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Role of endogenous salicylic acid in *Arabidopsis* response to elevated sulfur dioxide concentration

L. HAO[1]*, Y. WANG[1], J. XU[1], S.-D. FENG[2], C.-Y. MA[1], C. LIU[1], X. XU[1], G.-Z. LI[1] and S.J. HERBERT[3]

College of Chemistry and Life Sciences, Shenyang Normal University, 253 Huanghe North Street, Shenyang 110034, P.R. China[1]
College of Life Science and Technology, Harbin Normal University, Shida Nan Lu, 150025, Harbin, P.R. China[2]
Department of Plant, Soil, and Insect Sciences, University of Massachusetts, Amherst, MA 01003, USA[3]

Abstract

The response of *Arabidopsis thaliana* plants to elevated sulfur dioxide could be related to their endogenous salicylic acid (SA) content and signaling. The wild type (WT, ecotype Columbia) and its mutant *snc1* with high SA content, *npr1-1* with a blockage in SA signaling, transgenic line *nahG* with low SA content and double mutant *snc1nahG* plants were exposed to 0.5 mm^3 dm^{-3} SO_2 for 3 h d^{-1} for 14 d in a growth chamber. Under unstressed conditions, total SA contents in *snc1* and *npr1-1* were 7- and 2-fold higher than those in WT, respectively, but in *nahG* SA content was only 28 % of that in WT. The expression of *nahG* in *snc1* plants decreased SA content to the WT level. Increased SA contents were observed in *snc1*, *npr1-1* and WT after 12-h SO_2 exposure, whereas no major changes were detected in *nahG* and *snc1nahG* plants.The *snc1* plants exhibited higher tolerance to SO_2 exposure than *snc1nahG* plants and especially *nahG* and *npr1-1* plants according to plant biomass, total chlorophyll content and photosynthetic rate. The SO_2 exposure decreased net photosynthetic rate, maximum photochemical efficiency (F_v/F_m) and actual quantum efficiency of photosystem 2 (Φ_{PS2}). SO_2-induced oxidative damage in the tested plants was confirmed by increased malondialdehyde (MDA) content and electrolyte leakage. Increases in superoxide dismutase (SOD) and peroxidase (POD) activity, reduced glutathione (GSH) content and a ratio of reduced/oxidized glutathione (GSSG) might be responsible for the decreased contents of H_2O_2 and alleviation of oxidative injury in *snc1* plants compared with other lines exposed to SO_2. These observations implied that endogenous SA content and signaling may play an essential role in plant responses to SO_2 stress.

Additional key words: chlorophyll fluorescence, lipid peroxidation, net photosynthetic rate, redox homeostasis, stomatal conductance.

Introduction

Plants are continuously exposed to various biotic and abiotic stresses in their environment. In response to these stresses the expression of different but overlapping suites of genes is regulated, and different signaling pathways share one or more intermediates/components or have some common outputs, referred to as signaling crosstalk, in which plant hormones such as salicylic acid, jasmonic acid and ethylene have been demonstrated to play key roles (Fujita *et al*. 2006). In addition to its well-established role in plant responses to pathogen attack, salicylic acid has been extensively implicated in plant responses to a broad range of abiotic stresses such as low temperature, drought, salinity and osmotic stress (Horvath *et al*. 2007, Luo *et al*. 2009, Mutlu *et al*. 2009, Wang *et al*. 2009).

Sulfur dioxide, one of the first air pollutants recognized as harmful to humans and ecosystems, is rising progressively in the world, especially in developing countries and can reach concentration of 2 mm^3 dm^{-3} in heavily polluted regions (Legge and Krupa 2002). Although several of the mechanisms by which SO_2 could

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Abbreviations: CAT - catalase; c_i - internal CO_2 concentration; F_v/F_m - variable to maximum fluorescence ratio; g_s - stomatal conductance; MDA - malondialdehyde; *npr* - *nonexpressor of pathogenesis-relative gene*; P_N - net photosynthetic rate, POD - peroxidase; ROS - reactive oxygen species; SA - salicylic acid; *snc1* - *suppressor of npr*, *constitutive 1*; SOD - superoxide dismutase; Φ_{PS2} - quantum efficiency of photosystem 2.
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* Corresponding author; fax: (+86) 24 86593309, e-mail: haolinwj2001@yahoo.com.cn

possibly interfere with normal plant functions have been resolved, the essential mechanism of SO_2 toxicity is still unclear. Sulfite (SO_3^{2-}) and bisulfite (HSO_3^-), which have been shown to be toxic to many biochemical and physiological processes, are formed when SO_2 dissolves in cellular fluid (Pfanz and Heber 1986). The inhibition of photosynthesis and reactive oxygen species (ROS) formation are generally considered to be the first effects (Bowler *et al*. 1992, Nouchi 2002). ROS act as a messenger molecules (Dat *et al*. 2000), however, in excess cause membrane and DNA damage, inactivation of enzymes and even cell death (Valko *et al*. 2007). There is growing evidence of a direct link between antioxidants and stress defense (Ball *et al*. 2004).

