PHYTOACCUMULATION OF URANIUM BY *PHASEOLUS VULGARIS*

Background

Radioactive contamination of the environment surrounding facilities where uranium (U) has been mined and processed has occurred in many countries. If phytomanagement of uranium contaminated areas is envisaged, the impact of the contamination on the vegetation has to be investigated. Uranium is a radiotoxic and chemotoxic heavy metal. Mechanisms of toxicity have been predominantly studied on man and on some animal species. For plants, little information on uranium toxicity at the cellular level is available. In plants facing environmental stress (e.g. contamination by heavy metals) an increase in the formation of highly reactive oxygen species (ROS) is often observed. ROS are naturally produced in the plant cells and consequently, cells have developed several anti-oxidative defense mechanisms in order to control the redox state of the cell, an essential parameter for normal physiological and biochemical functioning. The defense system comprise antioxidative enzymes (superoxide dismutases, peroxidases, catalases, glutathione reductase) and antioxidants (e.g. glutathione, ascorbate,…). The presence of heavy metals (in particular uranium) results in an enhancement of the antioxidative defense mechanism.

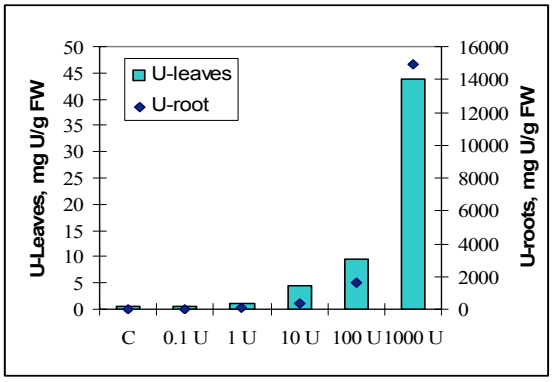
Objectives

The objective of the study was to analyze the biological effects (biometry, stress enzyme and antioxidant content, DNA integrity) induced by bioaccumulation of uranium in the bean *Phaseolus vulgaris,* to evaluate whether the various investigated biomarkers are related and to define possible dose-effect relationships.

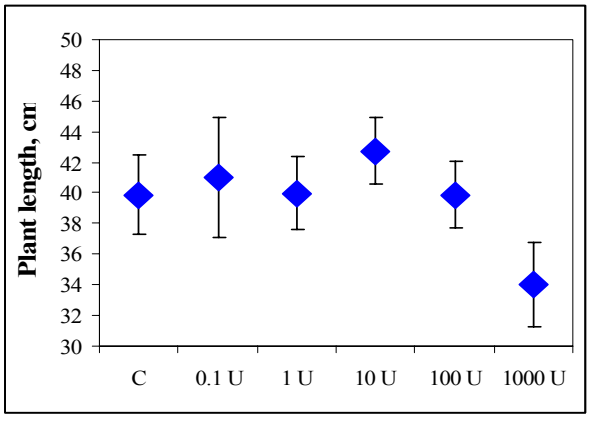
Principal results

We exposed ten-day-old bean seedlings to 0 to 1000 µM (micromolar=micromole/liter) U. Following a 7 day-exposure, plants were sampled for determination of U uptake, biometric parameters (shoot and root length and weight, leaf area index) activity of enzymes involved in the plant antioxidative defense mechanisms, level of the antioxidant glutathione and DNA integrity (comet assay). The enzymes studied cover enzymes for the ascorbate-gluthatione cycle (gluthatione reductase), enzymes capable of quenching reactive oxygen species (guaiacol, syringaldazine peroxidase and superoxide dismutase) as well as enzymes that catalyze reactions leading to the reduction of $NAD(P)^+$ (malic enzyme, glucose-6P-dehydrogenase).

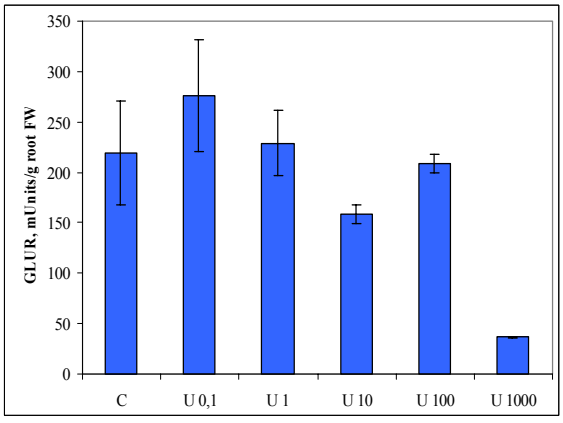
Beans (Phaseolus vulgaris) exposed to various U concentrations in hydroponics



Uranium concentrations (mg/g FW) in roots and primary leaves of beans exposed for 7 days to 0 to 1000 µM

