DOI: 10.1007/s11099-018-0806-8 PHOTOSYNTHETICA 56 (4): 1154-1160, 2018

H_2O_2-induced acclimation of photosystem II to excess light is mediated by alternative respiratory pathway and salicylic acid

Q.Z. HOU*, Y.P. WANG**, J.Y. LIANG*, L.Y. JIA*, H.Q. FENG*,+, J. WEN*, N. EHMET*, and J.Y. BAI*

*College of Life Science, Northwest Normal University, Lanzhou, 730070, Gansu Province, China**
*Institute of Modern Physics, Chinese Academy of Sciences, 730000 Lanzhou, China***

Abstract

Acclimation to excess light is required for optimizing plant performance under natural environment. The present work showed that the treatment of *Arabidopsis* leaves with exogenous H_2O_2 can increase the acclimation of PSII to excess light. Treatments with H_2O_2 also enhanced the capacity of the mitochondrial alternative respiratory pathway and salicylic acid (SA) content. Our work also showed that the lack in alternative oxidase (AOX1a) in AtAOX1a antisense line and the SA deficiency in *NahG* (salicylate hydroxylase gene) transgenic mutant attenuated the H_2O_2-induced acclimation of PSII to excess light. It indicates that the H_2O_2-induced acclimation of PSII to excess light could be mediated by the alternative respiratory pathway and SA.

Additional key words: excess light; hydrogen peroxide; photosystem II; salicylic acid.

Introduction

Light energy is absorbed by photosynthetic organs of plants and is used for photosynthetic CO_2 assimilation. In natural environments, however, plants often experience high irradiance stress generated by excess light (EL), which causes the amount of absorbed light energy to exceed that needed for photosynthesis. Excess light energy can lead to the overproduction of electrons, which may damage the reaction center of PSII and cause the perturbation and inhibition of photosynthetic electron transport, consequently, damaging plants and limiting crop production (Karpinski *et al*. 1999, 2000; Li *et al*. 2009).

Karpinski *et al.* (1999), Karpinska *et al.* (2000) found that treatment of leaves with H_2O_2 before EL can efficiently provoke plant acclimatory responses to subsequent EL. And, it is revealed that plant acclimatory responses to EL are controlled (at least in part) by the redox status of the Q_A (quinone A)–Q_B (quinone B)–PQ (plastoquinone) pool, and the H_2O_2-induced the acclimatory responses to EL could be related to the ability of H_2O_2 to regulate the redox status of Q_A–Q_B–PQ pool by increasing the oxidation of Q_A (Karpinski *et al*. 1997, 1999, Karpinska *et al*. 2000, Pfannschmidt *et al*. 1999).

Since EL exerts oxidative stress in both chloroplasts and cytosol, the chloroplastic and cytosolic antioxidant defenses play important roles in the plant acclimatory responses to EL (Karpinska *et al.* 2000; Mullineaux *et al*. 2000; Hernández *et al*. 2004). The data obtained from the current studies also suggest that the H_2O_2-induced EL acclimation could be attributed to the activation of cytosolic and chloroplastic antioxidant enzymes, such as cytosolic ascorbate peroxidase (Karpinski *et al*. 1997, 1999, Karpinska *et al*. 2000). However, the physiological processes or components that are involved in the plant EL acclimation are multiple. In the last decades, there has been increasing number of reports about the function of mitochondria in plant light acclimation, and these reports found that the mitochondrial alternative oxidase (AOX) is a main contributor to such a function of mitochondria (Yoshida *et al*. 2006, 2007; Zhang *et al*. 2010; Florez-Sarasa *et al*. 2011). Mitochondrial AOX is located in the mitochondrial inner membrane and catalyses the alternative respiratory pathway (or cyanide-resistant respiration). In higher plants, AOX branches from the main respiratory chain and makes the electrons flow from

Received 15 March 2017, *accepted* 18 August 2017, *published as online-first* 12 April 2018.
+Corresponding author; tel.: +86 13008762166; e-mail: fenghanq@nwnu.edu.cn
Abbreviations: AOX – alternative oxidase; Chl – chlorophyll; EL – excess light; *HEPES* – 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; LL – low light; *NahG* – salicylate hydroxylase gene; SA – salicylic acid; SHAM – salicylhydroxamic acid; TES – *N*-tris-hydroxymethyl-methyl-2-aminoethanesulphonic acid; V_{alt} – capacity of the mitochondrial alternative respiratory pathway.
Acknowledgements: This work was funded by the National Natural Science Foundation of China (31560070, 31360044, and 31260059), the Fundamental Research Funds for the Gansu Universities of Gansu Provincial Department of Finance, Science Research Funds for the Gansu Universities (2015A-007), Characteristic Discipline Construction Project of Gansu Province, and Innovation Team Fund of Northwest Normal University (Nwnu-lkqn-14-5).

ubiquinone directly to AOX conservation (complexes III and IV). Thus, the presence of AOX makes plants to dissipate the redox energy into heat instead of ATP production (Millenaar and Lambers 2003). Although AOX is not an antioxidant and thus bypasses two of the three sites of energy enzyme, it has been demonstrated that AOX has important benefits for dissipation of excess reduced equivalents in chloroplasts and thus functions in plant acclimatory responses to EL (Yoshida *et al*. 2006, 2007, Zhang *et al*. 2010, Florez-Sarasa *et al*. 2011). More importantly, it has been found that a treatment with exogenous H_2O_2 can enhance the capacity of the alternative respiratory pathway or induce the expression of AOX (Wagner 1995, Feng *et al*. 2008). These characteristics of AOX invoke a hypothesis that the alternative respiratory pathway, acting as a non-antioxidant component in mitochondria, might play a role in the H_2O_2-induced EL acclimation. However, whether such a role of the alternative respiratory pathway actually exists it has not been studied.

