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Responses of root growth and antioxidative systems of paddy rice exposed to antimony and selenium

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ABSTRACT

Antimony (Sb) is a toxic element for plant; however, its toxic mechanisms, in particular for its toxicity to plant root growth, are unclear. Selenium (Se) can detoxify Sb, but its associated detoxification mechanisms have not been fully clarified. The aim of the present study was to investigate the effects of Sb alone, or accompanying with Se, on the root growth, antioxidative systems and the uptake of Se and Sb in paddy rice. Two nested hydroponic experiments were performed based on a two-factor, five-level central composite design. The results showed that Sb and Se could each reduce the contents of the other in both the shoots and roots. With 0.8 mg L^{-1} Se in the solution, increasing Sb levels from 1 to 9 mg L^{-1} significantly reduced the shoot biomass but enhanced the root biomass; however, in the presence of 5 mg L^{-1} Sb in the solution, the opposite results were observed with increased Se levels from 0.1 to 1.5 mg L^{-1}. In the presence of 0.8 mg L^{-1} Se, the addition of Sb increased the values of most root growth parameters, such as root length, root area, root forks, and the numbers of fine roots, medium roots and thick roots, suggesting a counteracting effect on root growth already negatively affected by 0.8 mg L^{-1} Se. However, the increased values of the root growth parameters cannot explain the significantly inhibited Se uptake in both the shoots and roots, suggesting an unknown mechanism in this respect. The addition of Se counteracted the negative effects of Sb on the cell membrane lipids and shoot biomass, but reduced the values of the root growth parameters. Thus, the enzymes SOD, APX and CAT might play important roles in rebalancing the excess ROS resulting from exposure to Se and/or Sb. These results support the hypothesis that Se affects the root growth to inhibit Sb uptake and simultaneously regulate the antioxidant systems to reduce oxidative stress.

1. Introduction

Antimony (Sb) is a non-essential but toxic element for both humans and plants. Increasing anthropogenic activities have led to Sb contamination worldwide, particularly in China (Hou et al., 2013; Kuwae et al., 2013; Vaculík et al., 2013). The accumulation of Sb has been observed in plants growing in the soils in the vicinity of some mines, which has seriously threatened the food safety (Wu et al., 2011). Indeed, excess Sb accumulation in plants has pernicious effects on plant growth. Levels of 5–10 mg kg^{-1} of Sb in plant tissue are harmful to plants (Kabata-Pendias and Pendias, 2001). One well-known detrimental effect of excess Sb to plants is to trigger the burst of reactive oxygen species (ROS) and induce oxidative stress (Feng et al., 2009, 2011; Pan et al., 2011). The enhanced production of ROS can be well controlled by intrinsic defense systems in plants (Mittler, 2002), which primarily include low molecular weight antioxidants and enzymatic antioxidants. The enzymatic antioxidants primarily include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). SOD is responsible for the scavenging of superoxide anion ($O_2^{\bullet-}$), and other antioxidants can be used to quench hydrogen peroxide (H_2O_2) (Feng et al., 2009; Meharg and Hartley-Whitaker, 2002; Asada, 2006; Feng and Wei, 2012).

Other detrimental effects of Sb on plants have also been reported, such as (1) the inhibition of root growth and rice sprout (He and Yang, 1999); (2) the lipid peroxidation of cell membranes

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and impairment of the thylakoid system and cytoplasmic lipid droplets in the lichen *Xanthoria parietina* (Paoli et al., 2013); (3) the disruption of essential element uptake in plants, such as potassium (K), calcium (Ca) and copper (Cu) uptake in the wheat *Triticum aestivum* L. (Shtangeeva et al., 2011; Shtangeeva et al., 2014), and Ca, magnesium (Mg), K, iron (Fe), manganese (Mn) and Cu uptake in paddy rice (Fengmeizhan) (Feng et al., 2013c,d); (4) the disruption of plant photosynthesis (Pan et al., 2011; Zhang et al., 2010), specifically through the inhibition of chlorophyll synthesis and maximum photochemical efficiency(F_V/F_M) (Pan et al., 2011); and (5) the synthesis inhibition of soluble proteins (Paoli et al., 2013), soluble sugars and starch in sweet mustard (Feng et al., 2013c). Additional information regarding the toxicity of Sb to plants is still scarce.

Selenium (Se), an essential element for humans, is documented to be able to detoxify heavy metals (metalloids, HMs), including Sb (Feng et al., 2013d; Lin et al., 2012; Malik et al., 2012; Feng et al., 2013a; Kumar et al., 2013; Ding et al., 2014, 2015; Hu et al., 2014a); however, the associated mechanisms are not fully understood. Some potential mechanisms have been suggested (Feng et al., 2013a). A well-known detoxifying mechanism of HMs by Se is that proper doses of Se can regulate the antioxidant systems in plants to eliminate oxidative stress. However, to our knowledge, few studies have been conducted hitherto to investigate the responses of antioxidant systems in plants subjected to Sb, particularly in crops (Feng et al., 2009; Pan et al., 2011; Benhamdi et al., 2014; Corrales et al., 2014; Vaculíková et al., 2014), and little is known about the responses of plant antioxidant systems subjected to Sb and Se. It is not clear that whether antioxidative enzymes in Sb-stressed plants will be activated by Se to play important roles in detoxifying Sb.