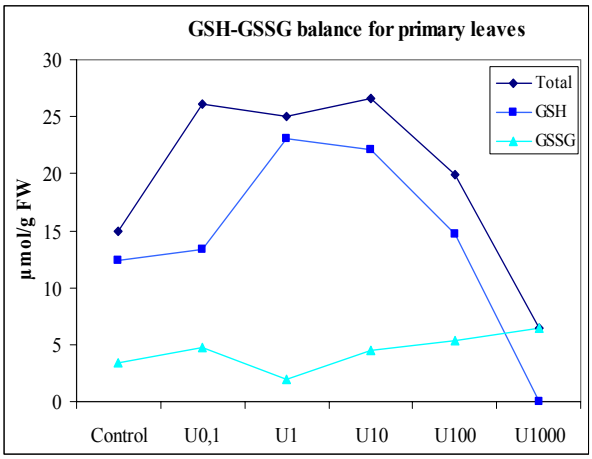


Total plant height of beans exposed to different U concentrations for 7 days (means ± sterr). Plant length is clearly affected at 1000 µM U

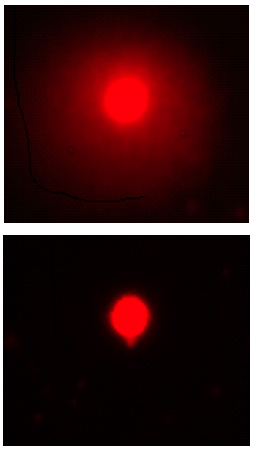


Glutathione reductase (GLUR) activity of bean roots exposed to different U concentrations for 7 days, is only significantly reduced for roots exposed to 1000 µM U

Generally we did not observe a significant difference in plant development between control and treated plants based on biometric parameters, except at 1000 µM U where growth was clearly hampered.
Antioxidative stress enzyme activity in roots was generally stimulated with increasing U concentrations (though not always significantly). However, for roots exposed to 1000 µM U, enzyme activity was generally significantly reduced.
Only at 1000 µM, DNA damage at the root level was observed (fully dispersed DNA in Comet assay) .
In primary leaves (leaves accumulate 1000 times less U than roots), no significant difference in activity of oxidative defense enzymes was observed nor any effect on the DNA integrity.



Levels of reduced GSH, oxidised GSSG and total glutathione for primary leaves of bean exposed for 7 days to different U concentrations.



Dispersion of DNA in COMET assay for bean roots exposed to to 0-100 µM U (top) or to 1000 µM U (bottom)

U treatment caused an accumulation of the antioxidant glutathione in the primary leaves (except at 1000 µM U). The ratio of reduced glutathione (GSH) to oxidised glutathione (GSSG) that plays an important role in the cellular redox status, was shown to be higher in exposed plants. Root glutathione content was not clearly affected by uranium treatment but the GSH/GSSG was higher than for the control. The observed increase in GSH and GSH/GSSG ratio points to an effective defence reaction to uranium induced stress.
For plants exposed to 1000 µM U the important reduction in total glutathione content (to zero in case of the roots) and the drop of reduced glutathione levels to zero indicates a complete disruption of the cellular redox status. This may explain the important drop in enzyme activity (GLUR activity was also importantly reduced) and the DNA damage observed for roots exposed to 1000 µM U.
These data indicate that uranium can cause oxidative stress and cellular redox imbalance. The reduction in enzyme activity and DNA integrity is clearly a marker for prospected significant reductions in plant development at the highest uranium concentrations. Toxicity thresholds for plant growth, DNA damage and enzyme activity hence correspond. Further research is needed to unravel the mechanisms by which the plants (in particular beans) defend themselves against high external uranium levels and the (indirect?) mechanisms by which oxidative stress is induced.
We were not able to deduce any dose-effect relationship for any of the biomarkers screened: a reason might be the limited sensitivity of beans to uranium.

Future work

Further study on the biological effects induced by external radiation or uranium accumulation by *Arabidopsis thaliana*, using a multi-biomarker approach is under progress. Furthermore, a thorough analysis of subtle effects (DNA damage, oxidative stress, metabolites, gene expression, …) viewed as early responses for individual disturbances (growth, reproduction) will be performed. A comparison between the effects observed for uranium (and external radiation) and the ones induced by a more conventional pollutant (Cd) will be completed and multiple stressor studies will also be performed.

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Main reference

Vandenhove H., A. Cuypers, M. Van Hees and J. Wannijn. 2005. *Effect of uranium and cadmium uptake on oxidative stress reactions for Phaseolus vulgaris. Uranium in the Environment: Mining Impact and Consequences*, Eds. B.J. Merkel and A. Hasche-Berger, Springer-Verlag, Berlin-Heidelberg. 175-182.