

Article Addendum

New insights into the roles of ethylene and jasmonic acid in the acquisition of selenium resistance in plants

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In a recent paper, we reported that both ethylene and jasmonic acid (JA) are important for selenium (Se) resistance in *Arabidopsis*.¹ Elevated levels of reactive oxygen species were associated with ethylene and JA production in a Se-resistant *Arabidopsis* ecotype. Here, we further discuss the functions of these phytohormones, and their possible interactions, in plant Se resistance and -accumulation, placing our data in a broader perspective of other recently published papers.

Selenium (Se) is a naturally occurring element commonly found in sedimentary rocks formed during the Carboniferous to Quaternary Periods.² Irrigation of Se-rich soils leads to selenate leaching in shallow groundwater.³ In addition, selenite is a common contaminant in oil-refinery wastewater.⁴ The accumulation of Se in surface water or soil can become a source of toxicity for plants.⁵ Se is chemically similar to sulfur (S) and can be metabolized by S metabolic pathways. Plants take up selenate, the most common soluble form of Se in soil, inadvertently via sulfate transporters, and assimilate it into selenocysteine and selenomethionine.⁵ Nonspecific replacement of the two essential S amino acids cysteine and methionine by these Se analogues in proteins is toxic.^{5,6} For this reason, much of the research on plant Se resistance has focused on Se interactions with S metabolism.

Recently, we obtained evidence for the involvement of defense-related phytohormones in the acquisition of Se resistance in plants.⁷ In a transcriptome study performed to identify selenate-responsive genes, the expression of many ethylene and/or jasmonic acid (JA) responsive genes was induced by selenate treatment. Induction of many of these same ethylene and/or JA responsive genes was also observed in selenite-treated plants.¹ These include the gene encoding

allene oxide synthase (AOS), a key enzyme in JA biosynthesis,⁸ and known to be induced by MeJA.⁹ Another selenite-induced gene was *PDF1.2* which encodes a plant defensin that requires concomitant triggering of the ethylene and JA pathway for its induction.¹⁰ Transgenic *AOS* promoter::*GUS* and *PDF1.2* promoter::*GUS* plants exhibited GUS activity in leaves of selenite-treated plants (Fig. 1B and D) but not in those of non-treated plants (Fig. 1A and C), confirming that these genes are Se-regulated. Furthermore, selenite treatment led to enhance ethylene generation and JA accumulation, particularly in the selenite-resistant ecotype Col-0.¹ Together, these results suggest that Se treatment triggers ethylene and JA production and responses. The importance of ethylene and JA for Se resistance in plants was further investigated using *Arabidopsis* mutants deficient in an aspect of ethylene- or JA-biosynthesis or signaling (*acs6*, *ein2* and *jar1*). These mutants showed less resistance to selenite and selenate than wildtype plants.^{1,7} Conversely, treatment of MeJA or 1-aminocyclopropane-1-carboxylic acid (ACC; precursor of ethylene) enhanced selenite resistance in a Se-sensitive *Arabidopsis* ecotype, *Ws-2*.¹ These results further suggest that ethylene and JA are important for Se resistance in plants.

In selenite-treated *Arabidopsis*, we also observed accumulation of salicylic acid (SA).¹ This plant hormone is a major phenylpropanoid compound whose biosynthesis is triggered by various biotic and abiotic stresses.^{11,12} Although SA levels were enhanced by selenite, our results suggest that SA production inhibits acquisition of plant Se resistance rather than enhancing it, like JA and ethylene. Treatment with SA increased selenite sensitivity in a Se-resistant *Arabidopsis* accession, Col-0.¹ The underlying mechanism for this negative effect of SA on Se resistance is still unclear, but one possible explanation is crosstalk between ethylene, JA and SA pathways. Many studies have shown that these hormones act in mutually antagonistic or coordinated ways in plants suffering biotic or abiotic stresses. For example, it was shown that JA accumulation is prevented by the NPR1-dependent SA-signaling pathway in plants infected by *Pseudomonas*.¹³ Also, SA is known to inhibit the activity of the last step in the ethylene biosynthesis pathway, ACC oxidase.¹⁴ Thus, the observed increased Se sensitivity in the presence of SA might act through inhibition of ethylene and/or JA signaling pathways.