Another important mechanism for the detoxification of HMs through Se involves the direct inhibition of the uptake of HMs, including Sb (Feng et al., 2011) and the regulation of the essential element uptake in plants exposed to Sb (Feng et al., 2013d). However, the question about why Se can directly regulate the uptake of HMs and essential elements in plants is still left to be resolved. Since the roots are the first part of the plant to contact HM-contaminated soil or soil solution (Lin et al., 2012; Ding et al., 2014; Li et al., 2012), the hypothesis that Se can influence plant root growth, thereby affecting Sb uptake, seems reasonable. In addition, it is unclear how plant root growth would respond to different levels of Sb. The resolution of these questions will provide information on whether and how Sb significantly affects the uptake of essential elements in plants.

Thus, the aim of the present study was to examine paddy rice (Shuangyou 998) in two nested hydroponic experiments based on a two-factor, five-level central composite design. Several aspects will be addressed, including: (1) the antioxidant responses of paddy-rice subjected to different levels of Se and Sb; and (2) the changes in the root growth of paddy rice. The results of the present study will contribute to the current understanding of the relationships among root growth, antioxidant responses, essential element uptake and the toxicity and detoxification of Sb.

2. Materials and methods

2.1. Experimental design

The experimental design and treatment concentrations of Se and Sb have been previously described (Feng et al., 2013d). Briefly, we selected 5 and 0.8 mg L^{-1} as the central values for Sb and Se, respectively, as 5 mg L^{-1} Sb induces oxidative stress and 1 mg L^{-1} Se is marginally toxic (Feng et al., 2013d). The other treatment levels of Sb and Se were determined according to Eq. (1)

$$x = \frac{(X - X_0)}{\Delta j} \tag{1}$$

where X, X_0, x and Δj denote the actual treatment levels, central values, coded values (see Supplementary Table 1) and scaling factors (2.829 mg L^{-1} for Sb and 0.495 mg L^{-1} for Se, respectively). Experiment I was used to investigate the single effects of Se on Sb uptake, root growth and antioxidant responses after fixing the Sb level at 5 mg L^{-1} and simultaneously increasing the Se levels from 0.1 to 1.5 mg L^{-1}. Similarly, the single effects of Sb on Se uptake, root growth and antioxidant responses were investigated after fixing the Se level at 0.8 mg L^{-1} while increasing the Sb levels from 1 to 9 mg L^{-1} (Supplementary Table 1). Experiment II contained 16 experimental runs and was performed to investigate the interactive effects of Se and Sb on their uptake, root growth and antioxidant responses. Because some treatments of Experiment I were the same as those of Experiment II, Experiment I was nested in Experiment II via the addition of Sb and Se treatments to Experiment II (runs 17-20, Supplementary Table 2), and the two experiments were conducted synchronously. There were three replications for each treatment, excluding run 9 of Experiment II, which included eight replications to minimize experimental error (Supplementary Table 2).

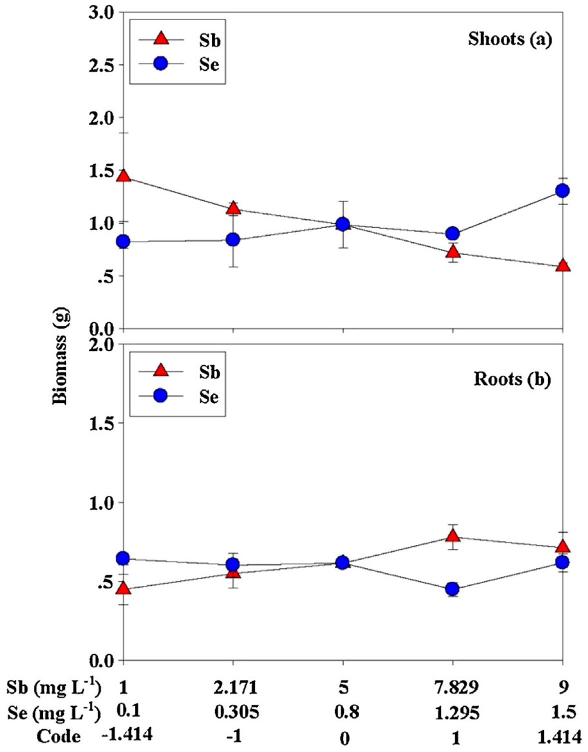

Fig. 1. Simple effects of Sb and Se on the biomass of paddy rice. Each of the curves was drawn based on the related single factor experiment. Symbols and vertical lines in the curves are means and standard error of means. Line —▲— Sb represents that Se was maintained at its central value of 0.8 mg L^{-1} while increasing the Sb concentration from 1 to 9 mg L^{-1}; line —●— Se represents the experiments in which Sb was maintained at its central of 5 mg L^{-1} while increasing the Se concentration from 0.1 to 1.5 mg L^{-1}.

2.2. Plant culture and treatment process

After sterilization using 2% NaClO for 10 min, the plump seeds (hybrid rice (Shuangyou 998) were thoroughly rinsed with tap water and de-ionized water and finally sown onto moist potting compost (1:1 volume ratio of clean perlite and vermiculite). The