Another question worth further studying is the cellular signaling cascade in the H_2O_2-induced EL acclimation. It is known that H_2O_2 can induce the accumulation of many important intracellular signaling molecules, including calcium, salicylic acid (SA), nitric oxide, and ethylene (Quan *et al*. 2008). However, the lack of knowledge about whether these downstream signals of H_2O_2 could be involved in the H_2O_2-induced EL acclimation is remarkable. Mateo *et al*. (2006) reported that SA is required for optimal photosynthesis and regulating redox homeostasis of plant cells. Thus, it is speculated that SA could act as a downstream signal of H_2O_2 and be involved in the H_2O_2-induced acclimation to EL. However, no data about the role of SA in H_2O_2-induced EL acclimation is given so far.

In the present work, by using AtAOX1a antisense line and *NahG* transgenic mutant, we investigated the effects of the alternative respiratory pathway and SA on the H_2O_2-induced acclimation of PSII to EL. This work may contribute to the understanding of the mitochondria-dependent mechanism and the cellular signaling cascade in H_2O_2-induced EL acclimation.

Material and methods

Plant materials and growth conditions: Seeds of *Arabidopsis* ecotype Columbia (Col-0) wild type, AtAOX1a antisense lines (AS-12), and *NahG* transgenic mutant, which encodes the enzyme salicylate hydroxylase that inactivates salicylic acid, were sown on a mixture of loam soil, vermiculite, and perlite (2:1:1, v/v) and were maintained at 4°C for 2 d. Then, the plants were grown in a growth chamber with 10-h day [100 ± 10 μmol(photon) m^{-2} s^{-1} at 22°C] and 14-h night (18°C) cycle and 70% relative air humidity. Eight-week-old plants were used for experimental treatments.

Treatment: Leaves of seedlings were detached and were vacuum infiltrated for 4 min with 0, 0.1, 1, or 10 mM H_2O_2 solutions prepared by dissolving in H_2O. Subsequently these leaves were floated on H_2O_2 solutions or H_2O for additional 2 h as described. All of these treatments were executed under low light [100 ± 10 μmol(photon) m^{-2} s^{-1} at 22°C]. Thereafter leaves were removed from the solutions, wiped, and left in the air. The leaves with the petiole were still placed in a small tube with some water to prevent desiccation and were exposed to excess light [1,500 ± 200 μmol(photon) m^{-2} s^{-1} PAR] for 1 h.

Capacity of mitochondrial alternative respiratory pathway: Mitochondria were isolated and purified as described by Keech *et al.* (2005). The isolated mitochondria were maintained in the dark for 1 h and the oxygen uptake by the isolated mitochondria was measured with a Clark-type oxygen electrode at 22°C in the dark. The reaction medium contained 10 mM TES (pH 7.5), 300 mM sucrose, 5 mM KH_2PO_4, 10 mM KCl, 2 mM $MgSO_4$, and 0.1% (w/v) bovine serum albumin. Malate (10 mM) and 1 mM glutamate were added into the reaction medium as substrates for mitochondrial respiration. To ensure complete activation of AOX, 10 mM dithiotreitol, and 1 mM pyruvate were supplemented. To measure the capacity of the mitochondrial alternative respiratory pathway (V_{alt}), 1 mM KCN was added and V_{alt} was defined as the stable O_2 uptake sensitive to salicylhydroxamic acid (SHAM) in the presence of 1 mM KCN; it was obtained when the rate of O_2 uptake was linear for at least 10 min after addition of KCN (Fig. 1S; *supplement available online*). V_{alt} were expressed as nmol(O_2) mg^{-1}(protein) min^{-1}. The residual respiration (O_2 uptake in the presence of both KCN and 5 mM SHAM) were undetectable. The concentrations of KCN and SHAM were used according to previous reports (Niewiadomska *et al*. 2004).

Chlorophyll (Chl) fluorescence parameters of the detached leaves treated with H_2O_2 or H_2O were measured by using a portable Chl fluorometer (*Walz, Effeltrich*, Germany), as described previously by Demmig-Adams *et al.* (1996). The Chl fluorescence parameters were calculated by the formulas according to Genty *et al.* (1989). In brief, the F_v/F_m, the maximal efficiency of PSII, was defined as $(F_m - F_0)/F_m$, where F_m is the maximum fluorescence emission from the dark-adapted state (30 min of dark adjustment by covering the leaf with a black cloth) measured with a pulse of saturating light [1-s saturating flash of 8,000 μmol(photon) m^{-2} s^{-1}] and F_0 is the minimal fluorescence emission from the dark-adapted state. Φ_{PSII}, the PSII operating efficiency, was defined as $(F_m' - F_s)/F_m'$, where F_s is the steady-state level of fluorescence emission at the given irradiance, and F_m' is the maximum fluorescence emission from the light-adapted state measured with

a pulse of saturating flash. Photochemical quenching (q_P) was defined as $(F_m' - F_s)/(F_m' - F_0')$, where F_0' is minimal fluorescence of the light-adapted state measured with a far red pulse.

