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Aluminum tolerance in *Arabidopsis thaliana* as affected by endogenous salicylic acid

D.Y. GUO, S.Y. ZHAO, L.L. HUANG, C.Y. MA, and L. HAO*

College of Chemistry and Life Sciences, Shenyang Normal University, Shenyang 110034, P.R. China

Abstract

Endogenous salicylic acid (SA) functions in plant response to an aluminum stress were assessed. We used different *Arabidopsis thaliana* genotypes including *snc1* with a constitutively high content of SA, *sid2* and *nahG* (transgenic lines) both with a low content of SA, SA insensitive mutant *npr1-1*, and *snc1/nahG* (*i.e.*, the *nahG* expression in the *snc1* background) with a similar SA content as in wild type (WT) plants. Results show that the *snc1* plants displayed obvious growth retardation of roots and shoots under the Al^{3+} stress, whereas the *sid2*, *nahG*, and *npr1-1* plants exhibited alleviated symptoms in comparison with the WT plants. The Al^{3+} content increased in all the tested genotypes with the increasing $AlCl_3$ concentration applied, but no significant variations were detected among the tested genotypes. The *snc1* had much higher superoxide dismutase and peroxidase activities, and a lower catalase activity and the ratio of reduced to oxidized glutathione accompanied by higher accumulations of H_2O_2 and malondialdehyde compared with the WT plants. These changes were largely reversed by the introduction of *nahG*; the *sid2*, *nahG*, and *npr1-1* plants were less affected than WT plants in all the above-mentioned parameters. The Al^{3+} stress significantly enhanced malate exudation in all the tested genotypes, but no significant correlation was observed between the SA-involved response to the Al^{3+} stress and the malate exudation. Based on these data, it was concluded that the SA-related functions in *Arabidopsis* response to the Al^{3+} stress were associated with the control of oxidative stress, but not of malate exudation.

Additional key words: catalase, glutathione, malate, lipid peroxidation, peroxidase, redox homeostasis, superoxide dismutase.

Introduction

Aluminum (Al) phytotoxicity is a major limiting factor of plant growth, development, and yield in acidic soils (pH < 5.0) which cover approximately 50 % of the world's potentially arable land (Kochian *et al*. 2004). The plant root apex is the foremost target of Al^{3+} toxicity thereby restricting the acquisition of water and nutrients from soils (Ma *et al*. 2001). In general, plants resist Al^{3+} toxicity by external detoxification and internal detoxification. The most representative finding in the former is the Al-activated exudation of organic acids from the root apex to rhizosphere where forming non-toxic complexes with Al^{3+} ions thereby preventing Al^{3+} entry into roots (Ma *et al*. 2000, Kinraide *et al*. 2005, Yang *et al*. 2013). The latter is associated with complicated but coordinated modifications in plant morphology, architecture, physiology, biochemistry, and gene expression (Ma *et al*. 2000, Kochian *et al*. 2004). A transcriptomic analysis has shown that more than 1 000 *Arabidopsis* genes may be involved in response to Al^{3+} stress, related to anti-oxidative defence, membrane transport, cell wall, energy metabolism, polysaccharide metabolism, signal transduction, transcriptional activation, and so on (Kumari *et al*. 2008).

As a signal molecule, salicylic acid (SA) has been extensively implicated in plant responses to adverse environmental stresses (*e.g.*, Horváth *et al*. 2007, Mutlu

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Abbreviations: CAT - catalase; MDA - malondialdehyde; *nahG* - naphthalene hydroxylase G; *npr1-1* - nonexpressor of pathogenesis-relative gene 1; POD - peroxidase; ROS - reactive oxygen species; SA - salicylic acid; *sid2* - SA induction deficient 2; *snc1* - suppressor of *npr1-1*, constitutive 1; SOD - superoxide dismutase.

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* Author for correspondence; fax: (+86) 24 86593309, e-mail: haolinwj2001@163.com

et al. 2009, 2013, Hao *et al*. 2011, Kang *et al*. 2013), including Al^{3+} stress. For instance, exogenous SA effectively alleviates Al phytotoxicity by decreasing the uptake of Al^{3+}, and increasing antioxidative defence and organic acid exudation (Liu *et al*. 2012, Pandey *et al*. 2013). In contrast, an SA treatment potentiates Al-induced damage to *Matricaria chamomilla* by elevating root-to-shoot transport of Al (Kováčik *et al*. 2012). These contrary findings may be due to different plant species tested as well as SA dosage, an application method, and timing. SA-altering mutants may be ideal experimental materials to reveal an SA role in plant response to Al^{3+} stress. Herein we employed *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) and its transgenic line *nahG* and mutant *sid2-1* both with SA deficiency, *snc1* with a constitutively high accumulation of SA, *npr1-1* with SA signal blockage, and *snc1/nahG* (*i.e*., the expression of *nahG* in the *snc1* background) to assess the effect of endogenous SA on plant response to an Al^{3+} stress. The detailed characteristics of these genotypes have been described in a previous report (Tao *et al*. 2013).

