STUDY OF SELENIUM INDUCED STRESS RESPONSES IN ARABIDOPSIS THALIANA L. AND PISUM SATIVUM L. PLANTS, THE POSSIBILITY OF BIOFORTIFICATION

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INTRODUCTION

The actuality of the present topic is based on the fact that research on selenium started just a few decades ago. Researchers pointed out whilst selenium is not essential for plants, it is a necessary microelement for animals and humans, furthermore, there is more and more research on this topic in order to study the possible role of selenium.

Selenium exposure, like other environmental stress factors, can induce stress-induced morphogenetic responses (SIMR) within the plant body, since plants control their organs’ growth and development adjusting to the current conditions of the environment (e.g. actual water- and mineral conditions, presence of organic and inorganic contamination). The stress-induced morphogenic responses can appear both in the root- and shoot systems. Not only the environmental conditions but the endogenous hormonal status of the root (e.g. auxin, cytokinin and ethylene) can control the morphological responses, which means that changing the metabolism and transport of plant hormones plays a role in the formation of SIMR. The active signal transduction between the exogen, environmental and endogen, hormonal regulatory elements via signal molecules, ensures the phasing of the growth and developmental signals.

A new group within the signal molecules, is the nitric oxide (NO) and its derivatives, so called reactive nitrogen species (RNS). The NO as a signal transductor, plays an important role in the developmental processes, and more recent studies confirm that NO and its derivatives are not specific, rather they can be considered as general and multifunctional signal molecules. However, during stress responses not only the RNS play signal transductive role but the reactive oxygen species (ROS) contribute to the morphogenetical changes. There is an active signal transduction between RNS and ROS, and researchers think today that we can speak about nitro-oxidative stress, during ROS and RNS formation, induced by biotic or abiotic stress, and they induce the changes together, of which targets are macromolecules.
OBJECTIVES

During my work, I aimed at focusing on the study of the selenium treatment induced hormonal and signal transductive processes and how these responses indicate the morphogenetic response in the *Arabidopsis* plants, furthermore the background mechanisms behind these phenomena. In addition to the model plant *Arabidopsis* (*Arabidopsis thaliana* L.), I also wanted to use a well-known, common food plant, the pea plant (*Pisum sativum* L.) in order to study selenium biofortification, since the quality famine causes serious health and ecological problems among Se-deficient areas worldwide.

During my experiments I aimed at finding answers to the following questions:

1. How do the applied selenium concentrations affect the development of the model plant *Arabidopsis*, and is there a morphogenetic response during selenium stress?

2. What kind of changes are induced by selenium in the hormonal system?

3. What kind of changes are induced by selenium exposure in the level of growth-regulating signal molecules (nitric oxide and hydrogen peroxide) within the root system of the *Arabidopsis* plants?

4. What kind of interactions exist between the development-regulating hormonal and signalling systems during selenium stress?

5. How does selenium affect the development and ripening of the crop of the pea plants during long-term treatment?

6. Would it be possible for me to use the applied method as a selenium biofortification method?
MATERIALS AND METHODS

Experiments on *Arabidopsis thaliana* L. plants

I used 2-4-7- and 14-day-old (DAG2/DAG4/DAG7/DAG14; days after germination) *Arabidopsis thaliana* L. plants to carry out my experiments. Beside the wild-type (*Col-0*), the nia1nia2 double mutant, the gsnor1-3 mutant, furthermore the β-glucuronidase (GUS) transgenic lines were used in order to examine the hormonal status (*DR5::GUS, ARR5::GUS* and the *ACS8::GUS/GFP*). During my experiments I also worked with three different *AtCKX::GUS* (*AtCKX4, AtCKX5, AtCKX6*) lines. I studied the *ipt-161* and *35S:CKX2 Arabidopsis* lines, as well. In addition, during my experiments the *aux1-7, hookless (hls1-1)* and *etr1-1 Arabidopsis* plants were also used. From the modified ascorbic acid (Asa) containing lines, the vtc2-1 and the *miox4* were used. I chose the *CYCB1;1::GFP Arabidopsis* line in order to study the cell proliferation. I applied sodium selenite (Na$_2$SeO$_3$) as main treatment at 10, 20 and 40 μM concentrations, added directly into the agar media, thus the plants germinated and grew on the Se-containing agar. As control, plants without Se treatment were used. I also applied the following treatments together with Se: S-nitroso-N-acetyl-penicillamine (SNAP) as NO donor at 10 μM concentration, and 6-benzylaminopurine (BA) as exogen CK at 0.1 μM concentration.

The selenium and sulphur contents were measured by inductively coupled plasma mass spectrometry (ICP-MS) in 4-day-old wild-type *Arabidopsis* plants root and shoot systems.

On the sample collecting days the following morphological parameters were recorded: cotyledon area, hypocotyl length, primary root length. The measurements were carried out on digital images, using Fiji and Zeiss Axiovision Rel. 4.8 softwares. Taking the digital images, Zeiss Axioscope 200-C stereomicroscope and Zeiss Axiovert 200M invert microscope were used. Based on the morphological parameters, selenium tolerance index was calculated.

In those transgenic *Arabidopsis* lines, which carry β-glucuronidase (GUS) activity, 5-bormo-4-chloro-3-indolil glucuronid (X-Gluc) staining was used. The samples
were detected by a Zeiss Axiovert 200M invert microscope. The X-Gluc staining of the *DR5::GUS* plants made it possible for me to localize and count the lateral roots, furthermore I could specify their developmental stage.