Salicylic acid analysis: Leaves were harvested and, after removal of the midribs, weighed and frozen in liquid nitrogen and stored at –80°C. Extraction of SA and quantification by HPLC with fluorescence detection (*Dionex*, *Sunyvale*, USA) were performed according to previously published methods (Surplus *et al*. 1998).

Statistical analysis: The results were expressed as the mean ± standard deviation (SD). The data were statistically evaluated with *t*-test method using *SPSS 16.0*. The difference was considered to be statistically significant when $P<0.05$.

Results

We investigated the effects of H_2O_2 treatment on the acclimation of PSII to EL (Fig. 1). The results showed that, under LL, treatment of Col-0 leaves with 0.1 or 1 mM H_2O_2 did not significantly affect the values of F_v/F_m, Φ_{PSII}, and q_P. However, 10 mM H_2O_2 significantly increased the values of Φ_{PSII} and q_P of Col-0 leaves under LL, although F_v/F_m was not significantly changed by 10 mM H_2O_2.

After the Col-0 leaves treated with H_2O_2 or H_2O under LL were exposed to EL for 1 h, the values of F_v/F_m, Φ_{PSII}, and q_P in these leaves decreased. Under EL, there was no significant difference in the F_v/F_m, Φ_{PSII}, and q_P between the H_2O-treated Col-0 leaves and the Col-0 leaves treated with either 0.1 or 1 mM H_2O_2. However, under EL, the values of F_v/F_m, Φ_{PSII}, and q_P in the 10 mM H_2O_2-treated Col-0 leaves were significantly higher than those in the H_2O-treated Col-0 leaves. And, the effects of 10 mM H_2O_2 on Φ_{PSII} and q_P under EL were more pronounced than those under LL. For example, under LL, Φ_{PSII} and q_P in the 10 mM H_2O_2-treated leaves increased by 14 and 6%, respectively, compared with those in the H_2O-treated leaves. In contrast, under EL, Φ_{PSII} and q_P in the 10 mM H_2O_2-treated leaves increased by 67 and 32%, respectively, compared with those in the H_2O-treated leaves. These observations indicate that 10 mM H_2O_2 can increase the acclimation of PSII to EL.

H_2O_2 enhanced the capacity of mitochondrial alternative respiratory pathway. We focused on the 10 mM H_2O_2-treated leaves to study the effect of H_2O_2 on the V_{alt} (Fig. 2). Under LL, 10 mM H_2O_2 treatment significantly increased the V_{alt} in the Col-0 leaves. After the Col-0 plants were exposed to EL for 1 h, the H_2O_2-treated Col-0 leaves also had significantly higher V_{alt} than that in the Col-0 leaves without the H_2O_2 treatment. The V_{alt} in the AS-12 leaves was significantly lower than those in the Col-0 plants with the same treatments and under the same conditions. H_2O_2 failed to enhance significantly the V_{alt} in AS-12 leaves under either LL or EL.

The H_2O_2-induced the acclimation of PSII to EL is mediated by the alternative respiratory pathway. There was no significant difference in the values of F_v/F_m, Φ_{PSII}, and q_P between Col-0 and AS-12 leaves under LL. Although 10 mM H_2O_2 treatment significantly increased the values of Φ_{PSII} and q_P of Col-0 leaves under LL, this treatment did not significantly affect the value of Φ_{PSII} and q_P of AS-12 leaves under LL (Fig. 3).

EL depressed the values of F_v/F_m, Φ_{PSII}, and q_P in both Col-0 and AS-12 leaves. In contrast, under EL, the values of F_v/F_m, Φ_{PSII}, and q_P in the AS-12 leaves were significantly lower than those in Col-0 leaves, suggesting that lack of AOX can decrease the acclimation of PSII to EL (Fig. 3).

Under EL, we compared the difference in the effect of H_2O_2 on the PSII photochemistry between the Col-leaves and AS-12 leaves. Under EL, the values of F_v/F_m, Φ_{PSII}, and q_P of Col-0 leaves were significantly increased by 22.2, 66.7, and 31.2%, respectively, by 10 mM H_2O_2 pretreatment. In contrast, however, there was no significant difference in the value of F_v/F_m between the H_2O_2- and H_2O-treated AS-12 leaves under EL. And, under EL, the effects of H_2O_2 on F_v/F_m, Φ_{PSII}, and q_P in the AS-12 leaves were more alleviative than those in the Col-leaves. Under EL, the values of Φ_{PSII} and q_P of AS-12 leaves significantly increased by 41.7 and 21.9%, respectively, by 10 mM H_2O_2 pretreatment. These observations indicate that the lack in AOX can attenuate the H_2O_2-induced acclimation of PSII to EL.