Materials and methods

Seeds of *Arabidopsis thaliana* L. ecotype Col-0 and its mutants *snc1* (Li *et al*. 2001), *npr1-1* (Cao *et al*. 1994), *sid2* (Wildermuth *et al*. 2001), transgenic line *nahG* (Gaffney *et al*. 1993), and *snc1/nahG* were kindly offered by Dr X. Li (the University of British Columbia), Dr X. Dong (Duke University), and Dr. L.A.J. Mur (the Aberystwyth University). After a cold treatment at 4 °C for 2 d, the seeds were surface sterilized with 75 % (v/v) ethanol for 1 min and 10 % (v/v) H_2O_2 for 5 min, then washed three times in sterile water, and sown in a self-made device (Hao *et al*. 2012) containing a half strength Murashige and Skoog (MS) solution with pH 6.2. Ten days after germination under a 14-h photoperiod, an irradiance of 150 μmol m^{-2} s^{-1} (provided by white fluorescent tubes), temperatures of 22/18 °C, and a relative humidity of 70 %, seedlings were thinned and left to grow for another 10 d. Then, they were transferred to a 1/2 MS solution with $AlCl_3$ at the final concentrations of 0, 0.5, and 1.0 mM, respectively. For acidic conditions, pH was adjusted to 4.5 with HCl. Unless otherwise indicated, samples were collected 7 d after the treatment and used for analyses.

A relative fresh mass (f.m.) increase was calculated by the formula (M1 - M0)/M0 × 100 %, where M0 is f.m. of roots or shoots at the beginning of the $AlCl_3$ exposure, and M1 is the corresponding value at the end of the $AlCl_3$ exposure. Collected fresh rosette leaves at the time points indicated in the text were used to quantify the total SA content according to Zawoznik *et al*. (2007). For detection of Al^{3+} content, root apices (terminal 2 - 3 mm of root) were collected, oven-dried overnight at 70 °C, weighed, then ground to a fine powder in a mortar and pestle, and digested with 5 cm^3 of concentrated HNO_3. The Al content in the homogenate was assayed by inductively coupled plasma-atomic emission spectrometry (ICP-AES, *iCAP 6000 Series*, *Thermo Electron Corporation*, MA, USA) according to the instrument specifications, and expressed per dry mass (d.m.) unit. Standard curves were prepared with $AlCl_3$ standard solutions. For determination of malate exudation, 20-d-old plants were transferred to a 1/2 MS solution (pH 4.5) with $AlCl_3$ at the final concentrations indicated in the text. Seven days after the treatment, the solution was collected to quantify malate exudation based on the method of Delhaize *et al*. (1993). Briefly, 1.35 cm^3 of the sample was incubated with 1.5 cm^3 of a buffer (0.5 M glycine, 0.4 M hydrazine, pH 9.0) and 0.1 cm^3 of 40 mM NAD. The reaction mixture was incubated for 30 to 60 min to obtain a stable absorbance (A_{340}) before the addition of 5 mm^3 of malate dehydrogenase (12 000 U cm^{-3}; *Boehringer*, Mannheim, Germany). For analysis of H_2O_2 content and lipid peroxidation, root samples were collected from the identically treated plants. The content of H_2O_2 was determined as described by Mukherjee and Choudhuri (1983). Lipid peroxidation was assessed based on the generation of malondialdehyde (MDA) according to Shalata and Tal (1998).

For determination of antioxidative capacity, fresh roots were harvested and homogenized in a mortar and pestle. The powder was suspended in 5 cm^3 of a pre-cooled extraction solution containing 50 mM phosphate-buffered saline (pH 7.8), 0.1 mM EDTA, 1 % (v/v) *Triton X-100*, and 4 % (m/v) polyvinylpyrrolidone. The homogenate was incubated on ice for 10 min, and centrifuged at 12 000 *g* and 4 °C for 15 min. The resultant supernatant was used to analyze antioxidant enzyme activities. A SOD (EC 1.15.1.1) activity was measured by the method of Beyer and Fridovich (1987). The reaction mixture contained a 50 mM phosphate buffer, pH 7.8, 9.9 mM L-methionine, 57 μM nitroblue tetrazolium (NBT), 0.025 % (m/v) *Triton X-100*, and 0.0044 % (m/v) riboflavin in a 3-cm^3 volume. The photoreduction of NBT (formation of purple formazan) was measured at 560 nm. One unit of SOD was defined as the amount of enzyme required to inhibit the photoreduction of NBT by 50 %. A CAT (EC 1.11.1.6) activity was measured according to Aebi (1983) by following the consumption of H_2O_2 at 240 nm for 5 min (a coefficient of absorbance 39.4 M^{-1} cm^{-1}). The reaction mixture contained a 100 mM potassium phosphate buffer (pH 7.0) and the plant extract in a 3-cm^3 volume. The reaction was initiated by adding 10 mm^3 of 30 % (m/v) H_2O_2. A POD (EC 1.11.1.7) activity was determined by the modified method of Hemeda and Klein (1990).

Briefly, the reaction mixture contained 16 mM guaiacol, a 100 mM potassium phosphate buffer (pH 6.5), and 10 mm^3 of 10 % (m/v) H_2O_2 in a 3-cm^3 volume. The reaction was initiated by adding the plant extract. An increase in absorbance due to guaiacol oxidation by H_2O_2 was measured at 470 nm (a coefficient of absorbance 26.6 M^{-1} cm^{-1}) at 30 s intervals up to 5 min. For all these measurements, a spectrophotometer *Spectronic 20* (*Bausch and Lomb*, MA, USA) was used. The total protein content in the plant extract was measured according to Bradford (1976) using bovine serum albumin as standard. The native polyacrylamide gel electrophoresis (PAGE) and staining SOD, POD, and CAT were performed as described previously (Hao *et al*. 2012). The content of the reduced form of glutathione (GSH) in roots was estimated following the method of Griffith and Meister (1979). The oxidized form of glutathione (GSSG) content was calculated from the difference between glutathione in 1,4-dithiothreitol (DTT)-treated samples and its non-DTT-treated samples.

