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REGULAR ARTICLE

Reducing basal salicylic acid enhances Arabidopsis tolerance to lead or cadmium

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Abstract
Aims Phytoremediation is an emerging strategy for the removal of heavy metal contaminants. However, one of the prerequisite is to understand adequately plant resistant mechanisms. The present study was performed to assess the role of endogenous SA in plant response to Pb or Cd using wild-type (wt) Arabidopsis and its SA-accumulating mutant *snc1*, SA-reducing transgenic line *nahG*, SA signal-blocking *npr1-1*, and *snc1/nahG* (i.e. expression of *nahG* in *snc1* plant) with a comparable level of SA to the wt.
Methods Plants were grown hydroponically in controlled conditions. For heavy metal exposure, Pb^{2+} or Cd^{2+} at final concentrations of 50 μM, 100 μM, and 150 μM, respectively, was added to the culture solution. Unless otherwise indicated, samples were harvested after 7 d of exposure, and used for analyses.
Results Compared to the wt level, the high endogenous SA significantly potentiated Pb- and Cd-induced plant growth retardation, whereas SA deficiency decreased the growth inhibition, and SA signaling blockage also had some protective effect. The expression of *nahG* in *snc1* plant mitigated effectively the growth inhibition. The SA-related mechanism was involved in redox homeostasis, photosynthetic process, and soluble matter accumulation.
Conclusions These results suggest that Pb- or Cd-induced phytotoxicity in Arabidopsis was intensified by elevated endogenous SA, whereas ameliorated by reduced SA.

Keywords Salicylic acid · Phytoremediation · Heavy metal · *Arabidopsis thaliana* · *nahG*

Abbreviations

CAT	catalase
Cd	cadmium
Fv/Fm	variable to maximum fluorescence ratio
MDA	malondialdehyde
nahG	naphthalene hydroxylase G
npr1-1	nonexpressor of pathogenesis-relative gene 1
Pb	plumbum
PN	net photosynthetic rate
POD	peroxidase
ROS	reactive oxygen species
SA	salicylic acid
snc1	suppressor of *npr1-1*, constitutive 1
SOD	superoxide dismutase
wt	wild type
ΦPS2	quantum efficiency of photosystem 2

Introduction

The heavy metal or metalloid pollution of soil or ground water is becoming more and more serious

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due to human activities and industrial processes. Because of their relative mobility, persistence and wide distribution in the environment, the removal of Pb and Cd contaminants is always a hard task. Phytoextraction of toxic metals has been intensely investigated mainly focusing on the identification and genetic modification of hyperaccumulators, heavy metal bioavailability, and the plant tolerant mechanisms (Bhargava et al. 2012; Memon and Schröder 2009; Rascio and Navari-Izzo 2011). Regarding the latter, increasing evidence has demonstrated that exposure to heavy metals increases plant endogenous SA contents, and exogenous SA can enhance heavy metal tolerance in a wide range of plant species, indicating that SA is involved in plant response to heavy metal stress (Horváth et al. 2007; Pál et al. 2006). However, due to the significant variation of basal levels of endogenous SA in different species of plants (Rivas-San Vicente and Plasencia 2011), it becomes difficult to evaluate the role of SA in plant responses to heavy metal stress by means of application of SA. Fortunately, the method of reverse genetics has been widely used to dissect the SA-related mechanisms, and a number of Arabidopsis mutants with gain-of-function or loss-of-function in SA-dependent signaling have been identified. Although *A. thaliana* is not a hyperaccumulator, it is a suitable target used to study metal accumulation and acclimation mechanisms. More importantly, because about a quarter of the recorded heavy metal hyperaccumulator species are the members of Brassicaceae (Memon and Schröder 2009), such as the model accumulator *Arabidopsis halleri* and *Thlaspi caerulescens* which has an average of 88.5 % DNA identity in coding regions to *A. thaliana* (Rigola et al. 2006), thus, the study results in *A. thaliana* could be extended to its relatives.

The aim of this study was to assess the role of endogenous SA in plant response to Pb or Cd stress, with special emphasis on its effects on plant growth, photosynthetic process, lipid peroxidation, electrolyte leakage, and soluble substance accumulation. Arabidopsis genotype *nahG* and *npr1-1* have been used extensively as SA deficiency and SA signaling blockage materials to dissect the SA-related role in plant responses to abiotic stresses. The *nahG* line is a transgenic plant which expresses the bacterial *nahG* (naphthalene hydroxylase G) gene encoding a SA-degrading enzyme, salicylate hydroxylase preventing the accumulation of SA in the host plants (Gaffney et al. 1993). The *npr1-1* plant has an ethyl-methanesulfonate (EMS)-mediated point mutation leading to a single aminoacyl substitution in the third ankyrin-repeat consensus sequence of NPR1, a known only positive regulator of systemic acquired resistance (SAR) to function downstream of SA and regulate *PR-1* genes through physical interaction with the TGA subclass of bZIP transcription factors in cell nucleus (Cao et al. 1994, 1997; Zhang et al. 1999). The mutant *npr1-1* completely blocks the induction of SAR by SA, displaying little expression of *PR* genes and increased susceptibility to bacterial and fungal infections (Cao et al. 1997). The *snc1* line is isolated from EMS-mutagenized M_2 progeny on the basis of the suppression of *npr1-1* phenotype (Li et al. 2001). This mutant has a high level of SA which is required for manifestation of the specific phenotype under stressed conditions such as pathogen infection (Li et al. 2001), NaCl exposure (Hao et al. 2012), thereby it is relevant to use *snc1* plants as a control of high SA in this study. In addition, to confirm that the high SA is responsible for the phenotype of *snc1* plants in the present experiment, we employed the *snc1/nahG* plant which was made by a cross between the *snc1* mutant and *nahG* transgenic line (Li et al. 2001).