The effects of H_2O_2 on the SA accumulation were studied. Under LL, 10 mM H_2O_2 treatment significantly increased the SA content in the Col-0 leaves. When the Col-0 plants were exposed to EL, the 10 mM H_2O_2-treated Col-0 leaves also showed a higher SA content than that of the Col-0 leaves without H_2O_2 treatment (Fig. 4).

The SA contents in the *NahG* leaves were significantly lower than those in the Col-0 plants with the same treatments and under the same conditions. H_2O_2 failed to significantly enhance the SA content in the leaves under LL and EL (Fig. 4).

The H_2O_2-induced acclimation of PSII to EL was mediated by SA. There was no significant difference in the values of F_v/F_m, Φ_{PSII}, and q_P between Col-0 and *NahG* leaves under LL. Under LL, H_2O_2 treatment did not significantly alter the value of these Chl fluorescence parameters in *NahG* leaves (Fig. 5).

When EL depressed the values of F_v/F_m, Φ_{PSII}, and q_P in either Col-0 or *NahG* leaves, the values of F_v/F_m, Φ_{PSII}, and q_P in Col-0 leaves were significantly higher than those in the *NahG* leaves, indicating that SA deficiency can decrease the acclimation of PSII to EL (Fig. 5).

We also compared the difference in the effect of H_2O_2 on the PSII photochemistry between the Col-leaves and *NahG* leaves. Under EL, the values of F_v/F_m, Φ_{PSII}, and q_P of Col-0 leaves significantly increased by 10 mM H_2O_2

pretreatment. In comparison, under EL, H_2O_2 pretreatment did not significantly change the values of F_v/F_m, Φ_{PSII}, and q_P of *NahG* leaves. The results showed that SA-deficiency in the *NahG* leaves impaired the capability of H_2O_2 to enhance the acclimation of PSII to EL.

Discussion

The present work showed that the Col-0 leaves pretreated with 10 mM (but not with 0.1 or 1 mM) H_2O_2 had significantly higher values of q_P and Φ_{PSII} before EL and had higher values of F_v/F_m, Φ_{PSII}, and q_P under EL, compared with the Col-0 leaves pretreated with H_2O (Fig. 1), suggesting that 10 mM H_2O_2 can initiate a change in PSII operation before EL and increase the acclimation of PSII to EL.

Previous work showed that the H_2O_2-induced EL acclimation is attributed to the ability of H_2O_2 to increase the oxidation of Q_A and the electron transport efficiency in PSII (Karpinska *et al*. 2000), both of which were reflected by the observed increases in q_P and Φ_{PSII} by H_2O_2 (Karpinska *et al*. 2000).Thus, based on these observations, we suggested that lower dosages of H_2O_2 were not effective enough to increase the oxidation of Q_A and PSII electron transport efficiency and thus failed to increase the acclimation of PSII to EL.

The H_2O_2-induced changes in the Chl fluorescence parameters under either LL or EL were accompanied by the increase of the V_{alt} (Fig. 2). Many works showed that the alternative respiration pathway can protect the photosynthetic electron transport chain from the harmful effects of high light (Yoshida *et al*. 2006, 2007; Noctor *et al*. 2007). The AOX1a is the predominant isoform in *Arabidopsis* cells (Zhang *et al*. 2010). The present work showed that the V_{alt} were greatly inhibited in AtAOX1a antisense lines (AS-12) (Fig. 2). The values of F_v/F_m, Φ_{PSII}, and q_P in Col-0 leaves were significantly higher than those in the AS-12 leaves under EL, but not under LL (Fig. 3). This indicate that AOX lack does not lead to a substantial disadvantage for the PSII photochemistry under non-stressed LL, but can decrease the acclimation of PSII to EL. We further investigated whether the H_2O_2-induced the acclimation of PSII to EL could also be associated with the alternative respiration pathway. The present observation showed that the increases of Φ_{PSII} and q_P values by 10 mM H_2O_2 under LL were abolished by lack of AOX in AS-12 leaves (Fig. 3), indicating that the ability of H_2O_2 to mediate the PSII photochemistry before EL is dependent on the AOX. Yoshida *et al*. (2006) showed that, even under LL, AOX inhibition by AOX inhibitors, such as SHAM and *n*-propyl gallate, can decrease the parameters of Chl fluorescence. Based on this observation, Yoshida *et al.* (2006) concluded that AOX plays important roles in PSII photochemistry even at LL. However, by using transgenic *Arabidopsis* with varying levels of AOX gene expression, AOX showed no effect on PSII photochemistry at LL (Zhang *et al*. 2010, Florez-Sarasa *et al*. 2011), a finding