Unless otherwise indicated, all data are expressed as means ± SD from at least three individual experiments, and they were subjected to the *ANOVA* using the *SAS* software (*SAS Institute*, Cary, NC, USA).

Results and discussion

The basal content of SA in the wild type (WT), *snc1*, *nahG*, *sid2*, *npr1-1*, and *snc1/nahG* plants was 1.58, 8.4, 0.43, 0.12, 2.82, and 1.4 μg g^{-1}(f.m.), respectively (Fig. 1*A*). Therefore, it was relevant that *snc1* was used as line with a high SA content, and *nahG* and *sid2* as lines with a low SA content. More importantly, the *snc1/nahG* plants included here should provide an insight into the role of SA in *snc1* plant response to the Al^{3+} stress, as the expression of *nahG* in the *snc1* plants reduced the SA content near to that in WT (Fig. 1*A*). The content of SA increased in the *snc1* plants at 6 h, in WT, *sid2*, and *npr1-1* at 12 h after the exposure to 1 mM $AlCl_3$, reached a maximum at 18 h, and then declined. The SA content in the *nahG* and *snc1/nahG* plants remained unaffected due to the nahG activity (Fig. 1*A*). Although the root Al^{3+} content in each genotype enhanced with increasing concentrations of $AlCl_3$, no statistically significant differences were detected among the tested lines (Fig. 1*B*). This implied that the changes in endogenous SA content or signalling did not affect the uptake and accumulation of Al^{3+}.

To assess the effect of the Al^{3+} stress on plant growth, we prolonged the exposure time to 14 d. The exposure to $AlCl_3$ reduced root and shoot growths of all the tested lines in a dose-dependent manner, to a greater extent in the *snc1* plants than in the *nahG*, *sid2*, and *npr1-1* plants as compared to WT (Fig. 2*A*,*B*). The Al^{3+}-induced growth retardation in the *snc1* plants was effectively alleviated by the introduction of *nahG*. The morphological phenotype also displayed that more severe damage occurred in the *snc1* plants, such as more extensive chlorosis than in the other tested lines, whereas the *nahG*, *sid2*, and *npr1-1* lines exhibited an alleviated symptom relative to the WT plants (Fig. 3). These data clearly demonstrate that endogenous SA played an important regulatory role in *Arabidopsis* responses to the Al^{3+} stress. More specifically, a reduced basal SA content or signal insensitive mutation alleviated Al^{3+} phytotoxicity, whereas a genetically constitutive accumulation of SA potentiated Al^{3+} toxicity. This is discrepant to previous observations where exogenous SA effectively mitigated Al-induced damage (*e.g*., Liu *et al*. 2012, Pandey *et al*. 2013). Nevertheless, some evidence also supports our conclusion. For instance, an application of 50 μM SA intensifies Al toxicity to *Matricaria chamomilla* plants by elevating root-to-shoot transport of Al^{3+} (Kováčik *et al*. 2012). In addition, *in vitro* cell cultures of *Arabidopsis*,

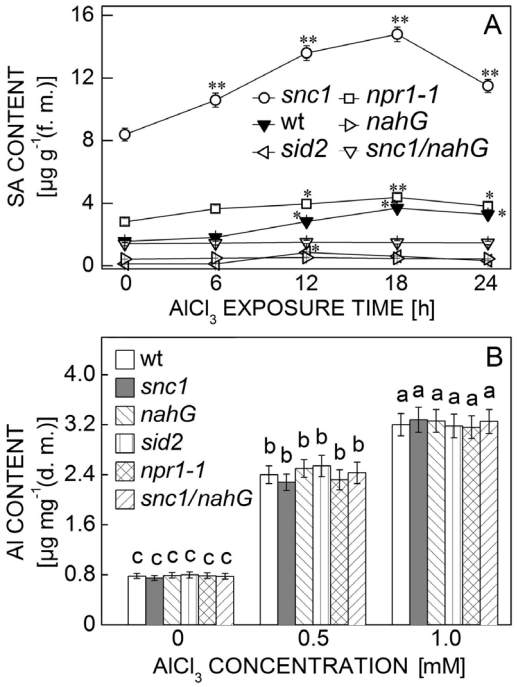

Fig. 1. The effect of exposure to 1 mM $AlCl_3$ on leaf SA content (*A*) and root Al^{3+} content (*B*) in different lines. Means ± SD, $n = 9$ (* and ** in the panel *A* indicate significant differences at $P < 0.05$ and $P < 0.01$, respectively, from the sample at the beginning of Al^{3+} exposure). Bars with different letters in the panel *B* indicate significant differences at $P < 0.05$ among lines. wt - wild type; *snc1* – a suppressor of *npr1-1*, constitutive 1; *nahG* - a transgenic line with salicylate hydroxylase gene; *sid2* - SA induction deficient, with a mutation in the gene encoding isochorismate synthase; *npr1-1* - a nonexpressor of pathogenesis-related gene 1; *snc1/nahG* -*nahG* expression in the *snc1* background.