In contrast to most of the SA-applying studies where treatments with SA can enhance Pb or Cd tolerance in a wide range of plant species (for a review, see Horváth et al. 2007), our data suggested that high endogenous SA intensified Pb- or Cd-induced phytotoxicity, whereas low SA ameliorated the toxicity, which was further confirmed by a reverted performance of *snc1/nahG* plants relative to the *snc1* plants.

Materials and methods

Plant material and treatment

Seeds of wild type (wt) of *A. thaliana* (L.) Heydn. (ecotype Columbia) and its mutants *snc1* (Li et al. 2001), *npr1-1* (Cao et al. 1994), transgenic line *nahG* (Gaffney et al. 1993), and *snc1/nahG* (Li et al. 2001) were sterilized in a 5 % (v/v) sodium hypochlorite solution for 15 min, followed by three washes with sterile distilled water. The seeds were sown in self-made devices according to a previous method (Hao et al. 2012), and placed at 4 °C in the dark for 48 h, then transferred to growth chambers under 10 h of light (150 μmol m^{-2} s^{-1}) at 22 °C and 14 h of dark at 18 °C, and 70 % humidity. Fifteen days after germination, the

seedlings were thinned to 1 seedling left in each seedholder, and grown for an additional 10 d.

For heavy metal exposure, plants were transferred to Hoagland solution containing either Pb^{2+} by adding $Pb(NO_3)_2$ or Cd^{2+} by adding $CdCl_2$ at final concentrations of 50 μM, 100 μM, and 150 μM, respectively. Except where mentioned, samples were harvested after 7 d of exposure, and used for analyses.

Determination of SA levels, Pb and Cd contents

According to the description of Zawoznik et al. (2007), total SA level was determined in pooled fresh rosette leaves (0.2 g) from 15 plants receiving the same treatment at the time points indicated in the text. For the determination of Pb or Cd content, dried plant material (0.2 g) from the same treatment was ground to a fine powder in a mortar and pestle and digested with 5 ml of concentrated HNO_3. Pb or Cd concentration in the extracts was estimated by inductively coupled plasma-atomic emission spectrometry (ICP-AES, iCAP 6000 Series, Thermo Electron Corporation). Standard curves were prepared with a Pb^{2+} or Cd^{2+} standard solution.

Estimation of plant growth

Plants were collected and weighed and their primary root length was measured. Plant growth was expressed by the relative increase in the biomass of aerial parts and in the primary root length during the exposure to heavy metals, and by the fresh weight of whole plant at the end of exposure. For biomass determination, the sample was dried at 80 °C for 48 h.

Measurement of biochemical parameters

CO_2 assimilation rate (PN) was determined using a portable photosynthetic system (LI-6200, LI-COR, Lincoln, NE, USA) with leaf chamber specific for Arabidopsis (LI-6400-17) at ambient climatic conditions, irradiance of 150 μmol m^{-2} s^{-1}, and 25 °C. Chlorophyll fluorescence parameters were analyzed using a portable fluorometer (Handy-PEA, Hansatech, Norfolk, UK) as described by Hao et al. (2012). The content of reduced form of glutathione (GSH) was detected according to the method of Griffith and Meister (1979). The oxidized form of glutathione (GSSG) was calculated from the difference between the amount of glutathione in 1,4-dithiothreitol (DTT)-treated samples and its non-DTT-treated samples. The content of proline was measured as described in Bates et al. (1973). For antioxidant enzyme activity determination, the fresh leaf cuts (0.5 g) were homogenized in liquid nitrogen using a mortar and pestle. The powder was suspended in 5 ml of cold extraction solution consisting of 50 mM phosphate-buffered saline (pH7.8), 0.1 mM EDTA, 1 % (v/v) Triton X-100, and 4 % (w/v) polyvinylpyrrolidone. The homogenate solution was incubated on ice for 10 min, and centrifuged at 12,000×g and 4 °C for 15 min. The supernatant was used as a crude enzyme extract to assay: (i) superoxide dismutase (SOD; EC 1.15.1.1) activity using the method of Beyer and Fridovich (1987); (ii) catalase (CAT; EC 1.11.1.6) activity according to the description by Aebi (1983), where the initial concentration of H_2O_2 was 0.04 % (v/v); and (iii) peroxidase (POD; EC 1.11.1.7) activity following Hemeda and Klein (1990). The total protein concentration of each extract was quantified by the method of Bradford (1976) using bovine serum albumin as the standard. The isozymes of SOD, POD, and CAT were analyzed by native polyacrylamide gel electrophoresis (PAGE) as described previously (Hao et al. 2012).

