Title: „The roots" of selenium toxicity: a new concept

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Addendum to:

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Abstract

Elevated levels of selenium (Se) causes toxicity in non-accumulator plant species. The primary reasons of toxic Se effect have been considered as selenoprotein accumulation and oxidative stress and the most characteristic symptom of Se exposure is the inhibited root elongation. In our paper published in Plant Cell Reports we presented that the meristem shortening and cell cycle arrest are the main reason of the Se-induced root growth inhibition. We also reported that selenium negatively regulates NO levels in a nitrate reductase-independent manner. This publication presents that selenium also affects cytokinin signalling and a mutually negative link between nitric oxide and cytokinin is involved in sensing of selenium toxicity.

Based on our recent publication and previous literature data we assume that disturbances in the homeostasis of both reactive oxygen and nitrogen species result in selenium-induced nitro-oxidative stress, contributing to toxicity. Additionally, the imbalance of hormonal homeostasis (cytokinin, auxin, ethylene) evoked by selenium is involved in the appearance of toxicity symptoms. Altogether, this Addendum proposes a new concept for the background mechanisms of plant selenium toxicity.

Key words: cytokinin, nitric oxide, phytohormones, reactive nitrogen species, selenium, toxicity

Selenium toxicity in non-accumulator plants

Selenium (Se) is a naturally occurring element essential for several organisms including bacteria, some green algae and mammals (Pilon-Smits and Quinn 2010). Although higher plants do not require selenium, they take it up thereupon its chemical and physical similarities to sulphur.[1] For higher plants, selenium is beneficial at low concentrations, but elevated levels of it cause toxicity.[2] For non-accumulator plants like *Arabidopsis thaliana*, tissue Se concentrations higher than 2 mg/kg cause toxicity[3] and these selenium levels result in approx. 10% reduction of yield without the appearance of visible symptoms.[4] However, in general the toxicity of selenium inevitably depends on the plant age, since seedlings are more sensitive to elevated Se levels than mature plants. The threshold value of toxicity depends also on the form of selenium and selenite reported to be more toxic than selenate.[5] In extreme cases, the large amount of accumulated selenium can drive to symptoms like chlorosis, necrosis, leaf withering and drying.[2] The remarkable inhibition of shoot and root development

is a more typical consequence of selenium exposure. As being the site of direct contact with the excess element, the root system is highly affected by selenium. The primary root (PR) elongation is markedly inhibited by excess Se and the effect proved to be concentration/time-dependent[6,7,8,9,10] and consequently being associated with selenium tolerance. Therefore, the degree of PR growth inhibition reflects the plants' resistance or susceptibility against selenium and can be presented as selenium tolerance index (%). [11, 12, 9]

Our recent paper[13] provided new information about the tissue- and cellular-level mechanisms of the effect of selenium (selenite) on primary root growth. We observed that both the length of the root apical meristem and its cell number are significantly smaller in selenite-exposed roots. Moreover, selenium treatment resulted in the notable reduction of cyclinB1 expression suggesting that the main reason for PR shortening is the inhibition of cell division in the apical meristem.

Known molecular mechanisms of selenium toxicity

The main reason for Se toxicity is the malformation of non-specific selenoproteins.[14] Selenoamino acids, selenocysteine or selenomethionine corporate into proteins in place of cysteine and methionine leading to alterations in protein structure and function.[15] Furthermore, the accumulation of these malfunctioning Se-containing proteins results in toxicity; although plants like the Se-hyperaccumulator *Stanleya pinnata* is able to decrease the amount of those by activating the ubiquitin-26S proteasome pathway[16]. Recently, the involvement of endoplasmic reticulum-assisted degradation (ERAD) in removal of misfolded selenoproteins and in selenium tolerance was proposed[17].