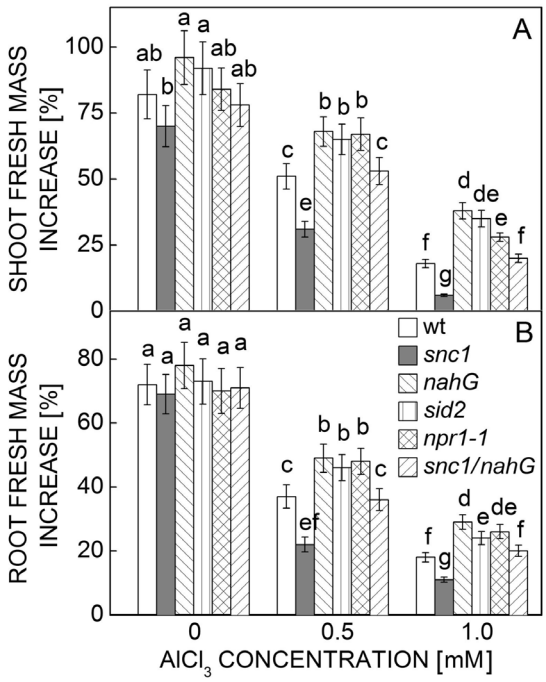

Fig. 2. The effect of exposure to $AlCl_3$ on plant growth. Relative fresh mass increases of shoots (*A*) and roots (*B*) during 14 d exposure are expressed as means ± SD, $n = 30$. See Fig. 1 for details.

sid2, *nahG*, and *npr1* display an enhanced tolerance to Al^{3+} stress relative to wild-type plants by inhibiting the expression of *AtrbohD*, a NADPH oxidase-coding gene, thus reducing ROS production and cell death (Kunihiro *et al*. 2011). In a previous study, we observed that the *snc1* plants were more sensitive to Cd or Pb stress than the *nahG* and *npr1-1* plants compared with the WT plants (Tao *et al*. 2013) suggesting that a common mechanism may exist in SA-mediated response to heavy metal stress.

To address the $AlCl_3$ concentration-dependent root malate exudation, we performed the experiment with an extended range of tested $AlCl_3$ concentrations from 60 μM to 1.5 mM. The results show that the malate exudation in each genotype significantly increased under the Al^3 stress and reached a peak at 1 mM $AlCl_3$ (Table 1). However, although the most sensitive performance of the *snc1* plants seemed to correspond to the least malate exudation under the Al^{3+} stress, a higher malate exudation at most of the $AlCl_3$ concentrations was not observed in the *nahG* or *sid2* line that exhibited a reduced growth inhibition relative to the WT plants (Figs. 2*A*,*B* and 3). This implies that the SA-related regulatory mechanism response to Al^{3+}, at least in *Arabidopsis*, was not tightly associated with malate exudation. This is indirectly supported by a previous finding that the *AtALMT1* (an Al-activated malate transporter, one of several genes critical for Al tolerance in *Arabidopsis*; Hoekenga *et al*. 2006) transcription is not induced by exogenous SA (Kobayashi *et al*. 2013). However, besides malate, other organic acids, such as oxalate and citric acid have also been repeatedly reported to facilitate external Al^{3+} detoxification in different plant species (Ma *et al*. 2001). For instance, exogenous SA alleviated Al toxicity in *Glycine max* is associated with citrate exudation (Liu *et al*. 2012). Therefore, more work will be necessary to evaluate a SA-related role in organic acid exudation-mediated external Al^{3+} detoxification.

One major event in Al^{3+} phytotoxicity is to trigger the production of reactive oxygen species (ROS) and thereby initiating lipid peroxidation. As main ROS, the H_2O_2 content increased in a positive correlation with $AlCl_3$ concentrations and the maximum amount was detected in the *snc1* line, whereas the H_2O_2 content was reduced to that in WT by the expression of *nahG*. The H_2O_2 content was less in *nahG*, *sid2*, and *npr1-1* lines than in WT (Fig. 4*A*). The pattern of malondialdehyde (MDA) content, a product of lipid peroxidation, among the tested lines was similar to that of H_2O_2 (Fig. 4B) suggesting a relationship between Al-induced H_2O_2 production and membrane lipid peroxidation.

The exposure to $AlCl_3$ increased SOD and POD activities in all the lines (Fig. 5*A*,*B*), and the highest activities of these two enzymes were always in *snc1* and lower or the same as in WT were in *nahG*, *sid2*, *npr1-1*,

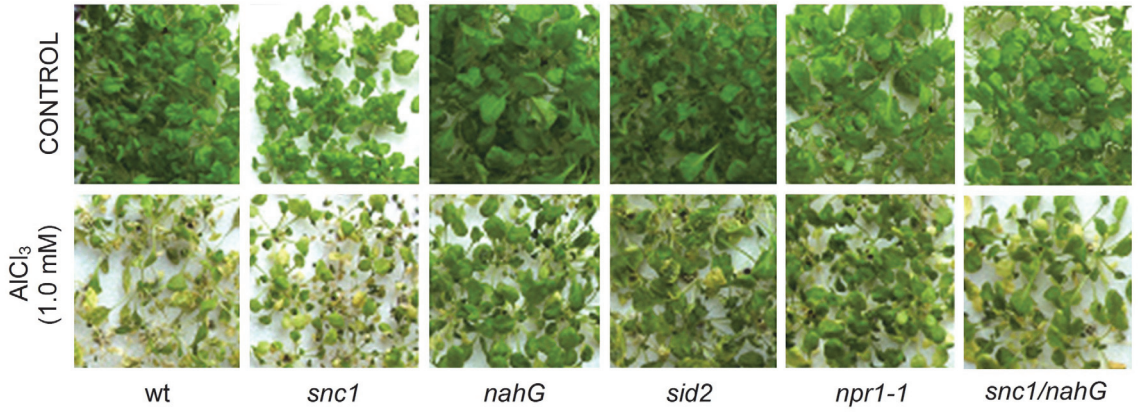

Fig. 3. Representative pictures of tested genotypes of *A. thaliana* exposed to 1 mM $AlCl_3$ for 14 d.