Hydrogen peroxide level was determined according to a method from Mukherjee and Choudhuri (1983). Lipid peroxidation was assessed by determining the malondialdehyde (MDA) production according to Shalata and Tal (1998). The electrolyte leakage was measured using conductivity meter (SA29-DDB-11A, Midwest Group, Beijing, China) as described by Hao et al. (2012).

Statistical analysis

Each datum point was the mean of at least three replicated experiments, and was analyzed by SAS software (SAS Institute, Cary, NC, USA), expressed as means ± SD.

Results

SA, Pb and Cd contents, and plant growth

The basal SA levels were 10.1, 2.86, 1.72, 0.43 and 1.3 μg g^{-1} fresh weight of plant, respectively in *snc1*,

npr1-1, wild type (wt), *nahG* and *snc1/nahG* plants under unstressed conditions (Fig. 1a). An increase level of SA was observed in *snc1* plants after 6 h of exposure, and in *npr1-1* and wt plants after 12 h of exposure to Cd (Fig. 1a) or Pb (data not shown) at 100 μM, respectively, whereas no change was detected in *nahG* and *snc1/nahG* plants. Pb or Cd was accumulated with a similar pattern in all the tested plants after exposure to Pb or Cd in a dose-dependent manner (Fig. 1b). In comparison with wt plants, *snc1* plant growth was inhibited by Pb or Cd exposure to a much greater extent, whereas *nahG* plants were more tolerant, and *npr1-1* plants also exhibited higher tolerance or at least a similar extent to all the applied doses

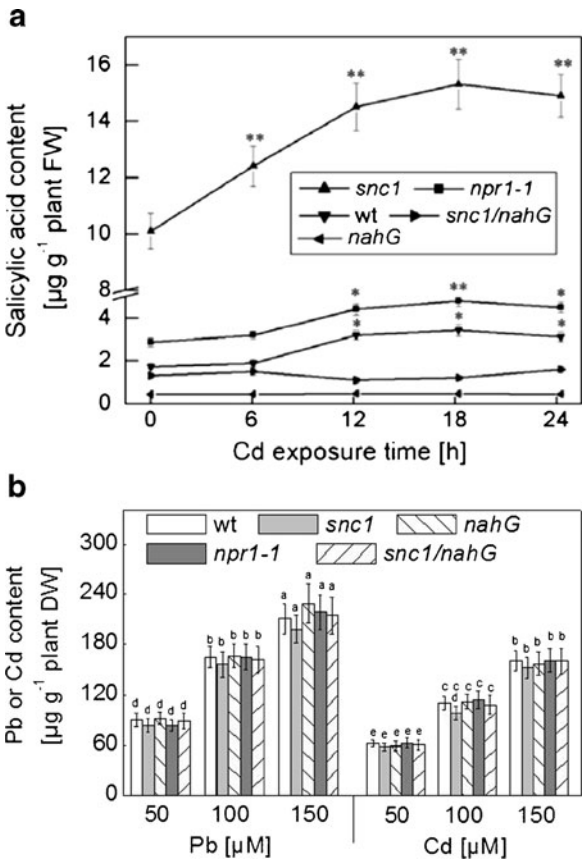

Fig. 1 Effect of exposure to Pb or Cd on SA levels and Pb or Cd contents in seedlings. **a** Effect of Cd at 100 μM on SA levels; (**b**) Pb or Cd contents. The data are from three replicated experiments (n=9), and represent means ± SD. "*" and "**" in the panel A respectively indicate significant difference at $P<0.05$ and $P<0.01$ from the sample at the beginning of Pb or Cd exposure. *Bars with different lower-case letters in the panel B* indicate significant differences at $P<0.05$, and the same below

of metals, as indicated by relative increases in the biomass of aerial parts of the plants (Fig. 2a) and in the root length (Fig. 2b) during the exposure, and by the fresh weight of whole plant at the end of exposure (Fig. 2c), as well as shown by the representative pictures of the tested plants exposed to Cd of 100 μM (Fig. 3). Interestingly, the growth suppression of *snc1* was effectively alleviated by the expression of *nahG* gene (i.e. *snc1/nahG* plants). In addition, these data in Fig. 2 also showed that the roots were more sensitive than the aerial parts to the heavy metal stress, and the phytotoxicity of Cd was greater than Pb considering the same applied dose. Based on the differential growth between wt plants and SA-altering mutants, it was concluded that Pb- or Cd-induced phytotoxicity in Arabidopsis was intensified by elevated endogenous SA, whereas ameliorated by reduced SA, which was further confirmed by the performance of *snc1/nahG* plants.

