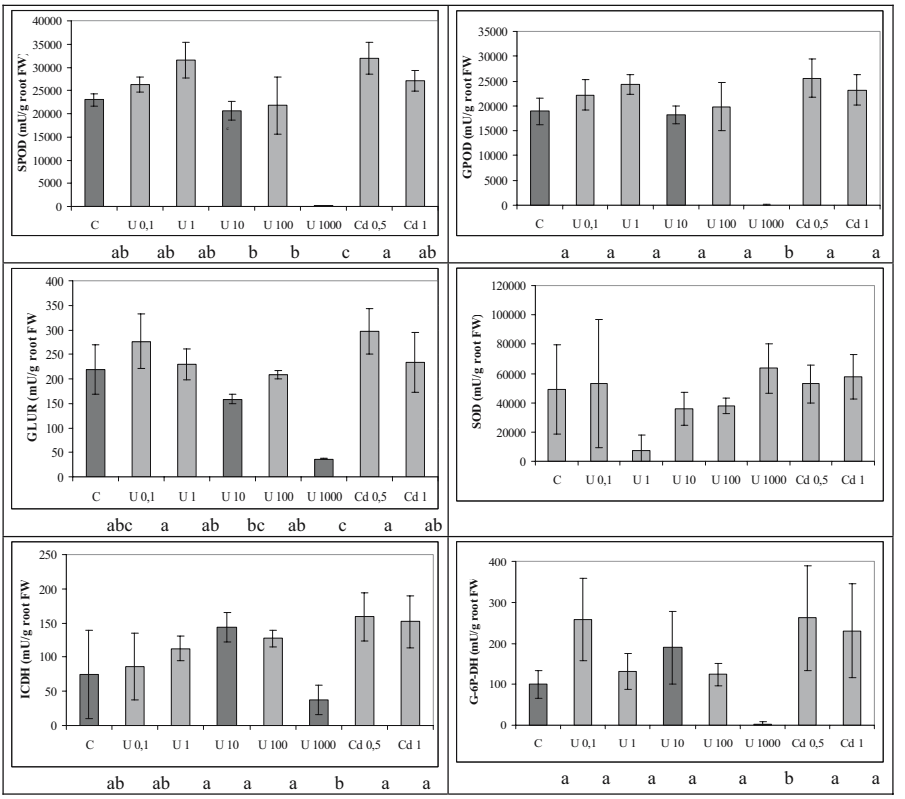

Fig. 2. Effect of exposure of beans (*Phaseolus vulgaris)* to different uranium and Cd concentrations on enzyme capacity of syringaldazine peroxidase (SPOD), guaiacol peroxidase (GPOD), glutathione reductase (GLUR), superoxide dismutase (SOD), isocitrate dehydrogenase (ICDH) and glucose-6-phosphate dehydrogenase (G-6P-DH),. Treatments with different letter annotations are significantly different. No significant differences observed for SOD capacity.

Discussion

Most of the effects observed following plant exposure to uranium are not significant, except for the significant reduction in capacity of enzymes involved in the plant's oxidative defense mechanism when exposed to 1000 µM uranium in the external solution. Plant development seemed also hampered at 1000 µM. The exposure of the plants (external exposure from uranium in the nutrient medium and internal exposure following uptake) was calculated using the dose conversion coefficients for external exposure from ^{238}U in water [1.3 X 10^{-7} (µG/h)/(Bq/kg)] and for internal exposure following uptake in vegetation [2.4 X 10^{-2} (µG/h)/(Bq/kg)] by Amiro (1996) and taken up by Taranenko et al. (2004). External exposure from uranium is negligible even with an external solution concentration of 1000 µM (3.85 x 10^{-4} µGy/h). The dose proposed at which no significant effects are expected at the level of the population as proposed in IAEA (2001) and reiterated in UNSCEAR (1996) are 10 mGy/d (or 416 µGy/h). This benchmark of ~400 µGy/h was not reached in the leaves, not even when exposed to 1000 µM U given the limited transfer to the leaves (concentration < 16 mg U/kg FW). For roots exposed to 100 µM U (root concentration ~1600 mg U/kg FW just exceeded the benchmark (477 µG/h). For roots exposed to 1000 µM U (root concentration ~14900 mg U/kg FW), the benchmark was exceeded 10-fold. Hence, only when the benchmark of 400 µGy/h is exceeded, effects are observed for the biometric and biochemical parameters investigated.

To the authors knowledge, no research has been performed so far on the effect of uranium uptake on the capacity of enzymes involved in the antioxidant defense system of plants. Zaka et al. (2002) studied the effects of low chronic doses of ionizing radiation on antioxidant enzymes in *Stipa capillata*. External gamma-radiation at a dose rate of 65 µSv/h induced an enhancement of the peroxidase (not specified), GLUR, SOD and G-6P-DH activities in plants originating from contaminated areas (Semipalatinsk) but not for control plants. This was interpreted by the authors that half a century of residence in led to a natural selection of the most adapted genotypes characterized by an efficient induction of the anti-oxidant enzyme activities.

The literature offers conflicting information on the phytotoxicity of uranium. For soils, levels as low as 5 mg/kg have been cited as toxic, whereas many studies reported no toxicity symptoms at levels 100-1000-fold higher (Sheppard et al. (1992). Sheppard et al. (2004) reported on ecotoxicity thresholds for uranium for freshwater plants (studies with algae). The effective concentrations at which a 25 % reduction of growth was observed ranged between 0.01 and 0.12 mg/L (0.05 and 0.5 µM), concentrations at which we did not observe any effects. From the study by Sheppard et al. (1992) beans were reported as being most resistant to high U levels, showing no effect in germination tests at 1000 mg/kg when germination rate for other crops was clearly reduced. Geometric means of uranium solid-liquid distribution coefficients (Kd) vary from 15 to 1600 ml/g for different textured soil (Thibault et al. 1990), resulting in soil solution uranium concentrations of respectively 3 and 300 µM. If we would apply this highest figure, no ef-

fects would be observed up to a uranium concentration in the solution of 300 μM, which is in agreement with our results.

Low Cd concentrations were used in our study, since this is similar to what is measured in the soil pore water of a slightly polluted sandy soil in the vicinity of an old Zn smelter in Belgium. Despite of the low concentrations, exposure of 10-day-old bean seedlings to 2 μM Cd resulted in a 20-40 ppm Cd concentration in the primary leaves after 24 h respectively 72 h (Smeets et al. 2005). Although in our experimental set-up, bean seedlings were exposed for 7 days to similar Cd concentrations, the uptake seems to be less pronounced. Probably the bioavailability of Cd in the nutrient solution is decreased and might be too low to impose a strong difference between control and treated plants.

Except for the SOD activities, the capacities of the enzymes studied showed an increased trend, although not significant. Elevation of environmental Cd concentrations has been reported to impose oxidative stress in plant cells (Chaoui et al. 1997, Smeets et al. 2005). An enhancement of the antioxidative defense mechanism can therefore be expected, while no visible damage nor changes in the biometric parameters will be observed.

Conclusions

Activity of stress enzymes was only significantly affected (decreased) at uranium concentrations of 1000 μM. Only at this soil solution concentration, the root uranium concentrations resulted in a dose clearly exceeding the set no-effect benchmark at population level. The reduction in enzyme activity is clearly a marker for prospected significant reductions in plant development at the highest uranium concentrations. Although the cadmium concentrations applied did not significantly affect the plant development nor the antioxidant enzyme capacities, the latter was slightly enhanced.

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