Table 1. The effect of exposure to different $AlCl_3$ concentrations on root malate exudation [nmol $plant^{-1}$] in different lines: WT - wild type; *snc1* – a suppressor of *npr1-1*, constitutive 1; *nahG* - a transgenic line with a salicylate hydroxylase gene; *sid2* - SA induction deficient, with a mutation in the gene encoding isochorismate synthase; *npr1-1* – a nonexpressor of pathogenesis-related gene 1; *snc1/nahG* - *nahG* expression in the *snc1* background. Means ± SD, $n = 9$. Different lower-case letters in each column indicate significant differences at $P < 0.05$.

Genotype	0 mM $AlCl_3$	0.06 mM $AlCl_3$	0.18 mM $AlCl_3$	1.00 mM $AlCl_3$	1.50 mM $AlCl_3$
WT	2.4 ± 0.12b	7.9 ± 0.45b	20.5 ± 1.16c	31.3 ± 2.20b	20.3 ± 1.20a
snc1	3.2 ± 0.26a	7.1 ± 0.42bc	13.2 ± 0.80d	22.9 ± 1.30c	17.4 ± 1.10b
nahG	2.5 ± 0.16b	7.8 ± 0.43b	26.8 ± 1.30b	29.8 ± 1.80b	18.9 ± 1.10ab
sid2	2.7 ± 0.18ab	7.5 ± 0.41b	31.9 ± 1.90a	32.6 ± 2.10b	19.8 ± 1.20a
npr1-1	2.6 ± 0.16b	10.3 ± 0.60a	31.1 ± 2.40a	38.6 ± 1.90a	18.4 ± 1.00ab
snc1/nahG	2.3 ± 0.11b	8.4 ± 0.52b	23.5 ± 1.40c	28.5 ± 1.80b	18.8 ± 1.2ab

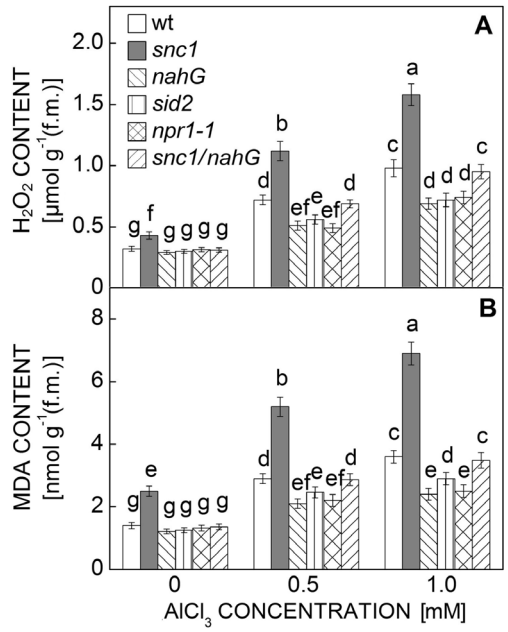

Fig. 4. The effects of exposure to $AlCl_3$ on root H_2O_2 content (*A*) and MDA content (*B*). Means ± SD, $n = 9$. For details see Fig. 1.

and *snc1/nahG* (Fig. 5*A*,*B*). In contrast, the Al^{3+} stress decreased CAT activities in all the tested lines in a dose-dependent manner (Fig. 5*C*), and to a greatest extent in the *snc1* line at each dose of $AlCl_3$. The CAT activity was largely restored by the expression of *nahG*. The CAT activity was less decreased in the *nahG*, *sid2*, and *npr1-1* lines compared with WT (Fig. 5*C*). To investigate the early effect of the Al^{3+} stress on isoforms of SOD, POD, and CAT, we conducted PAGE experiments using the crude enzyme extracts from the plants exposed to 1.0 mM $AlCl_3$ for 24 h. The results confirm that SOD and POD activities increased, whereas CAT activities decreased as indicated by the band intensities (Fig. 6). The isoform profiles of these three enzymes reflect that the antioxidative response started at the early stage of the Al^{3+} stress. A higher SOD activity and a lower CAT activity might contributed to the excessive H_2O_2 accumulation and lipid peroxidation in the *snc1* plants. Although the *snc1* plants also had a much higher POD activity than the other tested genotypes (Fig. 5*B*), this enzyme can only scavenge fine amounts of H_2O_2 in more specific locations (Mittler 2002). Furthermore, PODs