Net photosynthetic rate and chlorophyll fluorescence parameters

In line with the plant growth, the *snc1* had the lowest values in net photosynthetic rate (PN), ratio of variable to maximum chlorophyll fluorescence (Fv/Fm), and actual efficiency of PS2 (ΦPS2) under all the applied doses of heavy metals, whereas *nahG* plants performed much better than wt plants, and *npr1-1* plants were better than, or at least similar to wt (Fig. 2d–f). These reduced photosynthesis-related parameter values in *snc1* plants were recovered to the wt levels by the expression of *nahG* gene. This indicated that the SA-potentiated plant growth retardation under Pb- or Cd-exposure was associated directly with the impairment of photosynthetic process. Plant PN reduction is also often seen in the cases of exogenous applications of SA (leaf-infiltrated or via rooting medium) by inducing stomatal closure, slowing down PS2 electron transport, reducing RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) activity and chlorophyll contents and so on (Janda et al. 2012; Poór et al. 2011; Rivas-San Vicente and Plasencia 2011). Maintenance of CO_2 assimilation rate under heavy metal stresses is in favour of phytoremediation strategy because it sustains hyperaccumulators rapid growth and high biomass production.

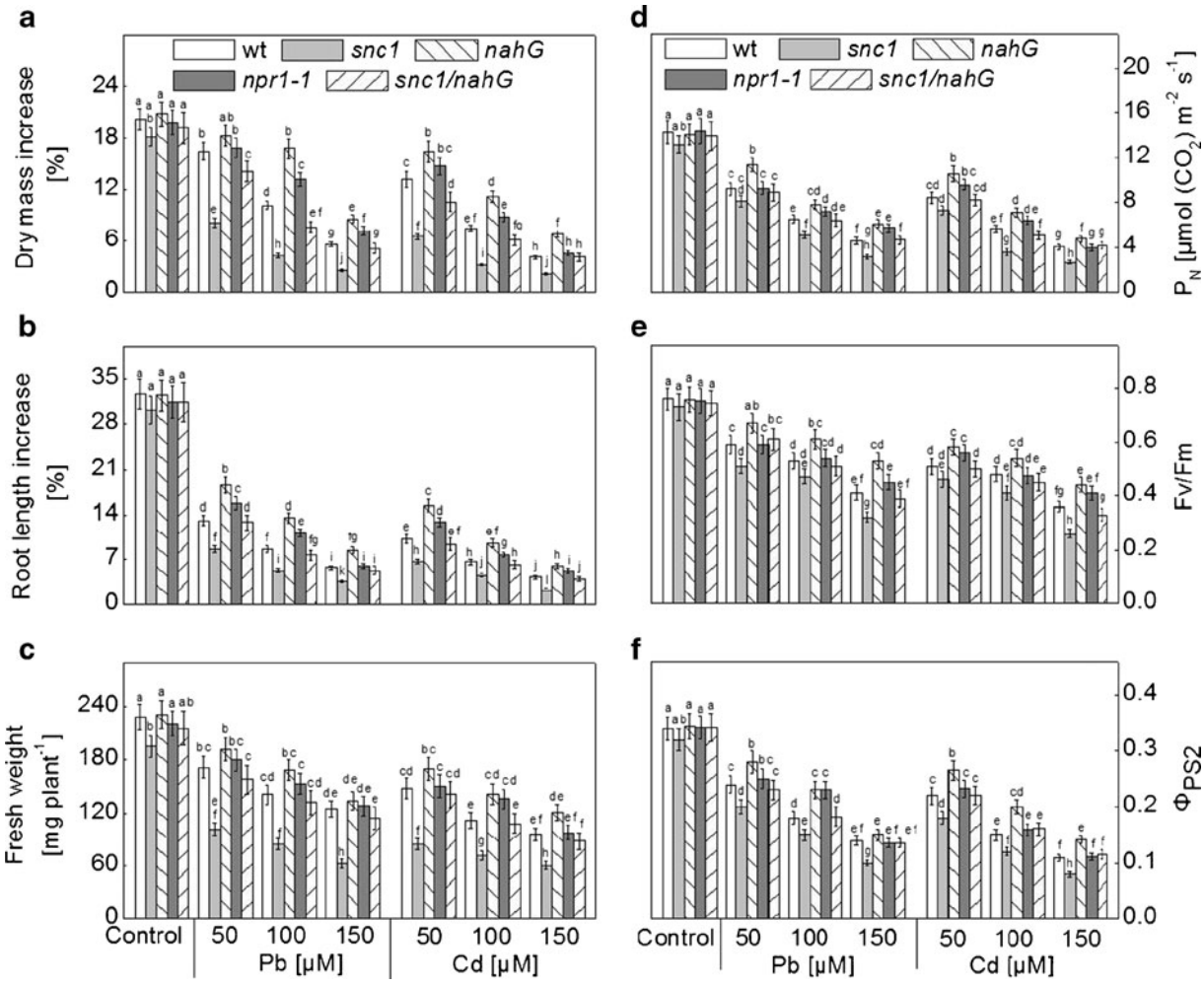

Fig. 2 Effect of Pb or Cd on the relative biomass increase of aerial parts (**a**) and relative length increase of primary roots (**b**) during the exposure, and the fresh weight of whole plant at the end of exposure (**c**), $n=60$; net photosynthetic rate (P_N) (**d**), ratio of variable to maximum fluorescence (Fv/Fm) (**e**), and actual photochemical efficiency (ΦPS2) (**f**), $n=12$