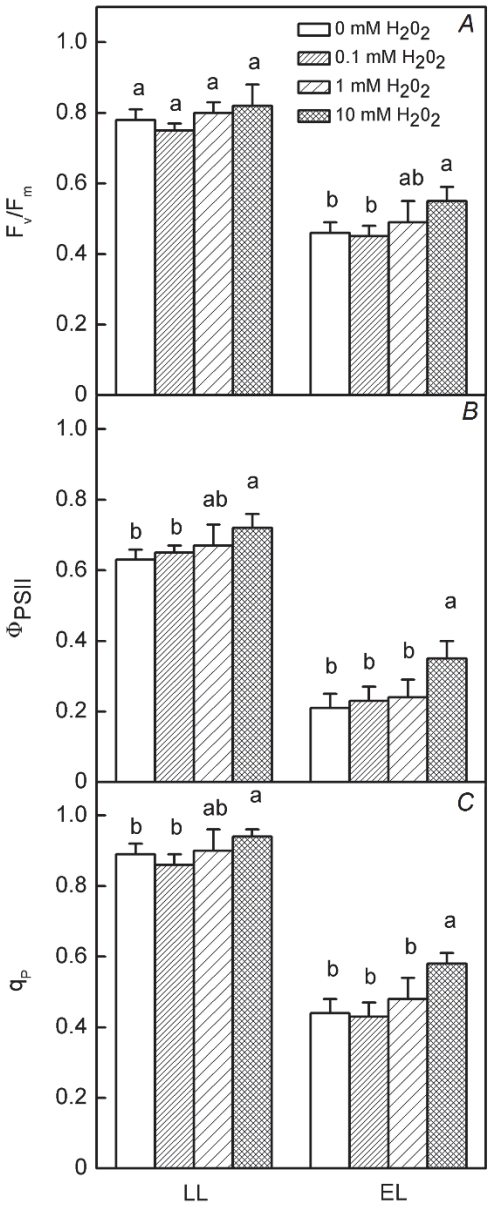

Fig. 1. The effects of 0.1, 1, or 10 mM H_2O_2 on the maximal efficiency of PSII (*A*), the PSII operating efficiency (*B*), and photochemical quenching (*C*) under low light and excess light conditions. The values represent means of at least four individual experiments. The means denoted by *the same letter* did not significantly differ at $P<0.05$ under the same light condition. H_2O_2 – hydrogen peroxide; F_v/F_m – the maximal efficiency of PSII; Φ_{PSII} – the PSII operating efficiency; q_P – photochemical quenching; LL – low light; EL – excess light.

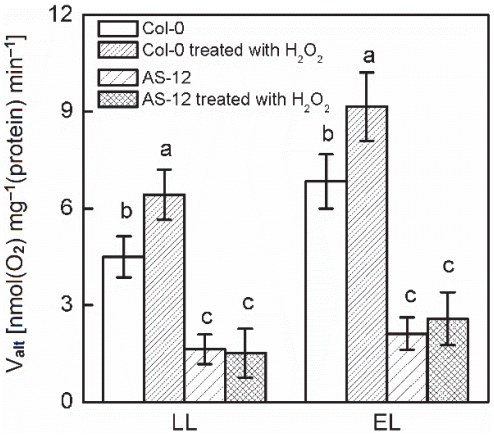

Fig. 2. The effect of 10 mM H_2O_2 on the capacity of mitochondrial alternative respiratory pathway (V_{alt}) under low light and excess light. The values of the V_{alt} represent means of at least four individual experiments. The means denoted by *the same letter* did not significantly differ at $P<0.05$ under the same light condition. H_2O_2 – hydrogen peroxide; V_{alt} – the capacity of mitochondrial alternative respiratory pathway; LL – low light; EL – excess light.

consistent with our present observation. The discrepancy between these results may originate from some side effects of these AOX inhibitors. For example, SHAM has been reported to inhibit the activity of peroxidase (Amor *et al.* 2000), while *n*-propyl gallate has been reported to inhibit the activity of plastid terminal oxidase (Yu *et al*. 2014). Peroxidase (Fryer *et al*. 2003) and plastid terminal oxidase (Joët *et al*. 2002) have been found to participate in the regulation of PSII photochemistry. Thus, it is possible that the application of AOX inhibitors under LL could affect PSII photochemistry by affecting peroxidase or plastid terminal oxidase, although the actual mechanism could be different or more complex than expected.

Under EL, 10 mM H_2O_2 pretreatment can effectively increase the values of F_v/F_m, Φ_{PSII}, and q_P of Col-0 leaves, whereas this H_2O_2 treatment failed to evoke an increase in F_v/F_m of AS-12 leaves. Furthermore, although the values of Φ_{PSII} and q_P of AS-12 leaves under EL significantly increased by 10 mM H_2O_2 pretreatment, the increase in the values of Φ_{PSII} and q_P by H_2O_2 was lower in AS-12 leaves, compared with the Col-0 leaves. These observations indicate that the ability of H_2O_2 to enhance the acclimation of PSII to EL can be dampened by the AOX lack. Thus, it is suggested that the H_2O_2-induced acclimation of PSII to EL could be associated with the alternative respiration pathway.