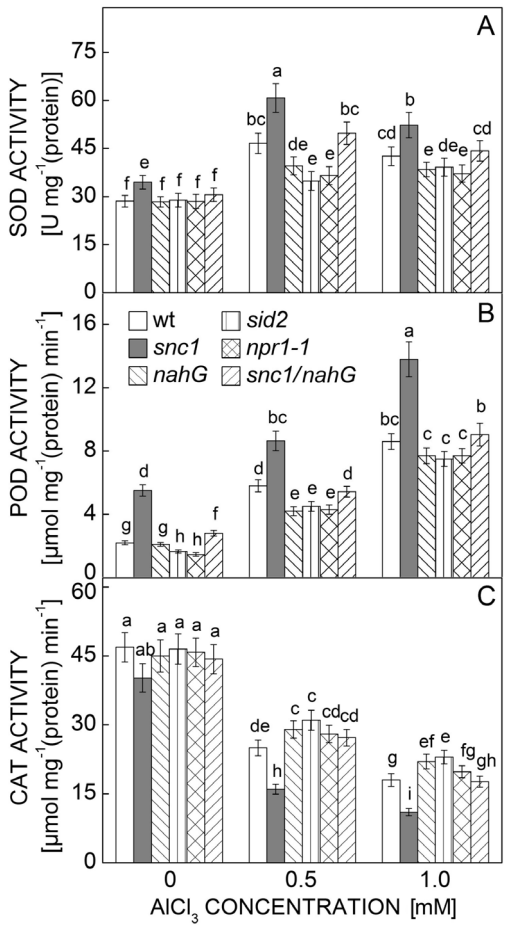

Fig. 5. The effect of exposure to $AlCl_3$ on root activities of antioxidative enzymes: SOD (*A*), POD (*B*), and CAT (*C*) after the 7-d exposure. Means ± SD, $n = 9$. For details see Fig. 1.

NADH-POD oxidase activity producing ROS, like $O_2^{\cdot -}$, $^{\cdot}OH$, and H_2O_2, and thereby triggering oxidative damage (Jouili *et al*. 2011, Kukavica *et al*. 2012). Al-induced increases in POD activity has been observed in other plant species. For instance, the POD activity is much higher in the Al-sensitive barley cultivar than in the Al-resistant cultivar under Al stress accompanying by a greater root growth inhibition than in the Al-resistant cultivar (Tamás *et al*. 2002). A short (6 h) exposure to Al increases the expression of multiple members of PODs (Kumari *et al*. 2008). PODs covalently bound to the cell wall inhibit pea root elongation by cell wall stiffening and lignification or suberization (Kukavica *et al*. 2012). Coincidentally, Al-induced inhibition of root elongation is likely to be caused by the inhibition of cell elongation (Ma 2000). Considering that there remains a large gap to adequately understand how Al causes a rapid inhibition of cell elongation, it would be desirable to investigate the correlation between Al-induced POD expression and Al-inhibited root elongation in future. SOD or POD activities are often used as biomarkers of metallic stress-induced oxidative damage (Jouili *et al*. 2011). We also noticed that the *snc1* line had a higher POD activity than the other tested lines under normal conditions when measured with a spectrophotometer (Fig. 5*B*) and by PAGE (Fig. 6), accompanied by the high content of H_2O_2 (Fig. 4*A*) and lipid peroxidation (Fig. 4*B*). This may explain the growth retardation of the *snc1* plants under normal conditions. The same thing also happens in other

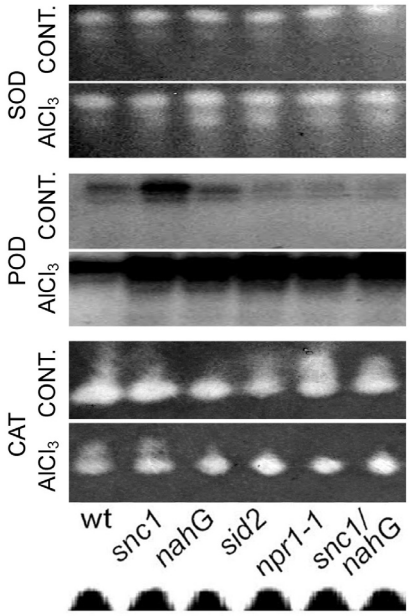

Fig. 6. The isoform profiles of SOD, POD, and CAT in the different lines. Crude enzyme extracts were prepared from plant roots exposed to 1 mM $AlCl_3$ for 24 h, then separated by PAGE (10 μg of protein per lane) and stained for enzyme activities. The loading amount was monitored by Coomassie brilliant blue G-250 staining gel.

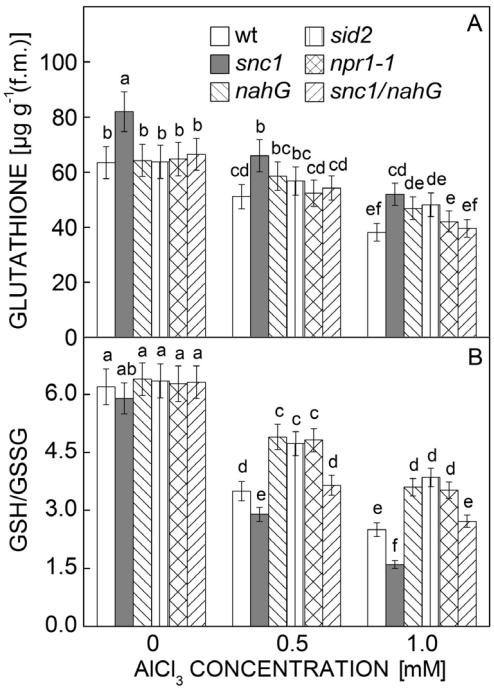

Fig. 7. The effect of exposure to $AlCl_3$ on root glutathione pool. The content of GSH (*A*) and the GSH/GSSG ratio (*B*) after the 7-d exposure. Means ± SD, $n = 9$. For details see Fig. 1.