Antioxidant enzymes

Exposure to Pb or Cd increased the activities of SOD and POD in all the analyzed plants with the exception of SOD in wt, *nahG* and *npr1-1* at Cd of 150 μM where no significant difference from their respective control plants (i.e. grown in the un-stressed condition) was detected. When compared to the activity of SOD and POD in wt plants, a much higher level was found in *snc1* plants, and a lower, or at most a similar extent was observed in *nahG* and *npr1-1* plants (Fig. 4a and b). However, the particularly high activity of SOD or POD in *snc1* plants was reduced by the expression of *nahG* gene to a comparable level to the wt at most of the applied doses of either metal, Pb or Cd. These were also demonstrated by their isoform profiles stained in gels (Fig. 4d). By opposite, the activities of CAT were decreased in all plants exposed to Pb or Cd with a dose-effect relationship except for the wt, *nahG*, *nar1-1* and *snc1/nahG* plants exposed to Cd of 50 μM where no significant difference was observed relative to their respective control (i.e. Cd=0) (Fig. 4c and d). The general changed trends of CAT activities among the genotypes were also opposite to SOD or POD (Fig. 4c versus Fig. 4a and b). Notably, the low level of CAT in *snc1* plants was up-regulated by the expression of

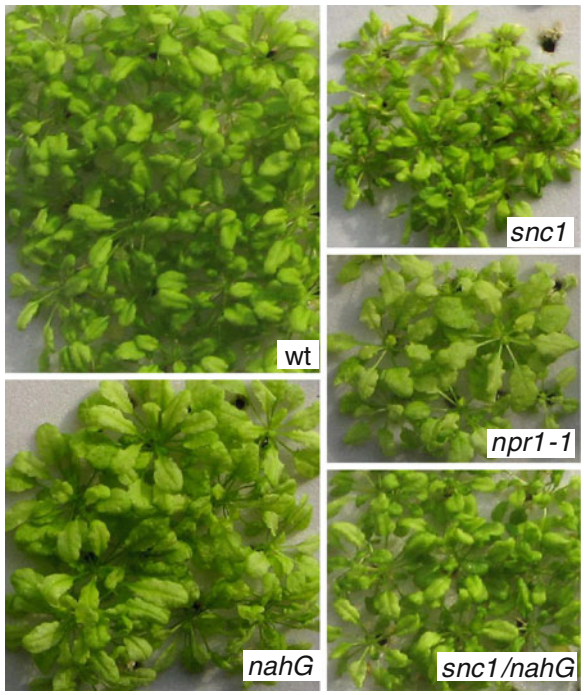

Fig. 3 Representative pictures of the tested genotypes at the end of exposure to Cd of 100 μM

nahG, especially in the metal-stressed conditions (Fig. 4c and d).

Glutathione and proline

Figure 5a clearly showed that although the highest level of glutathione was present in *snc1* plants under unstressed conditions, it declined to the lowest relative to other genotypes in the stressed conditions. Correspondingly, the ratio of reduced form of glutathione to its oxidized form (GSH/GSSG) was the lowest in *snc1* exposed to Pb or Cd (Fig. 5b). By contrast, *nahG* plants had higher levels, and *npr1-1* plants also possessed higher or at least similar levels, when compared to wt plants under the stressed conditions (Fig. 5a and b). Likewise, the expression of *nahG* in *snc1* plants effectively held the level of GSH and ratio of GSH/GSSG. Free proline was enhanced gradually in all the tested plants exposed to either of the two metals with increasing doses. However, the extent was lesser in *snc1* plants, whereas a greater or at least the same extent was observed in *nahG*, *npr1-1* or *snc1/nahG* plants compared to wt plants (Fig. 5c).