It is also well known that selenium exerts its toxic effect also through inducing oxidative stress.[14] Selenium-triggered production of reactive oxygen species (ROS) including hydrogen peroxide and superoxide was published in the root and shoot system by several authors.[11,7,8,18,19,13] Recently it was pointed out that superoxide generated by the glutathione-mediated reduction of selenite may partly be responsible for the Se-induced impairment of photosynthesis.[9]

The primary metabolism and the levels of some essential macro- and microelements are also affected by selenium and these changes are associated with selenium-induced growth inhibition.[18,20]

Novel mechanisms behind selenium toxicity: Reactive nitrogen species and phytohormones

In addition to ROS, selenium toxicity also involves changes in the level of reactive nitrogen species (RNS) such as nitric oxide (NO) and peroxynitrite. In our recent publication[13], selenite was shown to lead to the diminution of NO levels in a concentration-dependent manner in *Arabidopsis* roots. This selenite-induced NO decrease was observed also in the nitrate reductase-deficient *nia1nia2* mutant suggesting the NR-independence of the process (Fig 1). In pea root tips, selenite had an opposite effect on NO levels and significantly increased peroxynitrite formation and consequently protein tyrosine nitration as well.[19] Similarly, selenate exposure resulted in NO accumulation in *Brassica rapa* root tips and the effect proved to be concentration- and time-dependent.[8]

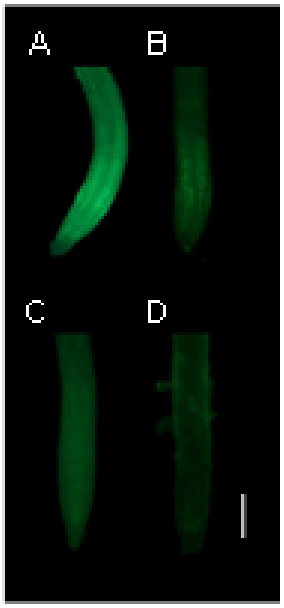

Fig 1 Root tips of wild-type (A,B) and *nia1nia2* (C,D) *Arabidopsis* treated with 0 (control, A,C) or 40 μM sodium-selenite for four days. Nitric oxide levels were visualized by staining the root samples with 4-amino-5-methylamino-2',7' difluorofluorescein diacetate. Bar=100 μm.

The above detailed published results clearly show that not only the homeostasis of ROS but the RNS are both greatly affected by selenium. Consequently besides selenium-

induced oxidative stress, the secondary nitrosative stress also contributes to selenium toxicity. Therefore, instead of oxidative stress, we urge to consider nitro-oxidative stress as an underlying mechanism of selenium phytotoxicity.

The actions of certain hormones like ethylene (ET), jasmonic acid (JA) and salicylic acid are related to stress resistance. Selenite exposure of *Arabidopsis* induced the production of ET and JA and the intensification of their signalling led to enhanced selenite tolerance.[11]

Besides stress hormones, phytohormones like auxin and cytokinin (CK) are major regulators of plant growth and development; ethylene is able to exert growth regulating effect as well. Therefore, the involvement of these morphogens in selenium-triggered growth arrest cannot be ignored. Previously, selenite was found to decrease auxin-inducible *DR5::GUS* activity in *Arabidopsis* root tips, while the *in situ* expression of ethylene biosynthetic *ACS8::GUS* notably enhanced.[7]

In our recent publication[13], a detailed examination of cytokinin levels and signalling was presented in selenite-exposed *Arabidopsis*. Our results clearly showed that cytokinin signalling intensified in the root tip while reduced in the above ground plant parts suggesting that the root-to-shoot translocation of CK is strongly inhibited by excess selenite. Additionally, the selenite-induced upregulation of cytokinin degrading enzymes (cytokinin oxidase 4 and 5) in the cotyledons and the downregulation of cytokinin oxidase 4 in the root tip may also contribute to the altered distribution of cytokinin. We also suggested that the accumulated cytokinin in the root tips may party be responsible for the root meristem shortening. Our experiments provided new evidences for the mutually negative relationship between cytokinin and NO in the root system of selenite-stressed *Arabidopsis*. It was also pointed out that overproduction of cytokinin or NO causes selenite insensitivity which implies the possibility that both molecules are involved in sensing of selenium toxicity in plant roots.