SA-accumulating *Arabidopsis* mutants, such as *cpr* and *acd* (for a review, see Rivas-San Vicente and Plasencia 2011).

The content of reduced glutathione (GSH) and the ratio of GSH to its oxidized form (GSSG) also revealed differential responses to the Al^{3+} stress among the tested lines. Although both the content of GSH and the GSH/GSSG ratio decreased by the Al^{3+} stress in a dose-dependent manner in all the tested lines, the *nahG*, *sid2*, and *npr1-1* lines had a higher or at least not lower GSH content and GSH/GSSG ratio than the WT plants. The GSH/GSSG ratio was always the lowest in the *snc1* line, but the GSH content was higher than in WT regardless of $AlCl_3$ concentration (Fig. 7*A*,*B*). These two parameters were restored to the WT levels by the expression of *nahG*. This suggests that the glutathione redox state (*i.e*., GSSG/GSH) might play an important role in Al^{3+} detoxification. Increasing evidence has shown that both GSSG/GSH ratio and GSH content play a central role in metal detoxification by chelation, antioxidative defence, and signal transduction regulation (Schaffer and Buettner 2001, Jozefczak *et al*. 2012). A positive correlation between the glutathione pool and Al tolerance has been established (*e.g*., Ma *et al*. 2012).

In conclusion, this study demonstrates that the constitutively high accumulation of SA intensified Al^{3+}-induced damage in *A. thaliana*, whereas reducing the basal SA content or signal blockage had some protective actions in plant response to the Al^{3+} stress. The SA-involved regulatory mechanism was associated with

the control of oxidative stress, but unrelated to the malate exudation. Future research on the correlation between Al-activated POD expression and Al-inhibited root elongation would provide an insight into understanding Al toxicity mechanism.

References

Aebi, H.: Catalase. - In: Bergmeyer, H.U. (ed.): Methods of Enzymatic Analysis. 3rd Ed. Pp. 273-282. Verlag-Chemie, Weinheim 1983.

Beyer, W.F., Fridovich, I.: Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. - Anal. Biochem. **161**: 559-566, 1987.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Cao, H., Bowling, S.A., Gordon, A.S., Dong, X.: Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. - Plant Cell **6**: 1583-1592, 1994.

Delhaize, E., Ryan, P.R., Randall, P.J.: Aluminum tolerance in wheat (*Triticum aextium* L.). II. Aluminum-stimulated excretion of malic acid from root apices. - Plant Physiol. **103**: 695-702, 1993.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., Ryals, J.: Requirement of salicylic acid for the induction of systemic acquired resistance. - Science **261**: 754-756, 1993.

Griffith, O.W., Meister, A.: Potent and specific inhibition of glutathione synthesis by buthionine sulfoximine (s-n-butylhomocysteine sulfoximine). - J. biol. Chem. **254**: 7558-7560, 1979.

Hao, L., Wang, Y., Xu, J., Feng, S.D., Ma, C.Y., Liu, C., Xu, X., Li, G.Z., Herbert, S.J.: Role of endogenous salicylic acid in *Arabidopsis* response to elevated SO_2 exposure. - Biol. Plant. **55**: 297-304, 2011.

Hao, L., Zhao, Y., Jin, D., Zhang, L., Bi, X.H., Chen, H.X., Xu, Q., Ma, C.Y., Li, G.Z.: Salicylic acid-altering *Arabidopsis* mutants response to salt stress. - Plant Soil **354**: 81-95, 2012.

Hemeda, H.M., Klein, B.P.: Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. - J. Food Sci. **55**: 184-185, 1990.

Hoekenga, O.A., Maron, L.G., Piñeros, M.A., Cançado, G.M.A., Shaff, J., Kobayashi, Y., Ryan, P.R., Dong, B., Delhaize, E., Sasaki, T., Matsumoto, H., Yamamoto, Y., Koyama, H., Kochian, L.: *AtALMT1*, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in *Arabidopsis*. - Proc. nat. Acad. Sci. USA **103**: 9738-9743, 2006.

Horváth, E., Pál, M., Szalai, G., Páldi, E., Janda, T.: Exogenous 4-hydroxybenzoic acid and salicylic acid modulate the effect of short-term drought and freezing stress on wheat plants. - Biol. Plant. **51**: 480-487, 2007.

Jouili, H., Bouazizi, H., Ferjani, E.E.: Plant peroxidases: biomarkers of metallic stress. - Acta Physiol. Plant. **33**: 2075-2082, 2011.

Jozefczak, M., Remans, T., Vangronsveld, J., Cuypers, A.: Glutathione is a key player in metal-induced oxidative stress defenses. - Int. J. mol. Sci. **13**: 3145-3175, 2012.

Kang, G.Z., Li, G.Z., Liu, G.Q., Xu, W., Peng, X.Q., Wang, C.Y., Zhu, Y.J., Guo, T.C.: Exogenous salicylic acid enhances wheat drought tolerance by influence on the expression of genes related to ascorbate-glutathione cycle. - Biol. Plant. **57**: 718-724, 2013.

Kinraide, T.B., Parker, D.R., Zobel, R.W.: Organic acid secretion as a mechanism of aluminium resistance: a model incorporating the root cortex, epidermis, and the external unstirred layer. - J. exp. Bot. **56**: 1853-1865, 2005.