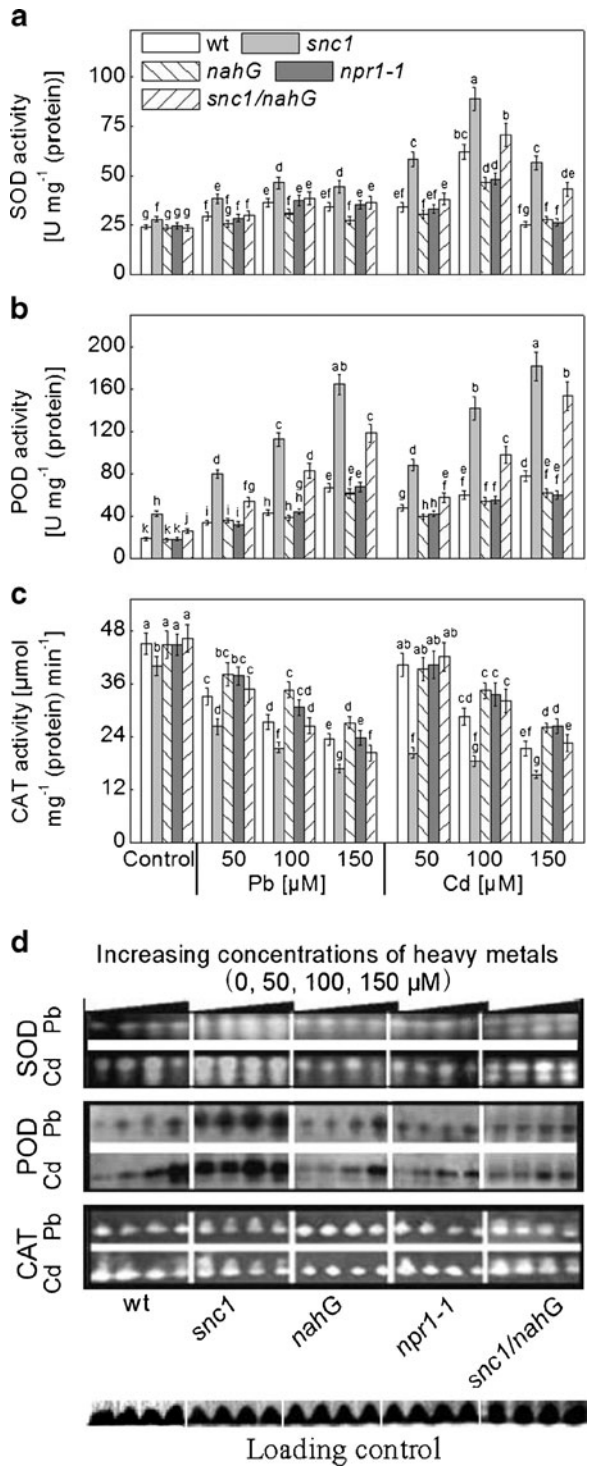

Fig. 4 Effect of Pb or Cd on the activities of antioxidative enzymes. **a** Superoxide dismutase (SOD), (**b**) Peroxidase (POD), and (**c**) Catalase (CAT). $n=9$. **d** The isoforms of SOD, POD and CAT in the representative plants stained in gels. Loading amount of protein per lane is 10 μg, which was confirmed by Coomassie brilliant blue G-250 staining in gel as shown by loading control