As described above, it appears that ethylene and JA play important roles in Se resistance in plants. Previous studies have shown that

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generation of reactive oxygen species (ROS) often precedes ethylene and JA production.^{15,16} Our recent work showed that ROS production is enhanced in the Se-resistant accession Col-0.¹ On the other hand, very high levels of ROS production decreased Se-resistance in plants. Such plants showed signs of high levels of SA production, which may have attenuated ethylene and/or JA function as described above. Thus, it appears that optimal levels of ROS production are necessary for acquisition of Se-resistance in Arabidopsis. The source of ROS in Se-treated plants is still unclear and will require further study. Incidentally, ROS production was also observed in a selenite-treated cell suspension of coffee.¹⁷

We would like to propose a simple model based on the results described in our recent papers (Fig. 2). According to our model, absorbed selenate or selenite generates ROS in the plant. Incidentally, an increase in cytosolic calcium concentration is also expected in these plants since many genes related to calcium signaling, such as calcium transporters, calcium binding proteins and calmodulin genes, were identified in the transcriptome analysis of selenate-treated plants.⁷ The Se-induced ROS mimic an oxidative burst in plant cells, and the perception of this change triggers a wide array of signaling cascades similar to those induced by plant pathogens. Furthermore, Se-induced changes in the levels of free calcium may phosphorylate one of the subunits of NADPH-oxidase known to generate ROS, or it may directly affect NADPH oxidase activity because this enzyme has an N-terminal sequence with two calcium-binding EF-hand motifs.^{18,19} The generated ROS activate the production of ethylene, JA and SA. JA signaling upregulates both stress responsive¹³ and S uptake/metabolism genes.⁹ Ethylene signaling also upregulates stress responsive genes, but the contribution of these genes to acquisition of Se resistance in plants is unclear. In this context, it is noteworthy that transgenic *Arabidopsis thaliana* that overproduce *Arabidopsis halleri* plant defensin (*AhPDF1.1*)²⁰ showed a slight but significant increase in tolerance to selenite compared to wild-type plants (Fig. 3). This same gene was shown earlier to confer Zn tolerance when overexpressed in Arabidopsis.²⁰ As the function of PDF proteins is still largely unknown, the mechanism of these positive effects of PDF1.1 on Zn and Se tolerance is unclear. Apart from possible functions of defense-related genes in the acquisition of Se resistance, the upregulation of S uptake/metabolism genes by JA likely is quite important for Se resistance. Several transgenic plants with enhanced Se accumulation and resistance have already been developed through overexpression of genes involved in S metabolism.²¹⁻²³ Higher plant S levels likely help prevent incorporation of Se into S compounds, particularly proteins. Moreover, higher levels of the reduced S compound glutathione may help alleviate Se-induced oxidative stress. Any involvement of ethylene signaling in upregulation of S uptake/metabolism genes has not been shown until now. At low level, SA might have no effect on these processes, and thus on Se resistance, but at a higher level SA decreases plant Se resistance, perhaps through inhibition of ethylene and/or JA synthesis or signaling.

From an applied perspective, it is interesting that it appears from this study that increasing plant ethylene and/or JA levels may be a useful approach to develop plants with enhanced Se resistance and/or content. In this

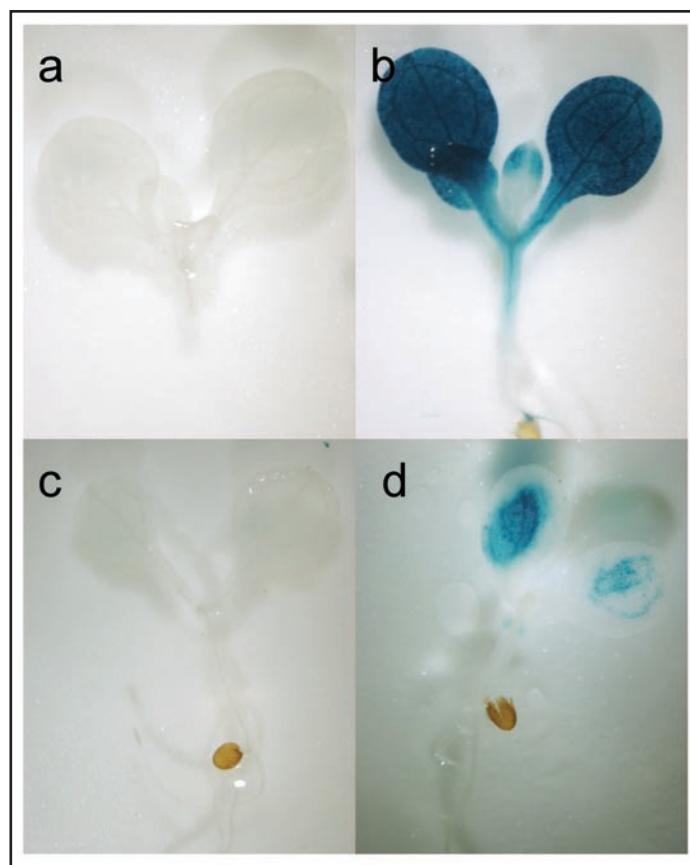


Figure 1. Induction of AOS and PDF1.2 gene expression with selenite treatment. Seedlings of transgenic plants carrying a AOS promoter::GUS insert were grown without (A) or with (B) 15 μ M selenite for 7 days, and stained with X-Gluc solution. Plants containing a PDF1.2 promoter::GUS insert were also grown without (C) or with (D) 15 μ M selenite, and seedlings were GUS-stained.