Kobayashi, Y., Kobayashi, Y., Sugimoto, M., Lakshmanan, V., Iuchi, S., Kobayashi, M., Bais, H.P., Koyama, H.: Characterization of the complex regulation of *AtALMT1* expression in response to phytohormones and other inducers. - Plant Physiol. **162**: 732-740, 2013.

Kochian, L.V., Hoekenga, O.A., Piñeros, M.A.: How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. - Annu. Rev. Plant Biol. **55**: 459-493, 2004.

Kováčik, J., Stork, F., Klejdus, B., Grúz, J., Hedbavný, J.: Effect of metabolic regulators on aluminium uptake and toxicity in *Matricaria chamomilla* plants. - Plant Physiol. Biochem. **54**: 140-148, 2012.

Kukavica, B.M., Veljović-Jovanović, S.D., Menckhoff, L., Lüthje, S.: Cell wall-bound cationic and anionic class III isoperoxidases of pea root: biochemical characterization and function in root growth. - J. exp. Bot. **63**: 4631-4645, 2012.

Kumari, M., Taylor, G.J., Deyholos, M.K.: Transcriptomic responses to aluminum stress in roots of *Arabidopsis thaliana*. - Mol. Genet. Genomics **279**: 339-357, 2008.

Kunihiro, S., Hiramatsu, T., Kawano, T.: Involvement of salicylic acid signal transduction in aluminum-responsive oxidative burst in *Arabidopsis thaliana* cell suspension culture. - Plant Signal Behav. **6**: 611-616, 2011.

Li, X., Clarke, J.D., Zhang, Y., Dong, X.: Activation of an EDS1-mediated R-gene pathway in the *snc1* mutant leads to constitutive, NPR1-independent pathogen resistance. - Mol. Plant Microbe Interact. **14**: 1131-1139, 2001.

Liu, N., You, J.F., Shi, W.L., Liu, W., Yang, Z.M.: Salicylic acid involved in the process of aluminum induced citrate exudation in *Glycine max* L. - Plant Soil **352**: 85-97, 2012.

Ma, B.H., Gao, L., Zhang, H.X., Cui, J., Shen, Z.G.: Aluminum-induced oxidative stress and changes in antioxidant defenses in the roots of rice varieties differing in Al tolerance. - Plant Cell Rep. **31**: 687-696, 2012.

Ma, J.F.: Role of organic acids in detoxification of aluminumin higher plants. - Plant Cell Physiol. **41**: 383-390, 2000.

Ma, J.F., Ryan, P.R., Delhaize, E.: Aluminium tolerance in plants and the complexing role of organic acids. - Trends Plant Sci. **6**: 273-278, 2001.

Mittler, R.: Oxidative stress, antioxidants and stress tolerance. - Trends Plant Sci. **7**: 405-410, 2002.

Mukherjee, S.P., Choudhuri, M.A.: Implications of water stress induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. - Physiol. Plant. **58**: 166-170, 1983.

Mutlu, S., Atici, Ö., Nalbantoğlu, B.: Effects of salicylic acid

and salinity on apoplastic antioxidant enzymes in two wheat cultivars differing in salt tolerance. - Biol. Plant. **53**: 334-338, 2009.

Mutlu, S., Karadağoğlu, Ö., Atici, Ö., Nalbantoğlu, B.: Protective role of salicylic acid applied before cold stress on antioxidative system and protein patterns in barley apoplast. - Biol. Plant. **57**: 507-513, 2013.

Pandey, P., Srivastava, R.K., Dubey, R.S.: Salicylic acid alleviates aluminum toxicity in rice seedlings better than magnesium and calcium by reducing aluminum uptake, suppressing oxidative damage and increasing antioxidative defense. - Ecotoxicology **22**: 656-670, 2013.

Rivas-San Vicente, M., Plasencia, J.: Salicylic acid beyond defence: its role in plant growth and development. - J. exp. Bot. **62**: 3321-3338, 2011.

Schaffer, F.Q., Buettner, G.R. : Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. - Free Radicals Biol. Med. **30**: 1191-1212, 2001.

Shalata, A., Tal, M.: The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. - Physiol. Plant. **104**: 167-174, 1998.

Tamás, L., Huttová, J., Mistrík, I.: Effect of aluminum on peroxidase activity in roots of Al-sensitive and Al-resistant barley cultivars. - Rost. Výroba **48**: 76-79, 2002.

Tao, S.Y., Sun, L.H., Ma, C.Y., Li, L.L., Li, G.Z., Hao, L.: Reducing basal salicylic acid enhances *Arabidopsis* tolerance to lead or cadmium. - Plant Soil **372**: 309-318, 2013.

Wildermuth, M.C., Dewdney, J., Wu, G., Ausubel, F.M.: Isochorismate synthase is required to synthesize salicylic acid for plant defence. - Nature **414**: 562-565, 2001.

Yang, L.T., Qi, Y.P., Jiang, H.X., Chen, L.S.: Roles of organic acid anion secretion in aluminium tolerance of higher plants. - BioMed Res. Int. 2013, Article ID 173682.

Zawoznik, M.S., Groppa, M.D., Tomaro, M.L., Benavides, M.P.: Endogenous salicylic acid potentiates cadmium-induced oxidative stress in *Arabidopsis thaliana*. - Plant Sci. **173**: 190-197, 2007.