Leon *et al.* (1995) reported that infiltration of tobacco leaves with H_2O_2 can increase the SA biosynthesis, indicating that SA could be involved in the signal transduction downstream of H_2O_2. The present work observed that the 10 mM H_2O_2-treated Col-0 leaves had the higher SA content than that in the Col-0 leaves without H_2O_2 treatment under both LL or EL (Fig. 4), suggesting that the H_2O_2 can increase the SA content in the

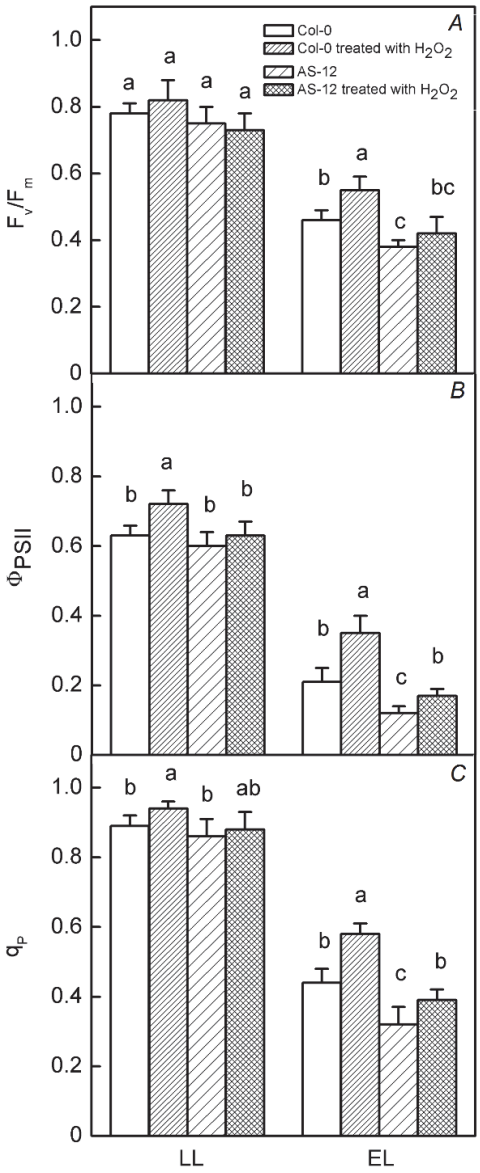

Fig. 3. The maximal efficiency of PSII (*A*), the PSII operating efficiency (*B*), and photochemical quenching (*C*) in H_2O_2- and H_2O-treated Col-0 and AS-12 leaves under low light and excess light conditions. The values represent means of at least four individual experiments. The means denoted by *the same letter* did not significantly differ at $P<0.05$ under the same light conditions. F_v/F_m – the maximal efficiency of PSII; Φ_{PSII} – the PSII operating efficiency; q_P – photochemical quenching; LL – low light; EL – excess light; H_2O_2 – hydrogen peroxide.; Col-0 – wild-type Columbia; AS-12 – AtAOX1a antisense lines.

Arabidopsis leaves. It is well known that SA is an important signaling molecule to enhance the plant resistance to pathogen infection and some abiotic stresses, such as drought, chilling, heavy metal toxicity, and heat (Gaffney *et al*. 1993, Shah 2003, Yuan and Lin 2008). Mateo *et al*. (2006) demonstrated that the acclimation to transient exposure to high light was impaired by the reduced SA content, suggesting that SA also is involved in

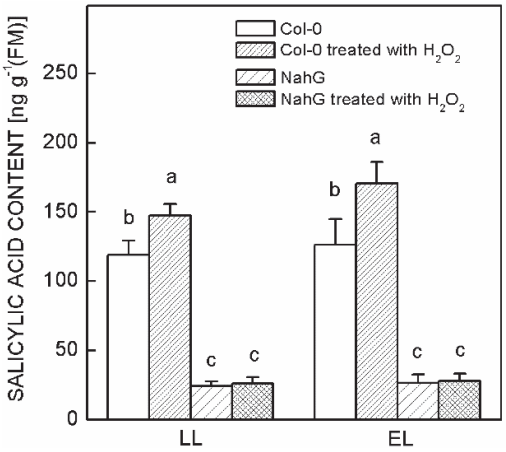

Fig. 4. The effects of H_2O_2 on salicylic acid contents under low light and excess light conditions. The values represent means of at least four individual experiments. The means denoted by *the same letter* did not significantly differ at $P<0.05$ at the same light condition. LL – low light; EL – excess light.

EL acclimation of plant. However, whether the H_2O_2-induced EL acclimation is associated with SA is still unknown.

Under LL, SA deficiency in *NahG* transgenic mutant did not significantly affect the Chl fluorescence parameters. Under EL, however, the values of these Chl fluorescence parameters in the *NahG* leaves were significantly lower than those in Col-0 leaves. This indicates that SA is not required for maintenance of the PSII photochemistry under nonstressing LL, but its deficiency can decrease the acclimation of PSII to EL (Fig. 5). SA deficiency in *NahG* leaves not only abolished the H_2O_2-induced increase of Φ_{PSII} and q_P before EL, but also dampened the ability of H_2O_2 to enhance the acclimation of PSII to EL (Fig. 5), indicating that the effects of H_2O_2 on PSII photochemistry under LL and EL could be dependent on SA. Thus, it is suggested that SA could act as a downstream signal of H_2O_2 and be involved in the H_2O_2-induced acclimation to EL.

