ARTICLE ADDENDUM

“The roots” of selenium toxicity: A new concept

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ABSTRACT

Elevated levels of selenium (Se) cause toxicity in non-accumulator plant species. The primary reasons for toxic Se effect have been considered to be selenoprotein accumulation and oxidative stress. However, based on our recent paper in Plant Cell Reports and previous literature data we suggest that disturbances in the homeostasis of both reactive oxygen and nitrogen species result in selenium-induced nitro-oxidative stress, contributing to toxicity. The most characteristic symptom of Se exposure is the inhibited root elongation which is partly caused by hormonal disturbances. Our recent paper suggests the involvement of cytokinin in selenium stress sensing of the root system. Altogether, the aim of this Addendum is to present reactive nitrogen species and phytohormones as new players in plant selenium toxicity.

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Selenium toxicity in non-accumulator plants

Selenium (Se) is a naturally occurring element essential to some organisms, including bacteria, some green algae and mammals. Although higher plants do not require selenium, they take it up due to its chemical and physical similarities to sulfur.¹ For higher plants, selenium is beneficial at low concentrations, but elevated levels of this element can cause toxicity.² For non-accumulator plants like *Arabidopsis thaliana*, tissue Se concentrations higher than 2 mg/kg cause toxicity³ and can result in an approximately 10% reduction of biomass yield without the appearance of visible symptoms.⁴ However, in general the toxicity of Se inevitably depends on the plant's 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 has been reported to be more toxic than selenate.⁵ In extreme cases, a large amount of accumulated selenium can give rise 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 it is the point of contact with the element in the environment, the root system is highly affected by Se. The primary root (PR) elongation is markedly inhibited by excess Se, and this effect has been shown to be concentration/time-dependent^{6,7,8,9,10} and consequently associated with selenium tolerance. Therefore, the degree of PR growth inhibition reflects the plants' resistance or susceptibility against Se and can be presented as selenium tolerance index (%).^{9,11,12}

Our recent paper¹³ 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

reduced in selenite-exposed roots. Moreover, Se treatment resulted in a 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- can be incorporated into proteins in place of cysteine and methionine residues, 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* are able to decrease the levels of these proteins by directing them to the ubiquitin-26S proteasome pathway.¹⁶ Recently, the involvement of endoplasmic reticulum-assisted degradation (ERAD) in removal of misfolded selenoproteins and in Se tolerance was proposed.¹⁷

It is also known that Se exerts its toxic effect also through inducing oxidative stress.¹⁴ Selenium-triggered production of reactive oxygen species (ROS) including hydrogen peroxide and superoxide has been observed in the root and shoot system by several research groups.^{7,8,11,13,18,19} 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 Se and these changes are associated with selenium-induced growth inhibition.^{18,20}

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

Novel mechanisms behind selenium toxicity: Reactive nitrogen species and phytohormones

In addition to ROS, Se 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

decrease NO levels in a concentration-dependent manner in *Arabidopsis* roots. This selenite-induced NO diminution was also observed in the nitrate reductase-deficient *nia1nia2* mutant, suggesting that nitrate reductase activity is not involved in NO level changes (Fig. 1). However, in pea root tips, selenite had the opposite effect on NO levels, and significantly increased peroxynitrite formation and consequently protein tyrosine nitration.¹⁹ Similarly, selenate exposure resulted in NO accumulation in *Brassica rapa* root tips and the effect proved to be concentration- and time-dependent.⁸ The diverse NO responses can be explained by the variable parameters such as the applied Se forms, concentrations, plant age and growth conditions.

These results show that the homeostasis of not only ROS but also RNS can be greatly affected by Se. Consequently, besides selenium-induced oxidative stress, the secondary nitrosative stress also contributes to selenium toxicity. Therefore, rather than simply oxidative stress, we urge the consideration of nitro-oxidative stress²¹ as an underlying mechanism of Se phytotoxicity.

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

Besides stress hormones, phytohormones like auxin and cytokinin (CK) are major regulators of plant growth and development; ethylene is also able to exert a regulatory effect on growth. Therefore, the involvement of these morphogens in Se-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* was notably enhanced.⁷

In our recent publication,¹³ a detailed examination of cytokinin levels and signaling was presented in selenite-exposed

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

Arabidopsis. Our results clearly showed that CK signaling intensified in the root tip, yet was reduced in the aboveground plant parts, suggesting that the root-to-shoot translocation of CK is strongly inhibited by excess selenite. Additionally, the selenite-induced upregulation of CK 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 partly be responsible for the root meristem shortening. Our results provided new evidence for the mutually negative relationship between cytokinin and NO in the root system of selenite-stressed *Arabidopsis*. They also pointed out that overproduction of CK or NO causes selenite insensitivity which suggests the possibility that both molecules are involved in sensing of Se toxicity in plant roots.

Based on the results of our recent paper¹³ and additional, data in the literature,^{7,19} we propose an updated concept for the background mechanisms of plant Se toxicity (Fig. 2). Since excess Se disturbs both ROS and RNS homeostasis, it causes secondary nitro-oxidative stress leading to macromolecule modifications and consequently cell death. Furthermore, phytohormones involved 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.

Despite the growing knowledge about plant responses to Se, a number of questions still need clarification. Future research must 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 Se toxicity.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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