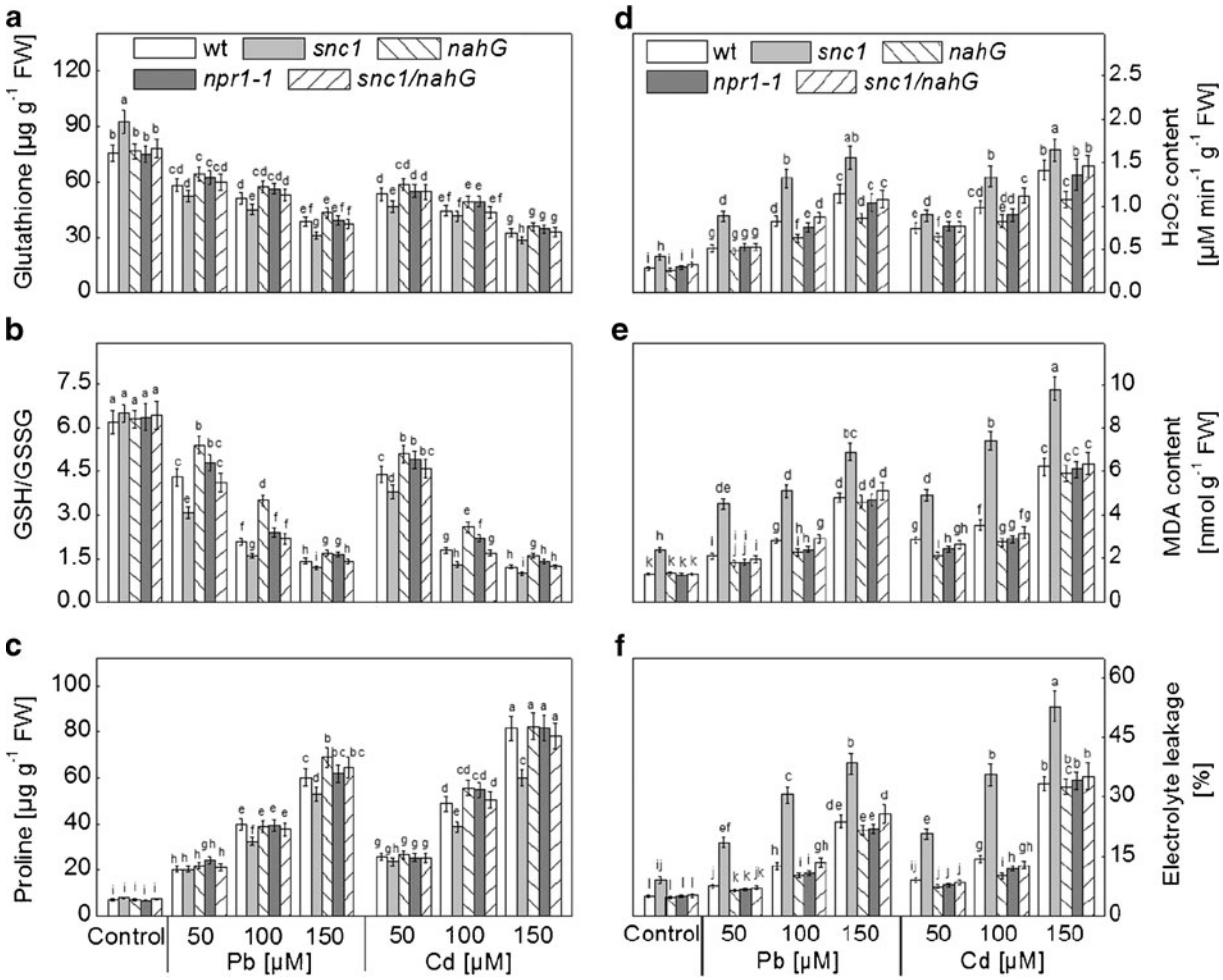

Fig. 5 Effect of Pb or Cd on reduced glutathione levels (GSH) (**a**), ratios of GSH to oxidized glutathione (GSH/GSSG) (**b**), proline levels (**c**), hydrogen peroxide (**d**), malondialdehyde (MDA) (**e**), and electrolyte leakage (**f**). $n=9$

Hydrogen peroxide, lipid peroxidation and electrolyte leakage

As expected, H_2O_2 was excessively produced in all the tested plants exposed to Pb or Cd with a dose-dependent manner, even though a difference existed among the tested genotypes, with the highest accumulation being observed in *snc1* plants, the least in *nahG* plants, a similar level to the wt being detected in *npr1-1* plants, and a significant reduction being found in *snc1/nahG* relative to *snc1* (Fig. 5d). In line with the level of H_2O_2, *snc1* plants had the highest accumulation of malondialdehyde (MDA) at all the applied doses of metals, whereas *nahG* and *npr1-1* produced lower MDA except the exposure to 150 μM of Pb or Cd where no statistical difference was observed between the mutants and wt plants (Fig. 5e). Strikingly, the heavy metal-induced MDA in *snc1* was reduced to the wt level by the expression of *nahG* gene. As a product of membrane lipid peroxidation, MDA has been widely used as an indicator of heavy metal phytotoxicity (Hasan et al. 2009; Pourrut et al. 2011). The change of electrolyte leakage was essentially consistent with the MDA (Fig. 5e). Electrolyte leakage is an important indicator to reflect the magnitude of cell death (Brosché and Kangasjärvi 2012).

Discussion

First of all, this study showed that the SA-accumulating *snc1* plants were more sensitive to Pb or Cd phytotoxicity

than other tested genotypes as indicated by plant growth and some physiological and biochemical parameters such as lipid peroxidation, electrolyte leakage, photosynthetic process, and soluble substance. The manifestation of the increased sensitivity to heavy metals was dependent on its high SA level, because the expression of *nahG* gene in *snc1* genetic background not only reduced the SA content to the wt level, but also recovered the wt-like phenotype in the metal-stressed conditions. This was in agreement with the performance of this genotype in other stressed conditions such as pathogen infection (Li et al. 2001) and NaCl exposure (Hao et al. 2012). On the other hand, the *nahG* transgenic line displayed higher tolerance than its wt plants, further demonstrating that the reduced endogenous SA was favourable for plant tolerance to heavy metals. Considerable evidence has demonstrated that exogenous SA can enhance Pb or Cd tolerance in a wide range of plant species (Horváth et al. 2007). Nevertheless, there also are a few studies showing that reduced level of SA in Arabidopsis increases Cd tolerance rather than decreases Cd uptake (Zawoznik et al. 2007), and SA signaling blockage (*npr1-1*) improves Ni tolerance also not affecting its absorption (Freeman et al. 2005). This discrepancy has also been observed in plant response to NaCl where a protective role of exogenous SA has been repeatedly evidenced in various plants, whereas a reduced endogenous level of SA has enhanced Arabidopsis tolerance to moderate salt stress (Cao et al. 2009; Hao et al. 2012). This suggests that the positive regulation of SA on plant response to environmental stresses may be related to its content, half-life in plants, or the timing of use. In fact, in most of the SA-applying studies, only a dozen of hours of SA pretreatment are sufficient to induce plant tolerance to various stresses, suggesting that the action of SA in that is an early event. However, SA-accumulating mutants have a constitutive SA level in their entire lives like the case in the *snc1*. The growth retardation like in *snc1* plants also occurs in other SA-accumulating Arabidopsis mutants such as *cpr* and *acd* series, which is totally or partially reverted by the expression of *nahG* or co-mutation of *npr1*, however, a growth promoting is observed in SA-deficient lines such as *nahG* and *sid2* (for a review, see Rivas-San Vicente and Plasencia 2011). In phytoremediation strategy of heavy metal contamination, a rapid growth and high biomass production are prerequisites to hyperaccumulators. In addition, based on the similar accumulated profile of either of the two metals, Pb or Cd, among the genotypes (Fig. 1b), it was proposed that the enhanced resistance in *nahG* plants or the re-acquisition of resistance in *snc1/nahG* plants was linked to a tolerant mechanism rather than an avoidant mechanism. A further work is needed to testify if the growth-promoting and the enhanced tolerance in SA-reducing (constitutive) Arabidopsis under the heavy metal stress also happen in *nahG*-transformed hyperaccumulators.