Based on the results of our recent paper[13] and additional, partly own literature data[7,19] we propose the completion of the present-day concept for the background mechanisms of plant selenium toxicity. Since excess selenium disturbs both ROS and RNS homeostasis, it causes secondary nitro-oxidative stress leading to macromolecule modifications and consequently cell death. Furthermore phytohormones participating in growth regulation and/or stress responses are also affected by selenium. Thus, it is easy to imagine that the imbalance of hormonal homeostasis contributes to the appearance of Se toxicity symptoms.

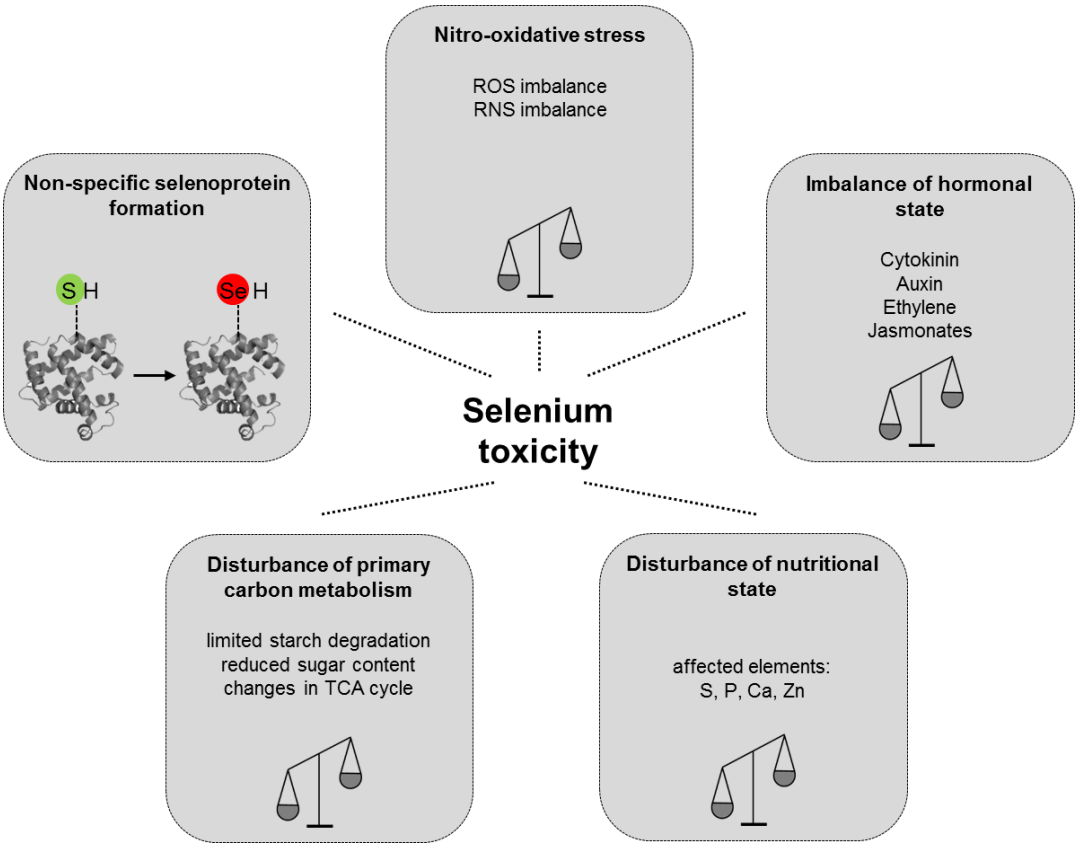

Fig 2 Schematic illustration representing a new concept of processes contributing plant selenium toxicity. See details in the text.

Despite the growing knowledge about plant responses to selenium, a number of questions still need clarification. Future research will have to focus on the underlying mechanisms of selenium-triggered nitrosative stress (e.g. nitration, S-nitrosylation) and the possible involvement of other growth hormones and their interactions with NO in sensing of selenium toxicity.

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