Can Se hyperaccumulation defend *Astragalus bisulcatus* against *Pseudomonas syringae* pv. siringae?

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Introduction

Heavy metal hyperaccumulation in plants could have an evolutionary sense because of the effect that metals may have in the interaction between the plants with other organisms. In particular, this could represent a way of chemical defence against pathogens and pests [Figure 1] **(Behmer et al., 2005)**.

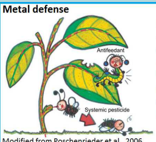

Figure 1: According to the metal defense hypothesis, the high metal concentrations that accumulate in leaf tissues of metal hyperaccumulators either deter (antifeedant) or intoxicate (plant systemic pesticide) herbivores or pathogens.

Modified from Poschenrieder et al., 2006

Tests of the elemental defence hypothesis for many elements such as As, Ni, Zn, Se and Cd have yet to be attempted **(Boyd R. S., 2007)**. So far, although some evidence has been provided that plants exposed to high metal concentrations have reduced susceptibility to various pathogens, but almost no studies have demonstrated that the metal itself was directly responsible for this effect **(Fones et. al, 2010)**.

Hiperaccumulation of selenium (Se) has been observed in the plant families *Asteraceae*, *Brasicaceae*, *Chenopodiaceae*, *Lecythidaceae*, *Fabaceae*, *Rubiaceae* and *Scrophulariaceae* **(Galeas et. al. 2008)**. To date, Se hyperaccumulation has been shown to protect plants from some mammalian herbivores as well as from several arthropod herbivores and some fungal pathogens **(Quinn et al., 2010)**.

Objectives

- **Analyze if heavy metal hyperaccumulation can offer protection against pathogens.**
  - **Research will be focused on *Astragalus bisulcatus* and *Pseudomonas syringae* pv. syringae B782a** [Figure 2]**.**

Figure 2: *P. syringae* pv. syringae B728a is a highly versatile foliar pathogen of bean that causes brown spot, a disease manifested as watersoaked lesions on bean leaves and pods.
Modified from Thakur et al. 2013

Expected results

- **Infection assays**

It is expected that the control group (growth without Se) will be the most significantly affected and, conversely, the group least affected would be the group treated with highest [Se].
Finally, according to Koch's Postulates, it is to be expected that the causal agent of the illness was *P. syringae* pv. syringae. This would determine that *P. syringae* pv. syringae is a pathogen of *A. bisulcatus*.

- **Pathogenicity assays**

On inoculation of *P. syringae* pv. syringae into *A. bisulcatus* plants treated with progressively higher [Se], is expected to decline both symptom development and *P. syringae* pv. syringae growth was significantly reduced as the [Se] treatment increases.
These results would confirm that Se hyperaccumulation inhibits bacterial growth *in planta* and defends *A. bisulcatus* against disease.

- **Bacterial growth in apoplast extracts**

With this assay one expects to see a decreased bacterial growth on apoplast extracts from high [Se] treated plants supported significantly less bacterial growth than those from plants grown without supplementary Se.
As such, it would be clear that Se hyperaccumulation by *A. bisulcatus* makes the apoplast a more hostile environment for the growth of *P. syringae* pv. syringae.

- **Se-tolerance assays of *P. syringae* pv. Syringae**

In these assays one is expected to see a decrease of viability of *P. syringae* pv. syringae to growth *in vitro* on LB media supplemented with Se at a range of concentrations. The higher [Se], the less growth will be seen.

- **Se content of whole-leaf and apoplast extract of *A. bisulcatus***

One can expect to see a high [Se] in whole-leaf and apoplast extracts from the plants treated with Se and not see Se concentration in whole-leaf and apoplast extracts from the plants treated only with nutritive solution. These high concentrations in whole-leaf tissue of *A. bisulcatus* plants growing at Se treatments would be sufficient to explain the observed reduction in bacterial growth *in planta*. Moreover, high apoplastic [Se] from the Se treatments would be also sufficient to explain bacterial growth reduction [Figure 3].

Methods

With this overall objective is proposed to carry out the following tests in the proposed patosystem:

- An infection assay in order to determine if *P. syringae* pv. syringae is a pathogen for *A. bisulcatus*.

- A pathogenicity assay in which *P. syringae* pv. syringae will infect to *A. bisulcatus* growth in progressive higher [Se] in order to evaluate if an inhibition of the disease is done with most [Se] in plant.

- A determination of [Se] in apoplastic spaces of the plant to determine the probability that the Se concentration in it phase causes an inhibition of pathogen growth *in vitro*.

- Se-tolerance assays for *P. syringae* pv. syringae to ascertain if it is possible that the hyperaccumulation has an inhibiting affect on the bacteria and, hence, if Se hyperaccumulation can defend *A. bisulcatus* against *P. syringae* pv. syringae.

- A final assay when Se concentration is measured in whole-leaf and in apoplasts of the different Se treatments of *A. bisulcatus* in order to confirm or refute that plants with a higher Se treatment accumulated a higher amount of Se.

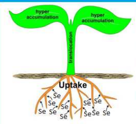

Figure 3: Hyperaccumulator plants actively take up large amounts of one or more heavy metals from the soil. The heavy metals are not retained in the roots but are translocated to the shoot and accumulated in aboveground organs.
Modified from Rascio et al., 2010

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