A large body of evidence has shown that SOD and/or POD play a crucial role in plants against Pb or Cd toxicity (e.g. Hasan et al. 2009; Pourrut et al. 2011). However, our data seemed to support another notion that SOD or POD can be used as a biomarker or monitor of metallic stress (Jouili et al. 2011; Passardi et al. 2005). Moreover, in view of the specific CAT profile relative to the POD among the genotypes (Fig. 4b versus c), this study implied that the CAT activity might be an essential complement to the peroxidative cycle of PODs responsible for the removal of cellular H_2O_2 and, accordingly, leading to lower lipid peroxidation and electrolyte leakage. Relative to other H_2O_2-decomposing enzymes, CATs are highly stable and active due to not require cellular reductants (Mhamd et al. 2010). In addition, in consideration of the extremely high POD activity and SA content, as well as the most sensitive phenotype in *snc1* plants relative to the other tested genotypes in the present study, we discussed the correlationship between POD activity, SA level and the sensitivity to heavy metals. Class III peroxidases (PODs) are heme-containing glycosylated proteins consisting of 73 members identified in *A. thaliana* participating in a broad range of physiological processes throughout the lifespan of the plant (Almagro et al. 2009). A body of evidence has definitely testified that the PODs are induced by heavy metals, but their role in phytoremediation is still equivocal (Passardi et al. 2005). Except for their regular peroxidative cycle catalyzing the reduction of H_2O_2, PODs have a separate oxidative (or hydroxylic) cycle, such as the NADH-POD oxidase activity, which can produce reactive oxygen species (ROS), like $^{\bullet}OH$, $HOO^{\bullet}$, H_2O_2 causing oxidative damage (Jouili et al. 2011; Kukavica et al. 2012). This might explain at least in part the *snc1* sensitivity to heavy metals. Because PODs are the members of pathogen-related (PR) family (Passardi et al. 2005) which is specifically induced by SA, it is a justifiable phenomenon that the *snc1* plants possess a high POD activity. Also, the recovery of a wt-like level of POD activity in

snc1/nahG plants further confirmed that the high POD activity was correlated to the high level of SA in *snc1* plants. In deed, earlier studies have shown that a part of the members of class III PODs are induced by SA (For a review, see Almagro et al. 2009). In turn, SA can be oxidized as electron (e^-) donor for POD generating SA free radicals ($SA^{\bullet}$), which may induce production of MDA and superoxide ($O_2^{\bullet -}$) (Kawano et al. 2004). Based on these reports, together with our observations in the present study, it was proposed that a synergistic action between SA and PODs may exist in the *snc1* plant leading to its sensitive phenotype under heavy metal stresses. On the other hand, it also supported the conclusion that the reducing basal SA can enhance Arabidopsis tolerance to Pb or Cd

Increasing evidence has shown that GSH plays a central role in metal chelation, compartment, homeostasis, antioxidative defense and signal transduction, therefore playing an important role in phytoextraction of toxic metals (Jozefczak et al. 2012; Seth et al. 2012). Glutathione exists in both reduced and oxidized forms, and its function relaying on the GSH/GSSG ratio and GSH level (Jozefczak et al. 2012). This was supported by our observation in the present study. It has been demonstrated that free proline enhances plant tolerance to a wide range of heavy metal species (Sharma and Dietz 2006). However, our observation indicated that even though the sensitivity of *snc1* to heavy metals may be associated with its lower levels of proline, and the re-acquisition of tolerance in *snc1/nahG* plants was also accompanied by the elevation of proline, the tolerance of *nahG* plants did not need higher proline levels (Fig. 5c), suggesting that proline level can not be used as a biomarker at least in evaluating the role of endogenous SA in Arabidopsis response to heavy metal stress.

Conclusion

Based on the performance of the tested genotypes under Pb or Cd stress, as indicated by plant growth, photosynthetic process, lipid peroxidation, electrolyte leakage and soluble substance accumulation, it is concluded that Pb- or Cd-induced phytotoxicity in Arabidopsis was intensified by elevated endogenous SA, whereas ameliorated by reduced SA. The high SA-caused sensitivity to heavy metals may be attributed to the synergistic action between POD activity and SA level. The SA-related role in the plant response to heavy metals is associated with a tolerant mechanism rather than an avoidant mechanism. It will be interesting to assess the performance of *nahG* genetransformed hyperaccumulators under heavy metal stresses in the future.

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