Previous work by Karpinska *et al.* (2000) showed that the concentrations of exogenous H_2O_2 to induce the acclimation of the plant to EL reached, even exceeded, the dose that has been suggested to induce cell death. Interestingly, Straus *et al.* (2010) found that in response to photooxidative stress the accumulation of SA can lead to runaway cell death by shifting the balance between superoxide anion (and possibly other oxygen radicals) towards H_2O_2. Thus, it is possible that the increase in SA content, which was induced by H_2O_2, could function as inhibition of cell death during H_2O_2-induced EL acclimation. On the other hand, some works showed that SA has potential to increase H_2O_2 production and, at the same time, enhance the activity of antioxidant enzymes (Mateo *et al*. 2006, Cao *et al*. 2012). Such characteristics of SA seems to fit well with the demand of the H_2O_2-induced EL acclimation, since both the sufficient intensity of H_2O_2 signals and activation of antioxidant defense are required for H_2O_2-induced EL acclimation.

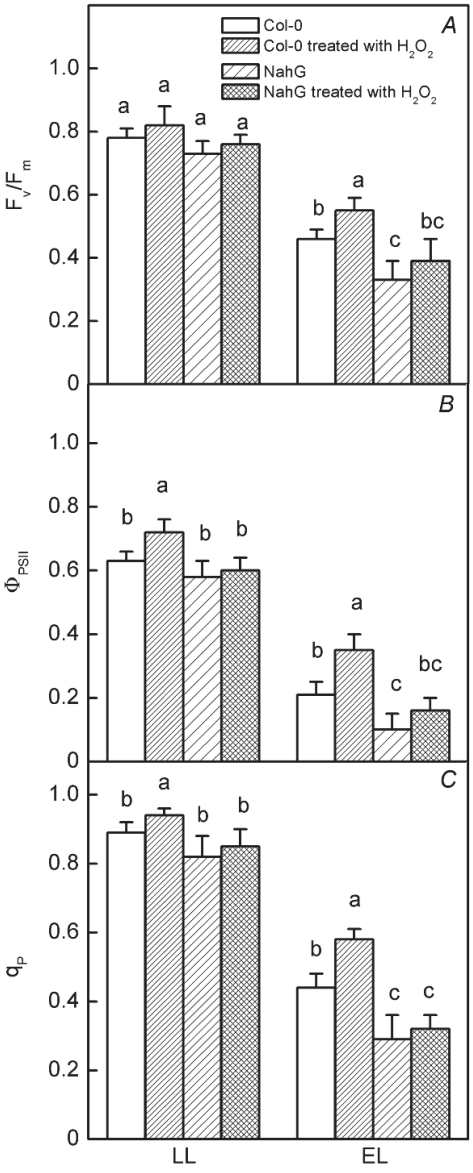

Fig. 5. The maximal efficiency of PSII (*A*), the PSII operating efficiency (*B*), and photochemical quenching (*C*) in H_2O_2- and H_2O-treated Col-0 and *NahG* leaves under low light and excess light conditions. The values represent means of at least four individual experiments. The means denoted by the same letter did not significantly differ at $P<0.05$ under the same light condition. F_v/F_m – the maximal efficiency of PSII; Φ_{PSII} – the PSII operating efficiency; q_P – photochemical quenching; LL – low light; EL – excess light.

Furthermore, previous work reported that exogenous SA can enhance the capacity of the alternative respiration pathway (Van Der Straeten *et al*. 1995). Simons *et al*. (1999) reported that the induction of AOX expression during the plant–pathogen combination was dependent on SA. These works indicate that SA, like H_2O_2, has potential to activate the alternative respiration pathway. Thus, it is possible that that a close link exists among H_2O_2, SA, and the alternative respiration pathway in the H_2O_2-induced acclimation of PSII to EL.

References

Amor Y., Chevion M., Levine A.: Anoxia pretreatment protects soybean cells against H_2O_2-induced cell death: possible involvement of peroxidases and of alternative oxidase. – FEBS Lett. **477**: 175-180, 2000.

Cao Y., Yuan R., Chai Y. *et al*.: Ultrasensitive luminol electrochemiluminescence for protein detection based on *in situ* generated hydrogen peroxide as coreactant with glucose oxidase anchored AuNPs@MWCNTs labeling. – Biosens. Bioelectron. **31**: 305-309, 2012.

Demmig-Adams B., Adams W.W. III., Barker D.H. *et al.*: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. – Physiol. Plantarum **98**: 253-264, 1996.

Feng H.Q., Li X., Duan J.G. *et al*.: Chilling tolerance of wheat seedlings is related to an enhanced alternative respiratory pathway. – Crop Sci. **48**: 2381-2388, 2008.

Florez-Sarasa I., Flexas J., Rasmusson A G. *et al*.: *In vivo* cytochrome and alternative pathway respiration in leaves of *Arabidopsis thaliana* plants with altered alternative oxidase under different light conditions. – Plant Cell Environ. **34**: 1373-1383, 2011.

Fryer M.J., Ball L., Oxborough K. *et al*.: Control of Ascorbate Peroxidase 2 expression by hydrogen peroxide and leaf water status during excess light stress reveals a functional organisation of *Arabidopsis* leaves. – Plant J. **33**: 691-705, 2003.

Gaffney T., Friedrich L., Vernooij B. *et al*.: Requirement of salicylic acid for the induction of systemic acquired resistance. – Science **261**: 754-756, 1993.