The *snc1* (*suppressor of npr1-1, constitutive 1*) mutant, isolated in a screen to restore the inducible pathogenesis-relative (*PR*) gene expression in the *npr1-1* (*nonexpressor of PR gene 1*) background, contains high contents of salicylic acid (SA), displays constitutive *PR-1* gene expression and significantly enhanced resistance to pathogens (Li *et al*. 2001). However, to our knowledge, there are not yet reports on *snc1*-mediated abiotic stress responses. The bacterial *nahG* (*naphthalene hydroxylase G*) gene encodes a SA-degrading enzyme, salicylate hydroxylase, and its expression in transgenic plants prevents the accumulation of SA and completely eliminates the onset of systemic aquired resistance (Gaffney *et al*. 1993). The *nahG* transgenic *Arabidopsis* lines have also been used intensely to establish the essential role of SA in plant responses to abiotic stresses (Munné-Bosch *et al*. 2007). In addition, many other SA-altering *Arabidopsis* mutants, isolated and characterized in pathogen defense research, have been used to study the role endogenous SA in plant response to biotic and abiotic stresses. For example, *cpr* (*constitutive expresser of pathogenesis related genes*) with high-accumulating SA, *eds* (*enhance disease susceptibility*) with low foliar SA content, as well as SA signaling deficient mutants were used (Scott *et al*. 2004, Mateo *et al*. 2006). Our previous study indicated that exogenous application of SA increased wheat tolerance to elevated SO_2 stress (Hao *et al*. 2005). However, little is known about the effect of endogenous SA content on plant response to SO_2-caused stress. To address the physiological and biochemical mechanism of the plant response to SO_2, we use the *Arabidopsis* mutants, *snc1*, *npr1-1*, *nahG* and *snc1nahG* double mutants to investigate the role of endogenous SA in plant response to elevated SO_2.

Materials and methods

Arabidopsis thaliana (L.) Heydn. ecotype Columbia (WT) and its mutants *snc1* (Li *et al*. 2001), *npr1-1* (Cao *et al*. 1994), *nahG* transgenic line (Delaney *et al*. 1994) and *snc1nahG* double mutant were grown in pots containing a mixture of peat + *Perlite* + *Vermiculite* (1:1:1, v/v/v) in a growth chamber under 10-h photoperiod (irradiance of 100 μmol m^{-2} s^{-1}), day/night temperature of 22/18 °C and relative humidity 80 %. For SO_2 exposure, three-week-old plants were transferred to a glass chamber (0.8 × 0.8 × 0.8 m) and SO_2 gas was supplied directly from cylinders, into a dilution reservoir into which charcoal filtered air was drawn. Mean concentration of the chamber SO_2 was monitored during treatment by measurement every 30 min using pararosaniline hydrochloride spectrophotometry (Goyal 2001). For the control, charcoal-filtered air was supplied. To reduce the chamber effects, each fumigation experiment comprises of 6 chambers (3 for SO_2 fumigation, 3 for control). Plants were fumigated during the light period for 3 h d^{-1} for 14 d. The mean concentration of SO_2 was 0.5 mm^3 dm^{-3} chosen according to an earlier report (Kubo *et al*. 1995). Except where mentioned in the text, all physiological and biochemical analyses were carried out immediately following the 14 d exposure to SO_2.

For total SA (free and conjugated forms) quantification, pooled rosette leaves from three plants receiving the same treatment (0.2 g fresh mass) were analyzed following the procedure described by Newman *et al*. (2001). Total chlorophyll content was determined following the method described by Zhang and Qu (2003) and expressed as a percentage of the parallel control (SO_2-unstressed plants). For dry mass determination, the excised rosettes were dried for 3 d at 80 °C and then relative dry mass increase was calculated.

Net photosynthesis rate (P_N), intercellular CO_2 concentration (c_i) and stomatal conductance (g_s) were measured 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. During the measurement (10:00 - 12:00) the irradiance was 250 μmol m^{-2} s^{-1} and temperature 25 °C. Chlorophyll fluorescence parameters were measured at 25 °C using a portable fluorometer (*Handy-PEA*, *Hansatech*, Norfolk, UK). Plants were kept for 15 min in darkness to determine minimum fluorescence F_0 under a weak irradiance from a light-emitting diode, following by an 1.0-s saturating pulse (1 200 μmol m^{-2} s^{-1}) to determine maximum fluorescence F_m and then exposed for 15 min to actinic light (30 μmol m^{-2} s^{-1}) to determine steady state fluorescence (F_s') and F_m'. Maximum quantum efficiency of photosystem 2 [$F_v/F_m = (F_m - F_0)/F_m$] and actual quantum efficiency of PS 2 [$\Phi_{PS2} = (F_m' - F_s')/F_m'$] were calculated as described by Baker *et al*. (2001).

Hydrogen peroxide content was determined according to Mukherjee and Choudhuri (1983). The content of reduced glutathione (GSH) was determined as described by Griffith and Meister (1979). Oxidized glutathione (GSSG) contents were calculated from the difference between total GSH in 1,4-dithiothreitol (DTT)-treated samples and GSH in non-DTT-treated samples.

For enzyme activity assays a fresh material (0.5 g) was homogenized to a fine powder in a mortar under liquid

nitrogen. The powder was added to a test-tube containing 5 cm^3 of ice-cold extract solution consisting of 50 mM phosphate buffer saline (PBS, pH 7.8), 0.1 mM EDTA, 1 % (v/v) *Triton X-100*, 4 % (m/v) polyvinylpyrrolidone. After mixing, the solution was incubated for 10 min on ice. The homogenate was centrifuged at 12 000 *g* for 15 min at 4 °C and the supernatant was collected. Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed using the photochemical nitroblue tetrazolium (NBT) method described by Beyer and Fridovich (1987). Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the decomposition of H_2O_2 directly at 240 nm for 3 min, as described by Aebi (1983), where the initial concentration of H_2O_2 was 0.04 % (v/v) in PBS, pH 7.0. Peroxidase (POD; EC 1.11.1.7) activity was estimated according to Hemeda and Klein (1990). For all specific enzyme activity calculations, the protein content of the leaf extract was estimated by the method of Bradford (1976) using bovine serum albumin as the standard.

The lipid peroxidation was determined by measuring the content of malondialdehyde following method of Shalata and Tal (1998). To estimate electrolyte leakage, 20 leaf discs (each 8 mm in diameter) were placed in a glass vial and rinsed three-times with 20 cm^3 deionized water to remove any electrolytes released during leaf disc excision. The vials were then filled with 20 cm^3 deionized water and incubated in the dark for 6 h at room temperature. The electrolytic conductivity (EC_1) of the solution was then measured using a conductivity meter (*SA29-DDB-11A*, *Midwest Group*, Beijing, China). The solution was then heated to 100 °C, cooled to room temperature, and the electrolytic conductivity (EC_2) was measured once again. The percentage electrolyte leakage (EL) of the leaf discs was calculated as $EL = EC_1/EC_2 \times 100$.

Each data point shown in figures was the mean of at least three replicated treatments. Data were subjected to analysis of variance (*ANOVA*) using *SAS* software (SAS Institute, Cary, NC, USA).

Results and discussion

In control (SO_2-unstressed) conditions total SA contents in *snc1* and *npr1-1* were 7- and 2-fold higher than that in WT, respectively, but in *nahG* only 28 % of that in WT. The expression of *nahG* in *snc1* plants (double mutant) decreased SA content to a comparable level to that in WT. Increased SA contents were observed in *snc1*, *npr1-1* and WT after 12-h SO_2 exposure, while no major changes were detected in *nahG* and *snc1nahG* (Fig. 1). SO_2 exposure

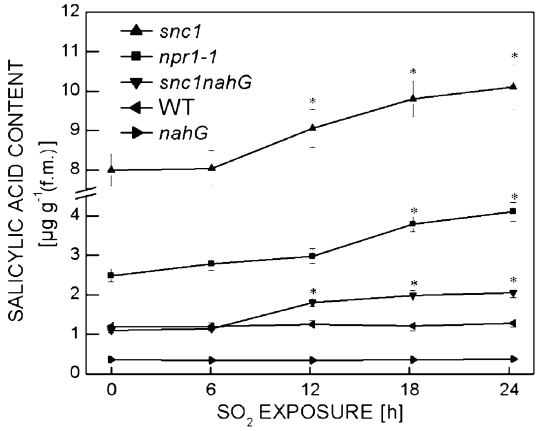

Fig. 1. SO_2-induced accumulation of total SA in *A. thaliana*. Plants were exposed to 0.5 mm^3 dm^{-3} SO_2 for 0, 6, 12, 18 and 24 h. Means ± SD, $n = 9$ (* indicates significant difference at $P < 0.05$).

decreased chlorophyll content in all analyzed lines in a time-dependent manner, however, the decrease magnitude was lower in *snc1* plants, but higher in *nahG* and *npr1-1* plants when compared to WT (Fig. 2). Consistent with the chlorophyll change trends, SO_2 exposure resulted in large areas of necrosis (plant tissue death) on the leaves of *nahG* and *npr1-1* plants, while only small necrotic spots were observed on *snc1* plants (data not shown). Under control conditions, relative increase in dry mass was 58 % in *snc1*, while the value varied between 76 and 82 % in other lines (Fig. 3*A*), indicating that the high SA content in *snc1* inhibited plant growth. However, under SO_2 exposure, the dry mass increase in *snc1* plants decreased only slightly, while a dramatic reduction occurred in other lines (Fig. 3*A*), suggesting that the high accumulation of SA in

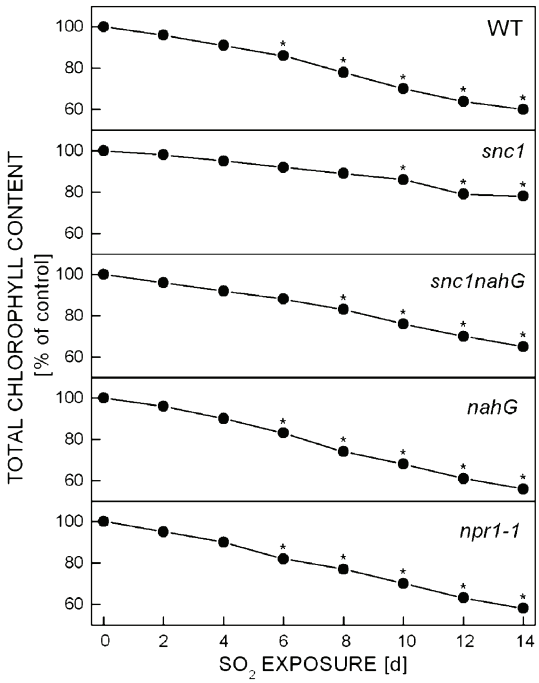

Fig. 2. Effect of SO_2 exposure on total chlorophyll content. Means ± SD, $n = 9$ (* indicates significant difference at $P < 0.05$).

snc1 appeared to prevent SO_2-induced growth inhibition.