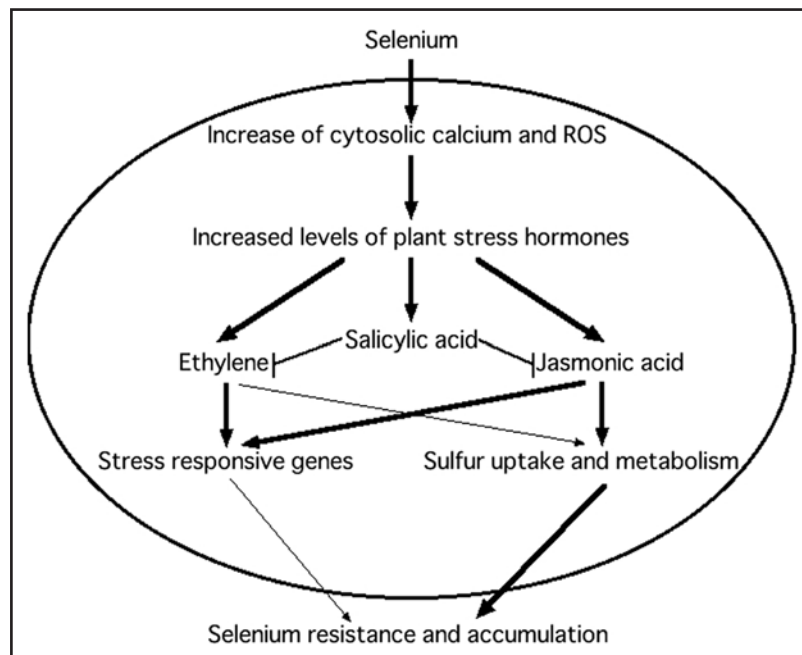


Figure 2. Schematic model for plant selenium responses.

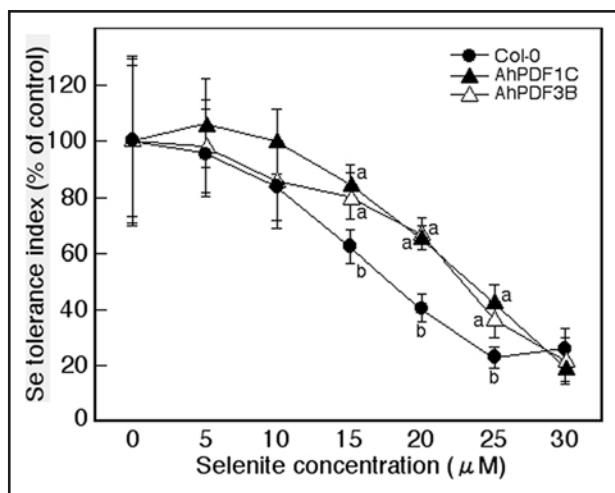


Figure 3. Selenite tolerance index for Col-0 (filled circles) and two lines of transgenic *Arabidopsis thaliana* expressing the *Arabidopsis halleri* PDF1.1 gene (AhPDF1C; filled triangles, AhPDF3B; open triangles). Plants were grown on control medium or on medium with various concentrations of sodium selenite for 10 days and measured for root length. Shown are the means \pm SD ($n = 20$). Lower case letters indicate significant differences between Col-0 and transgenic plants for a particular selenite concentration ($p > 0.05$).

context it is interesting to note that providing plants with ethylene or JA precursor resulted in not only higher Se tolerance but also higher accumulation (unpublished results). From an evolutionary perspective, the finding that higher levels of ethylene and JA correlate with higher Se tolerance and accumulation may give insight into the evolution of Se hyperaccumulation. Indeed, a Se hyperaccumulator plant, *Stanleya pinnata*, (Brassicaceae) shows constitutive high levels of JA production (unpublished results). Production of plants with enhanced Se tolerance and accumulation will be useful both for producing Se-fortified food to avoid Se deficiency in humans and livestock, and to clean up excess Se from polluted soil and water.

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