Genty B., Briantais J.M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Hernández J.A., Escobar C., Creissen G. *et al*.: Role of hydrogen peroxide and the redox state of ascorbate in the induction of antioxidant enzymes in pea leaves under excess light stress. – Funct. Plant Biology **31**: 359-368, 2004.

Karpinska B., Wingsle G., Karpinski S.: Antagonistic effects of hydrogen peroxide and glutathione on acclimation to excess excitation energy in *Arabidopsis*. – IUBMB Life **50**: 21-26, 2000.

Karpinski S., Reynolds H., Karpinska B. *et al*.: Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. – Science **284**: 654-657, 1999.

Karpinski S., Escobar C., Karpinska B. *et al*. Photosynthetic electron transport regulates the expression of cytosolic ascorbate peroxidase genes in *Arabidopsis* during excess light stress. – Plant Cell **9**: 627-640, 1997.

Keech O., Dizengremel P., Gardeström P.: Preparation of leaf mitochondria from *Arabidopsis thaliana.* – Physiol. Plantarum **124**: 403-409, 2005.

Leon J., Lawton M.A., Raskin I.: Hydrogen peroxide stimulates salicylic acid biosynthesis in tobacco. – Plant Physiol. **108**: 1673-1678, 1995.

Li Z., Wakao S., Fischer B.B. *et al*.: Sensing and responding to excess light. – Annu. Rev. Plant Biol. **60**: 239-260, 2009.

Mateo A., Funck D., Mühlenbock P. *et al*.: Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. – J. Exp. Bot. **57**: 1795-1807, 2006.

Millenaar F.F., Lambers H.: The alternative oxidase: *in vivo* regulation and function. – Plant Biol. **5**: 2-15, 2003.

Mullineaux P., Ball L., Escobar C. *et al*.: Are diverse signalling pathways integrated in the regulation of *Arabidopsis* antioxidant defence gene expression in response to excess excitation energy? – Philos. T. R. Soc. B **355**: 1531-1540, 2000.

Niewiadomska E., Karpinska B., Romanowska E. *et al*.: A salinity-induced C3-CAM transition increases energy conservation in the halophyte *Mesembryanthemum crystallinum* L. – Plant Cell Physiol. **45**: 789-794, 2004.

Noctor G., De Paepe R., Foyer C.H.: Mitochondrial redox biology and homeostasis in plants. – Trends Plant Sci. **12**: 125-134, 2007.

Pfannschmidt T., Nilsson A., Allen J F.: Photosynthetic control of chloroplast gene expression. – Nature **397**: 625-628, 1999.

Quan L.J., Zhang B., Shi W.W. *et al*.: Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. – J. Integr. Plant Biol. **50**: 2-18, 2008.

Joët T., Genty B., Josse E.M. *et al*.: Involvement of a plastid terminal oxidase in plastoquinone oxidation as evidenced by expression of the *Arabidopsis thaliana* enzyme in tobacco. – J. Biol. Chem. **277**: 31623-31630, 2002.

Shah J.: The salicylic acid loop in plant defense. – Curr. Opin. Plant Biol. **6**: 365-371, 2003.

Surplus S.L., Jordan B.R., Murphy A.M. *et al*.: Ultraviolet-B-induced responses in *Arabidopsis thaliana*: role of salicylic acid and reactive oxygen species in the regulation of transcripts encoding photosynthetic and acidic pathogenesis-related proteins. – Plant Cell Environ. **7**: 685-694, 1998.

Simons B.H., Millenaar F.F., Mulder L. *et al*.: Enhanced expression and activation of the alternative oxidase during infection of *Arabidopsis* with *Pseudomonas syringae* pv tomato. – Plant Physiol. **120**: 529-538, 1999.

Straus M.R., Rietz S., Ver Loren van Themaat E. *et al*.: Salicylic acid antagonism of EDS1-driven cell death is important for immune and oxidative stress responses in *Arabidopsis*. – Plant J. **62**: 628-640, 2010.

Van Der Straeten D., Chaerle L., Sharkov G. *et al*.: Salicylic acid enhances the activity of the alternative pathway of respiration in tobacco leaves and induces thermogenicity. – Planta **196**: 412-419, 1995.

Wagner A.M., Krab K.: The alternative respiration pathway in plants: Role and regulation. – Physiol. Plantarum **95**: 318-325, 1995.

Yoshida K., Terashima I., Noguchi K.: Distinct roles of the cytochrome pathway and alternative oxidase in leaf photosynthesis. – Plant Cell Physiol. **47**: 22-31, 2006.

Yoshida K., Terashima I., Noguchi K.: Up-regulation of mitochondrial alternative oxidase concomitant with chloroplast over-reduction by excess light. – Plant Cell Physiol. **48**: 606-614, 2007.

Yu Q., Feilke K., Krieger-Liszkay A. *et al*.: Functional and molecular characterization of plastid terminal oxidase from rice (*Oryza sativa*). – BBA-Bioenergetics **1837**: 1284-1292, 2014.

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

Zhang D.W., Xu F., Zhang Z.W. *et al*.: Effects of light on cyanide resistant respiration and alternative oxidase function in *Arabidopsis* seedlings. – Plant Cell Environ. **33**: 2121-2131, 2010.