Exposure to SO_2 inhibited photosynthetic rate in all tested lines, but more in *nahG*, *npr1-1, snc1nahG* and WT plants than in *snc1* plants (Fig. 3*C*). This was in accordance with the growth responses to SO_2 exposure in these lines (Fig. 3*A*). In addition, a comparison between Fig. 3*A* and 3*C* also suggested that the lowered P_N in *snc1* plants under unstressed conditions may be at least partially responsible for the plant growth retardation. Intercellular CO_2 concentration (c_i) was relevant to the P_N in all the lines (Fig. 3*C vs.* 3*E*).

The stomatal conductance (g_s) of *snc1* plants was much lower than that of WT, while no obvious difference was observed in the other mutants (Fig. 3*D*), suggesting that high content of SA suppressed g_s. This was consistent with the exogenous application of SA causing stomatal closure (Mateo *et al*. 2006). SO_2 exposure significantly reduced g_s of all lines except the *snc1* where it was affected only slightly (Fig. 3*D*). The fact that both SO_2 and CO_2 enter mesophyll *via* stomata, might explain that the SO_2-induced phytotoxicity and photosynthetic rate were comparable in mutants *snc1* and *npr1-1* having almost the same g_s (Fig. 3*D*). However, no strong correlation between the g_s or P_N and with phytotoxicity was observed, indicating that other factors may also play an important role in SO_2 responses. In addition, it remains to be elucidated whether the partial closure of stomata was a direct response to SO_2 insult or a reaction to an increased internal CO_2 concentration due to the impairment of photosynthesis (Fig. 3*E*).

To address whether the SO_2-induced growth retardation and the protective effect of SA were relevant to photosynthetic processes, the chlorophyll fluorescence parameters were measured. The ratio of variable to maximum fluorescence (F_v/F_m), as an indicator of damage to the PS 2 reaction centre, has been widely used in estimation of the maximum quantum yield of PS 2 photochemistry. Under unstressed conditions, F_v/F_m in

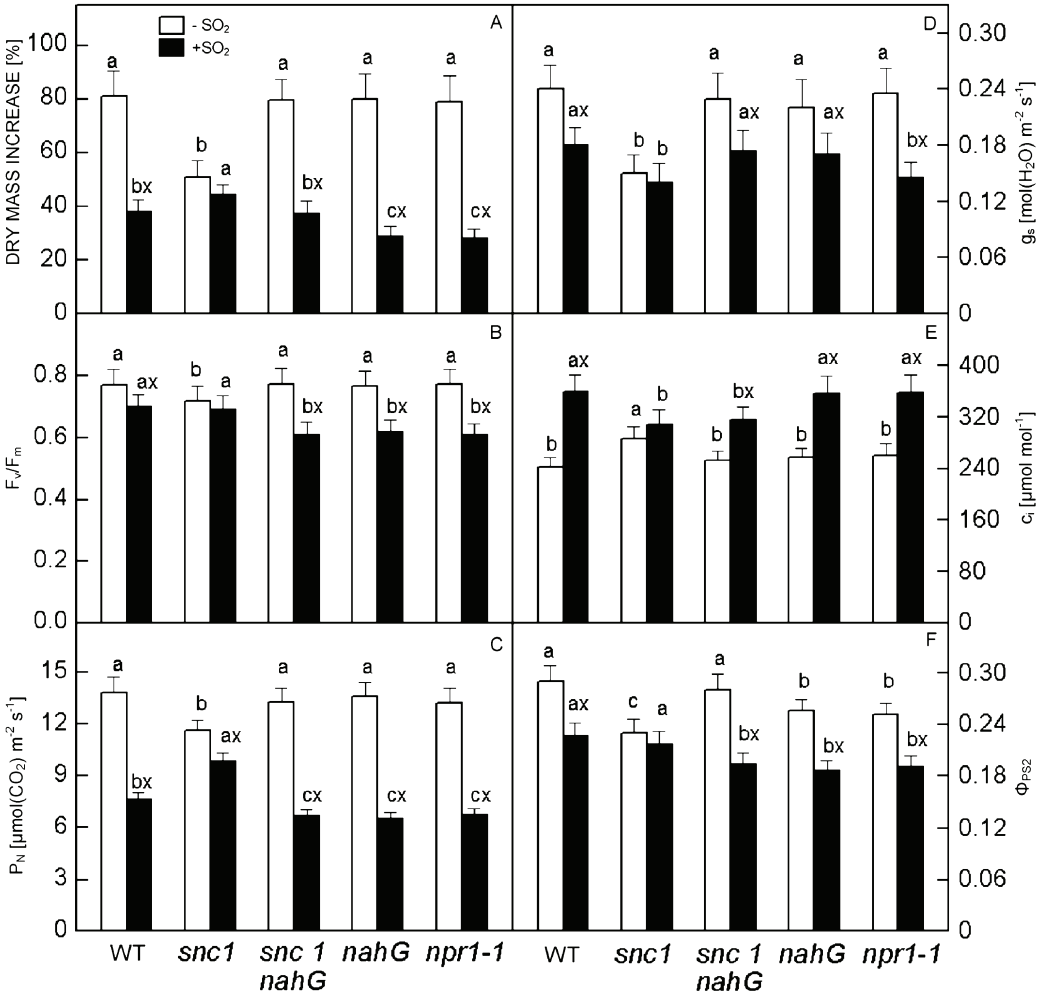

Fig. 3. Effect of SO_2 exposure on plant growth (*A*), maximum photochemical efficiency (F_v/F_m) (*B*), net photosynthetic rate, P_N (*C*), stomata conductance, g_s (*D*), intercellular CO_2 concentration, c_i (*E*), and actual photochemical efficiency, Φ_{PS2} (*F*). See Material and methods for detail. Means ± SD, $n = 9$ (different letters indicate significant difference at $P < 0.05$ between WT and mutants, x indicates significant differences between SO_2-stressed plant and its unstressed control).

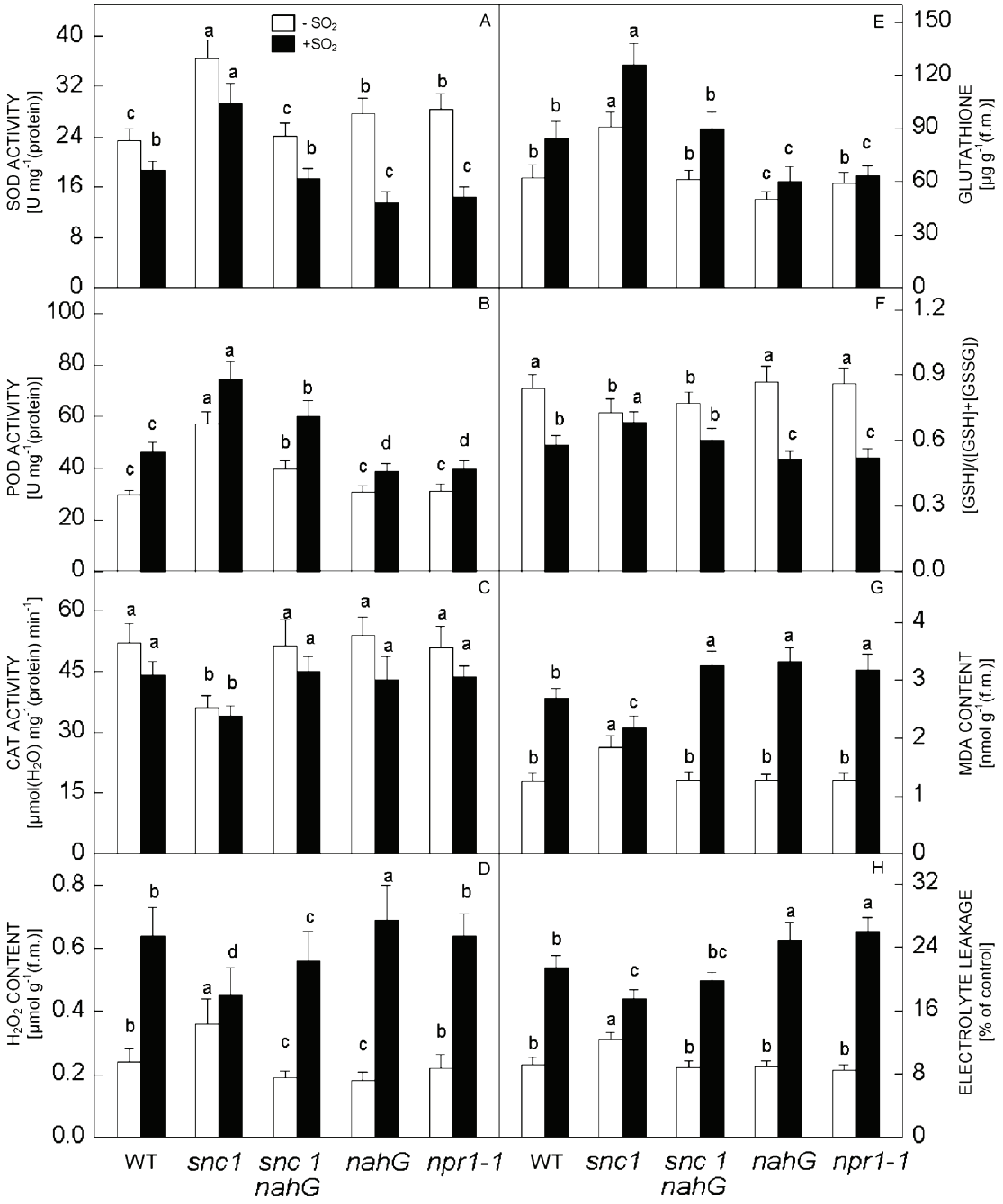

Fig. 4. Effect of SO_2 exposure on antioxidant capacity, hydrogen peroxide content and lipid peroxidation: *A* - superoxide dismutase (SOD) activity, *B* - peroxidase (POD) activity, *C* - catalase (CAT) activity, *D* - H_2O_2 content, *E* - reduced glutathione (GSH) content, *F* - ratio of reduced glutathione (GSH)/oxidized glutathione (GSSG), *G* - malondiadehyde (MDA) content, *H* - electrolyte leakage. Means ± SD, $n = 9$ (different letters indicate significant difference at $P < 0.05$).

snc1 was lower than in other lines (Fig. 3*B*). However, SO_2 exposure obviously reduced F_v/F_m in all lines except *snc1* (Fig. 3*B*). Likewise, SO_2 exposure markedly decreased actual efficiency of PS 2 (Φ_{PS2}) in the tested lines, with the exception of *snc1* where the Φ_{PS2} had no major difference between SO_2-stressed sample and unstressed control (Fig. 3*F*). These data indicated that SO_2 exposure impaired the capability of plants to use photon energy and thus altered photosynthetic processes, and the performance of the photosynthetic machinery in *snc1* plant may contribute at least in part to its SO_2 tolerance.

SO_2 can promote ROS production and cause oxidative stress (Dat *et al*. 2000), therefore, H_2O_2 content and antioxidative capacity were determined in this study. In unstressed conditions, content of H_2O_2 was much higher in *snc1* plants relative to WT, whereas considerably lower in

snc1nahG and *nahG* plants (Fig. 4*D*), indicating a positive correlation between SA and H_2O_2 contents in the cells. SO_2 exposure induced H_2O_2 accumulation in all the lines, but minimum increase was observed in *snc1* plants (Fig. 4*D*) indicating that the high content of SA alleviated the SO_2-induced H_2O_2 accumulation. The *snc1* plants had higher SOD activity than other lines under unstressed conditions (Fig. 4*A*), which was in agreement with higher H_2O_2 content in this line. Although the SO_2 exposure suppressed SOD activity in all lines tested, greater reduction occurred in *nahG* and *npr1-1* than in other lines (Fig. 4*A*). Likewise, the *snc1* had the highest POD activity in both unstressed and stressed conditions (Fig. 4*B*). In contrast to SOD, POD activity was increased by SO_2 exposure in all lines. The CAT activity was the lowest in *snc1* under unstressed conditions (Fig. 4*C*), indicating that the high content of SA repressed the CAT activity. SO_2 exposure inhibited CAT activity in all lines, although the difference was not significant in *snc1* (Fig. 4*C*). These data suggested that POD rather than CAT may be responsible for the H_2O_2 removal under SO_2 exposure (Fig. 4*D*).

The GSH content was much higher in *snc1* plants than in WT and *snc1nahG* under unstressed conditions (Fig. 4*E*), suggesting that SA may promote glutathione biosynthesis, which has been described previously (Mateo *et al.* 2006). The SO_2 exposure increased GSH accumulation in all lines compared to their unstressed controls. Glutathione exists in both a reduced (GSH) and oxidized form (glutathione disulphide; GSSG), and its influence on cellular redox status depends on both GSH/GSSG ratio and GSH content (Schaffer and Buettner 2001). Under unstressed conditions, the GSH/GSSG ratio was decreased in *snc1* and *snc1nahG* compared to other lines. Under SO_2 exposure, however, further decrease of the ratio was not significant in *snc1*, while a dramatic decrease was observed in other lines, especially in *nahG* and *npr1-1* (Fig. 4*F*). Taken together with other indices tested here, it was proposed that the maintenance of a redox potential in cells may be important to plant tolerance to SO_2 stress.

Since SO_2 exposure resulted in excessive H_2O_2 accumulation in the analyzed plant lines (Fig. 4*D*), a measurement of membrane damage was made as indicated by the formation of MDA, a product of lipid peroxidation, and by electrolyte leakage. Under unstressed conditions, the content of MDA in *snc1* plants was much higher than that in WT and other mutants (Fig. 4*G*), indicating that the SA constitutive accumulation caused oxidative injury. As expected, SO_2 exposure promoted MDA production in all the lines. However, maximum accumulation occurred only in lines with SA deficiency or the SA signal block, whereas only a 19 % increase was detected in *snc1* plants. This confirmed that the constitutive accumulation of SA protected strongly the cell membrane against SO_2-caused damage. Completely consistent with the MDA patterns was the electrolyte leakage (Fig. 4*H*).

As *snc1* plants were dwarfed and with curled leaves, we investigated if the SO_2-tolerant phenotype exhibited in this mutant was related to its reduced growth. To address this issue, we cultured the *snc1* plants at higher irradiance of 500 μmol m^{-2} s^{-1}. The hypothesis that the inhibitory effect of high SA on growth may be overcome at higher irradiance came from a previous report where the dwarfed and curled leaf phenotype of *cpr1-1* mutant (with high SA and constitutive expression of *PR1* similar to *snc1*) was reverted to almost normal growth in high irradiance (Mateo *et al*. 2006). Our experiment confirmed the above mentioned results (data not shown).

In addition to elevated SA content and its positive role in plant responses to pathogen infection, growing evidence indicates that increased SA levels (*e.g*. exogenous application or mutation with high-accumulated endogenous SA) promote plant tolerance to various abiotic stresses (Mateo *et al*. 2006, Yuan and Lin 2008). In this study, we used the *Arabidopsis* mutants, *snc1*, *npr1-1*, *nahG*, all isolated or constructed in pathogen defense research, and *snc1nahG* double mutant to reveal that the endogenous SA content may be involved in *Arabidopsis* plant responses to elevated SO_2 exposure.

SA was increased in *snc1*, *npr1-1* and wild type plants after 12 h of SO_2 exposure (Fig. 1), indicating that it is involved in the early response to SO_2 stress, consistent with a previous report where ozone treatment causes a rapid accumulation of SA within the first hours (Sharma *et al*. 1996). However, in *nahG* or *snc1nahG* plants, the content of SA did not change in response to the SO_2 exposure. The comparison between controls and SO_2-stressed plants showed that the SA accumulation has dual effects on plant growth, *i.e*., under unstressed conditions, high content of endogenous SA dramatically suppressed *snc1* plant growth, however, the *snc1* plant exhibited a strong tolerance to SO_2 as indicated by chlorophyll content (Fig. 2), plant growth (Fig. 3*A*), photosynthesis (Fig. 3*C*), as well as other indices tested compared with other analyzed lines. The SA-accumulating *Arabidopsis* mutants always display growth retardation, such as *cpr* mutants (Bowling *et al*. 1997) and *mapk4* (Petersen *et al*. 2000). However, little is known about the mechanisms (Mateo *et al*. 2006). The data in this experiment indicated that the growth retardation of *snc1* plants grown in unstressed conditions was tightly linked to the reduction of net photosynthetic rate (Fig. 3*C*) and chlorophyll fluorescence parameters (Fig. 3*B,F*), the elevation of H_2O_2 (Fig. 4*D*) and oxidative damage indicated by MDA content (Fig. 4*G*) and electrolyte leakage (Fig. 4*H*). Similar results have been also obtained in other SA-accumulating mutants such as *dnd1-1* and *cpr* series (Mateo *et al*. 2006).

The inhibition of photosynthesis is generally considered to be one of the first effects of SO_2 stress (Nouchi 2002). SO_2 exposure resulted in decreased F_v/F_m (Fig. 3*B*) and Φ_{PS2} (Fig. 3*F*) in all lines, however, maximum decrease occurred only in lines with low content of SA (*nahG* and *snc1nahG*) and line with a blockage in SA signal pathway (*npr1-1*), while the least difference was observed in *snc1*. This essentially correlated with the net photosynthetic rate and plant growth in these plants. The double mutant *snc1nahG* plant

was similar to WT plants in many indices tested here in both SO_2-unstressed and stressed conditions.

Increasing evidence indicates that SA-improved plant resistance to biotic and abiotic stresses is associated with the maintenance of redox homeostasis in cells (Mou *et al*. 2003, Mateo *et al*. 2006, Tada *et al*. 2008), which is dependent on a balance between ROS formation and removal (Dat *et al*. 2008). Our previous study showed that the early accumulation of H_2O_2 is favorable to naked oat plant's adaption to NaCl stress (Xu *et al*. 2008). In this experiment, the SA-accumulating mutant *snc1* had higher H_2O_2 content than other analyzed lines (Fig. 4*D*), and this might be as a signal to activate the antioxidant defensive mechanism of cells. SOD and POD activities were much higher in *snc1* than in other lines in both SO_2-unstressed and stressed conditions (Fig. 4*A*,*B*), suggesting that high content of SA could increase SOD and POD activity independent of the altered environmental conditions. Simultaneously, the high SA content decreased CAT activity in *snc1* plants (Fig. 4*C*). It was shown previously that exogenous SA activates antioxidant enzymes in plants in response to different abiotic stresses such as paraquat (Ananieva *et al*. 2004), heavy metals (Popova *et al*. 2009), and salinity (Xu *et al*. 2008, Mutlu *et al*. 2009). The inhibition of CAT activity by SA has also been described (*e.g*. Chen *et al.* 1993).

When compared to WT, the GSH content increased in *snc1*, whereas decreased in *nahG* and *npr1-1* under both SO_2-unstressed and stressed conditions (Fig. 4*E*), further indicating a tight link between SA content and/or signaling and glutathione biosynthesis proposed by Mateo *et al*. (2006). Interestingly, the GSH/GSSG ratio in *snc1* was lower in unstressed conditions, while higher under SO_2 stress in comparison with other lines (Fig. 4*F*). As an important component of the ascorbate-glutathione cycle, GSH participates in the removal of H_2O_2 (Foyer *et al*. 1997).

In summary, this experiment shows that the high accumulation of SA in *snc1* plants on one hand may exert oxidative stress through initiating ROS generation under unstressed conditions, but on the other hand may contribute to a protective function through maintaining high antioxidative capacity under SO_2 exposure.

References

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

Ananieva, E.A., Christov, K.N., Popova, L.P.: Exogenous treatment with salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. - J. Plant Physiol. **161**: 319-328, 2004.

Ball, L., Accotto, G.P., Bechtold, U., Creissen, G., Funck, D., Jimenez, A., Kular, B., Leyland, N., Mejia-Carranza, J., Reynolds, H., Karpinski, S., Mullineauxc, P.M.: Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in *Arabidopsis*. - Plant Cell **16**: 2448-2462, 2004.

Baker, N.R., Oxborough, K., Lawson, T., Morison, J.I.: High resolution imaging of photosynthetic activities of tissues, cells and chloroplasts in leaves. - J. exp. Bot. **52**: 615-621, 2001.

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

Bowler, C., Van Montagu, M., Inzé, D.: Superoxide dismutase and stress tolerance. - Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 83-116, 1992.

Bowling, S.A., Clarke, J.D., Liu, Y., Klessig, D.F., Dong, X.: The *cpr5* mutant of *Arabidopsis* expresses both NPR1-dependent and NPR1-independent resistance. - Plant Cell **9**: 1573-1584, 1997.

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.

Chen, Z., Silva, H., Klessig, D.F.: Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. - Science **262**: 1883-1886, 1993.

Dat, J., Vandenabeele, S., Vranová, E., Van Montagu, M., Inzé, D., Van Breusegem, F.: Dual action of active oxygen species during plant stress responses. - Cell. Mol. Life Sci. **57**: 779-795, 2000.

Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E., Ryals, J.: A central role of salicylic acid in plant disease resistance. - Science **266**: 1247-1250, 1994.

Foyer, C.H., Lopez-Delgado, H., Dat, J.F., Scott, I.M.: Hydrogen peroxide- and glutathione-associated mechanisms of acclamatory stress tolerance and signaling. - Physiol. Plant. **100**: 241-254, 1997.

Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., Shinozak, K.: Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. - Curr. Opin. Plant Biol. **9**: 436-442, 2006.

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.

Goyal, S.K.: Use of rosaniline hydrochloride dye for atmospheric SO_2 determination and method sensitivity analysis. - J. Environ. Monitoring **3**: 666-670, 2001.

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., Zhang, H., Xu, X., Tao, S., Yu, L.: SO_2-caused oxidative stress and modulation of some signal molecules in wheat. - Chin. J. appl. Ecol. **16**: 1038-1042, 2005.

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

Horvath, E., Pal, M., Szalai, G., Paldi, 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.

Kubo, A., Saji, H., Tanaka, K., Kondo, N.: Expression of *Arabidopsis* cytosolic ascorbate peroxidase gene in response to ozone or sulfur dioxide. - Plant mol. Biol. **29**: 479-489, 1995.

Legge, A.H., Krupa, S.V.: Effects of sulphur dioxide. - In: Bell, J.N.B., Treshow, M. (ed.): Air Pollution and Plant Life. Pp. 135-162. Wiley, Chichester 2002.

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.

Luo, M.H., Yuan, S., Chen Y.E., Liu, W.J., Du, J.B., Lei, T., Wang, M.B., Lin H.H.: Effects of salicylic acid on the photosystem 2 of barley seedlings under osmotic stress. - Biol. Plant. **53**: 663-669, 2009.

Mateo, A., Funck, D., Mühlenbock, P., Kular, B., Mullineaux, P.M., Karpinski, S.: Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. - J. exp. Bot. **57**: 1795-1807, 2006.

Mou, Z.L., Fan, W.H., Dong, X.: Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. - Cell **113**: 935-944, 2003.

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.

Munné-Bosch, S., Peñuelas, J., Llusià, J.: A deficiency in salicylic acid alters isoprenoid accumulation in water-stresses *NahG* transgenic *Arabidopsis* plants. - Plant Sci. **172**: 756-762, 2007.

Mutlu, S., Atici, Ö., Nalbantoglu, 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.

Newman, M.A., Van Roepenack-Lahaye, E., Parr, A., Daniels, M.J., Dow, J.M.: Induction of hydroxycinnamoyl-tyramine conjugates in pepper by *Xanthomonas campestris*: a plant defense response activated by *hrp* gene-dependent and -independent mechanisms. - Mol. Plant Microbe Interact. **14**: 785-792, 2001.

Nouchi, I.: Responses of whole plants to air pollutions. - In: Omasa, K. (ed.) Air Pollution and Plant Biotechnology. Pp. 4-8. Springer-Verlag, Berlin - Tokyo 2002.

Petersen, M., Brodersen, P., Naested, H., Andreasson, E., Lindhart, U., Johansen, B., Nielsen, H.B., Lacy, M., Austin, M.J., Parker, J.E., Sharma, S.B., Klessig, D.F., Martienssen, R., Mattsson, O., Jensen, A.B., Mundy, J.: *Arabidopsis* MAP kinase 4 negatively regulates systemic acquired resistance. - Cell **103**: 1111-1120, 2000.

Pfanz, H., Heber, U.: Buffer capacities of leaves, leaf cells, and leaf cell organelles in relation to fluxes of potentially acidic gases. - Plant Physiol. **81**: 597-602, 1986.

Popova, L.P., Maslenkova, L.T., Yordanova, R.Y., Ivanova, A.P., Krantev, A.P., Szalai, G., Janda, T.: Exogenous treatment with salicylic acid attenuates cadmium toxicity in pea seedlings. - Plant Physiol. Biochem. **47**: 224-231, 2009.

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

Scott, I.M., Clarke, S.M., Wood, J.E., Mur, L.A.J.: Salicylate accumulation inhibits growth at chilling temperature in *Arabidopsis*. - Plant Physiol. **35**: 1040-1049, 2004.

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.

Sharma, Y.K., León, J., Raskin, I., Davis, K.R.: Ozone-induced responses in *Arabidopsis thaliana*: the role of salicylic acid in the accumulation of defense-relative transcripts and induced resistance. - Proc. nat. Acad. Sci. USA **93**: 5099-5104, 1996.

Tada, Y., Spoel, S.H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., Zou, J., Dong, X.: Plant immunity requires conformational charges of NPR1 via S-nitrosylation and thioredoxins. - Science **321**: 952-956, 2008.

Valko, M., Leibfritz, D., Moncola, J., Cronin, M.T.D., Mazura, M., Telser, J.: Free radicals and antioxidants in normal physiological functions and human disease. - Int. J. Biochem. Cell. Biol. **39**: 44-84, 2007.

Wang, D.H., Li, X.X., Su, Z.K., Ren, H.X.: The role of salicylic acid in response of two rice cultivars to chilling stress. - Biol. Plant. **53**: 545-552, 2009.

Xu, Q., Xu, X., Zhao, Y., Jiao, K., Herbert, S.J., Hao, L.: Salicylic acid, hydrogen peroxide and calcium-induced saline tolerance associated with endogenous hydrogen peroxide homeostasis in naked oat seedlings. - Plant Growth Regul. **54**: 249-259, 2008.

Yuan, S., Lin, H.H.: Role of salicylic acid in plant abiotic stress. - Z. Naturforsch. **63c**: 313-320, 2008.

Zhang, Z.L., Qu, W.J.: Guide to Plant Physiology Experiment. 3rd Ed. - Higher Education Press, Beijing 2003.