Fluorescently stained samples were *in situ* and *in vivo* measured by a Zeiss Axiovert 200M type invert microscope. The pixel intensity was determined both in the root and shoot. The level of NO was visualized by 4-amino-5-methylamino-2′7′-difluorofluorescein diacetate (DAF-FM DA), while the level of hydrogen peroxide of the plants by Ampliflu™ (or 10-acetyl-3,7-dihydroxyphenoxazine or Amplex Red). In order to study the viability of root tips and cotyledons, I used the fluorescein diacetate (FDA) dye.

The GFP expression of the *CYCB1;1::GFP* plants was measured using Zeiss LSM 700 Axio Observer.Z1 and Olympus LSM 700 laser scanning confocal microscopes. I used propidium iodide (PI) staining on the 4-day-old plants in order to make the cell walls visible. The intensity and localization of the GFP signal was analyzed on digital images using Zeiss Zen2010, Olympos Fluoview FV100, and Fiji softwares. The distance between the quiescent centre (QC) and the beginning of the transition zone (TZ), where the elongation of the cells is powerful, was determined. The size of the root meristem was measured on digital images using the Fiji software.

**Experiments on *Pisum sativum* L. plants**

During my experiments in the biofortification topic, I used *Pisum sativum* L. Petit provencal pea plants. The seedlings were transferred to perlite-filled 5-litre pots (4 plants/pot and 6 pots/treatment) and grew under greenhouse conditions. Watering of the plants was carried out applying Hoagland solution. The pea plants were grown under control conditions for 35 days, then they got 10, 50 and 100 μM Na₂SeO₃ treatment, added into the Hoagland solution, for 50 (50 and 100 μM Se) or 56 days (10 μM Se). During the experimental period, seeds were collected three times, using two-week breaks.

The following morphological parameters were measured manually: shoot length, shoot fresh weight, leaf length, primary root length and root fresh weight. The
crop-related parameters were the following: number of the pods/plant, fresh and dry weight of the seed containing pod, fresh weight of the seeds, number of the seeds/pod.

Beside selenium, other micro- (Zn, Mn, Fe, Co, Cu, Mo) and macro elements (K, Mg, Ca) were determined in the shoot, root and crop of the plants by ICP-MS method.

RESULTS AND DISCUSSION

During my Ph.D. studies, I investigated the developmental, hormonal and signalling responses of the model organism *Arabidopsis thaliana* L. with the help of genetical and biochemical methods under different concentrations of selenite treatment. Furthermore, I studied in our experimental system whether selenium accumulates in the seeds of pea plants during the biofortification experiments and how this long-term Se exposure affects the development of *Pisum sativum* L. plants. With the use of these experimental systems, it became possible to widen our understanding not only in the field of plant physiology but also add useful information to the opportunity of biofortification.

Based on my results, I can summarise:

1. The higher Se concentrations (20 and 40 µM) caused the **growth inhibition of the shoot and primary root** however, long-term (14 days), mild Se exposure (10 µM) resulted in stress induced morphogenetic response. These developmental responses are considered **elements of the adaptation process** since the re-orientation of means from development for protection mechanisms ensures better survival. Besides the growth inhibition, the death of cells is also possible, which may happen **via** incorporation of Se into proteins (as selenocystein and selenomethionine).

2. I pointed out that Se exposure can cause significant **changes in hormone homeostasis**: Se decreases the auxin response (DR5-dependent GUS activity), increases the biosynthesis of ethylene (ACS8::GUS expression). The activity and spatial distribution of the cytokinin response promoter (ARR5)-dependent
GUS change under selenite stress as well, presumably via the inhibition of root-to-shoot translocation and the regulation of AtCKX4 and AtCKX5.

3. During the early development of the seedlings, selenium excess decreased the amount of nitric oxide (which happens independently from NR), and increases the hydrogen peroxide level in the root, which implicates the antagonistic relationship between these two molecules. Furthermore, using biochemical (NO donor treatment) and genetical (gsnor1-3 and nia1nia2 mutants) methods, I proved, that high NO levels contribute to the induction of selenium tolerance, while the optimal level of H2O2 is necessary for the Se endurance.

4. It can be hypothesized, that the Se induced H2O2 decreases the auxin-dependent genexpression during early development, whereas in older roots NO inhibits the transport of auxin, resulting in the reduction of total auxin level and growth inhibition of the root. The selenium-induced intensified ethylene biosynthesis (ACS8::GUS activity) takes part in cell death induction, thus in the inhibition of growth and the H2O2 is a downstream component of the signalling. Furthermore, my results show, that in selenium treated roots there is no regulatory relationship between NO and ethylene. Under control conditions, there is a mutual negative relationship between CK and NO in Arabidopsis roots. In case of Se exposure, CK influences the metabolism of NO, and the decrease of NO level proved to be necessary for the activation of ARR5 promoter. This result shows a negative CK-NO cross talk under selenium stress.

5. Selenium affects negatively the long-term development of pea plants, and in the reproductive phase plants survival strategy focuses on the producing and ripening of the seeds.

6. The development of seeds was also limited by selenium treatment, however, the biofortification proved to be successful because selenium accumulated in the new seeds.
As a result, it is clear, that the elements of the hormonal (auxin, cytokinin and ethylene) and signalling (NO and H₂O₂) networks act together and are in a relationship, they regulate the Se exposure induced developmental changes. Our research group reported the changes in NO metabolism caused by selenium for the first time, and the role of NO in selenium tolerance. Our new and important result is the investigation of the relationship between NO and cytokinin, and its nature under an abiotic (selenium) stress condition.

In our opinion, with this work, I could contribute to the understanding of the role and interactions of nitric oxide in plants.
PUBLICATION LIST

Topic-related publications:


Other publications:


**Total impact factor: 19.873**
Scientific report:


Scientific lectures:


Scientific posters:


