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RESEARCH ARTICLE

Enhancing tolerance of rice (*Oryza sativa*) to simulated acid rain by exogenous abscisic acid

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Abstract Abscisic acid (ABA) regulates much important plant physiological and biochemical processes and induces tolerance to different stresses. Here, we studied the regulation of exogenous ABA on adaptation of rice seedlings to simulated acid rain (SAR) stress by measuring biomass dry weight, stomatal conductance, net photosynthesis rate, nutrient elements, and endogenous hormones. The application of 10 μM ABA alleviated the SAR-induced inhibition on growth, stomatal conductance, net photosynthesis rate, and decreases in contents of nutrient (K, Mg, N, and P) and hormone (auxin, gibberellins, and zeatin). Moreover, 10 μM ABA could stimulate the Ca content as signaling molecules under SAR stress. Contrarily, the application of 100 μM ABA aggravated the SAR-induced inhibition on growth, stomatal conductance, net photosynthesis rate, and contents of nutrient and hormone. The results got after a 5-day recovery (without SAR) show that exogenous 10 μM ABA can promote self-restoration process in rice whereas 100 μM ABA hindered the restoration by increasing deficiency of nutrients and disturbing the balance of hormones. These results confirmed that exogenous ABA at proper concentration could enhance the tolerance of rice to SAR stress.

Keywords Simulated acid rain · ABA application · Hormone · Mineral nutrient · Rice seedlings

Introduction

Abscisic acid (ABA) is one of the hormones in plants, playing roles in stress perception and response pathways as a messenger (Cao et al. 2014, Wang et al. 2013a). ABA is involved in many plant physiological processes such as seed maturation, seed dormancy, growth, and developmental regulation, and its role in adaptive responses to abiotic stresses is well established as plant stress hormone (Apel and Hirt 2004, Wasilewska et al. 2008). In addition, ABA plays an important role in plant response to abiotic stresses either as a result of its endogenous concentration or through exogenous application (Haisel et al. 2006, Wang et al. 2007). The application of exogenous ABA can enhance tolerance in plants to extreme temperatures, heavy metals, drought, and salinity by increasing contents of proline and soluble sugar, enhancing water retention, reducing membrane lipid peroxidation, and maintaining membrane integrity and photosynthetic characteristics (Han et al. 2016, Kaur et al. 2015, Li et al. 2014b, Liu et al. 2013, Minardi et al. 2014, Pattanagul 2011, Zheng et al. 2015).

Acid rain, a serious global environmental issue, exerts deleterious effects on the phenotype and physiological characteristics of plants by destructing chloroplast ultrastucture, blocking the synthesis of chlorophyll and decreasing photosynthesis, disturbing cation homeostasis, and accelerating the accumulation of reactive oxygen species (Kováčik et al. 2011, Liang and Wang 2013, Liang et al. 2005, Singh and Agrawal 2008, Sun et al. 2013, Wang et al. 2013b, Zhou and Huang 2002). In addition, acid rain accelerated the leaching of

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nutrients (Ca, Mg, K, and Na) from leaves which leads to the abnormal or restricted growth (Wang et al. 2014a). In response to acid rain stress, plants have complex sensing and signaling mechanisms to regulate the ion homeostasis. For example, hormones participate in the control of these regulatory networks to improve the transportation and distribution of nutrient (Rubio et al. 2009). ABA increases the vacuole Na^+ content but inhibit the xylem transport and the plasma membrane influx in barley roots (Behl and Jeschke 1981). Chen et al. (2001) also found that the increase in Ca^{2+} uptake may be associated with the increase in ABA to regulate uptake and transport of salt ions (Na^+, Cl^-) under high levels of external salinity. However, few studies have considered the role of hormones (ABA in particular) in plant response to acid rain stress (Qiu et al. 2004, Wu and Liang 2016), and no evidence is provided for confirming how the application of exogenous ABA affect the tolerance of plants to acid rain stress. Based on the information, finding feasible ways for eliminating the negative effects of acid rain on plants will be possible, and it is meaningful for us to face the challenge of food security.

Therefore, our study is aimed at (1) revealing the tolerance in rice seedlings to simulated acid rain (SAR) with or without exogenous ABA and (2) exploring the role of exogenous ABA in plant tolerance to SAR stress by regulating nutrient contents and endogenous hormone. To achieve these, we studied the effect of exogenous ABA on biomass dry weight, stomatal conductance, net photosynthesis rate, contents of nutrient, and endogenous hormone in rice seedlings exposed to SAR during exposure and recovery periods. These results will provide basic information for alleviating the damage to plants caused by acid rain.

Materials and methods

Plant material and growth conditions

Rice (*Oryza sativa*) seeds "Huaidao 8" were supplied by Xishan Seed Company (Wuxi, China). The seeds were surface disinfected with $HgCl_2$ (0.1%, *w/v*) for 10 min and washed three times with deionized water. After soaking in distilled water for 12 h, the seeds were placed in a dish (90 mm) underlaid with three layers of filter paper and germinated in an incubator at 25 ± 1 °C. After 2 days, germinated rice seeds were cultured in plastic box (6.88 L) filled with vermiculite for 25 days. When two leaves appeared, rice seedlings were cultivated in routine nutrition solution (pH 5.5) prepared by the method provided by the International Rice Research Institute (Zhu et al. 2009) in a growth chamber with irradiance of 300 μmol m^{-2} s^{-1} photosynthetically active radiation (400 to 700 nm) at 25 °C and 70% relative humidity during day time and at 20 °C and 80% relative humanity at night.

Simulated acid rain and exogenous ABA treatment

The solution of simulated acid rain (Yang et al. 2014) at pH values of 4.5 and 3.5 was prepared by adjusting the pH of the control rain with the addition of concentrated H_2SO_4 and HNO_3 at a ratio of 3:1 (*v/v*, by chemical equivalents) (Chen et al. 2010). Simulated acid rain solution (pH 4.5 and 3.5) was sprayed at 24-h intervals on the leaves of rice seedlings till drops began to fall. In our preliminary experiments (data not shown in the manuscript), we studied the effect of different concentrations of exogenous ABA (0.1, 1, 10 and 100 μM) on growth of rice seedlings. We found that 10 μM ABA could promote obviously the growth of rice seedlings whereas the application of 100 μM ABA inhibited the growth of rice seedlings. Based on the results, we used the exogenous ABA at the concentration of 10 and 100 to study effects of the combination ABA and SAR on growth and physiology in rice seedlings in this paper. At the seedling stage (the fourth leaf of rice developed completely), the rice seedlings were separated to nine groups, and treated as described in Table 1. (1) For the control group, rice seedlings were cultured with the nutrition solution at pH 5.5 and sprayed with distilled water (pH 7.0) on leaves. (2) For the single SAR group, rice seedlings were cultured with the nutrition solution at pH 4.5 or 3.5 and sprayed with SAR at pH 4.5 or 3.5 on leaves. (3) For the single exogenous ABA group, rice seedlings were cultured with the nutrition solution at pH 5.5 and sprayed with exogenous ABA at different concentrations (10 or 100 μM) on leaves. (4) For the combined treatment of SAR and exogenous ABA groups, rice seedlings were cultured with the nutrition solution at pH 4.5 or 3.5, sprayed with SAR at pH 4.5 or 3.5 on the leaves in the morning and then sprayed with exogenous ABA at different concentrations (10 and 100 μM) on the leaves in the afternoon. The application of exogenous ABA by leaf spray was better because the addition of exogenous ABA in the nutrition solution could be influenced by the pH of the nutrition solution adjusted by acid rain. The nutrition solution was renewed every day to stabilize pH. All of the treatments were performed in triplicates. For each replication, three rice seedlings were analyzed. After the treatment with SAR and exogenous ABA for 5 days, half of the rice seedlings were collected to determine the indices. The rest

Table 1 Overview of all experiments with SAR and ABA

ABA concentration	pH 7.0	pH 4.5	pH 3.5
0	Control	pH 4.5	pH 3.5
10 μM	10 μM	pH 4.5 + 10 μM	pH 3.5 + 10 μM
100 μM	100 μM	pH 4.5 + 100 μM	pH 3.5 + 100 μM

of the rice seedlings were moved to be cultured under the control conditions for another 5 days and then were collected for analysis.

Biomass dry weight determination

Fresh rice seedlings were collected and washed three times with distilled water, and the water on the leaf surface was absorbed by filter paper. Biomass dry weight was determined after at 60 °C for 24 h (Rusak et al. 2009).

Determination of gas exchange parameters

Stomatal conductance (G_s) and net photosynthesis rate (P_n) were measured with a portable gas exchange system (CIRAS-1, PP Systems International Ltd., UK) under the cultured condition of rice seedling at 25 ± 2 °C, CO_2 concentration 340 μmol mol^{-1} and a photosynthetically active photon flux density of 500 μmol·m^{-2} s^{-1} (Liang et al. 2011).

Determination of mineral element content

Fresh plants were collected, cleaned, and washed three times with distilled water. The leaves were dried in an oven and crushed into pieces of 1 mm in diameter. For each sample, 0.1 g crushed leaves was then digested in 8 mL oxidizing solution (15 M HNO_3 and 9 M H_2O_2, *v/v*) for 30 min at 2600 kPa (80 psi) in a MDS-2000 microwave oven (CEM Corp., Matthews, NC). The samples were diluted to a final volume of 25 mL with deionized water for further analysis (Wang et al. 2008). The contents of mineral elements (K, Ca, Mg, and P) in each sample were determined by AAS (atomic absorption spectroscopy, 3110, Perkin Elmer, Norwalk, CT, USA) (Hermans et al. 2010).

Nitrate content determination

Nitrate content was measured according to the method described by Miranda et al. (2001). Rice leaves were collected and washed three times with distilled water, and the water on the surface of the leaves was absorbed by filter paper. Fresh leaves (1 g) were put into graduated test tube with 20 mL deionized water. Test tubes blocked with plugs were put into boiling water for 30 min, and then cooled with flowing water. Extraction was filtered and diluted to 25 mL with deionized water, and then the extracting solution (0.1 mL) was transferred to test tubes with 0.4 mL 5% (*w*/*v*) salicylic acid–H_2SO_4 solution. The mixture was shaken for 20 min at ambient temperature. After adding 9.5 mL 8% NaOH, the mixture was shaken, cooling to room temperature. The absorbance was measured at 410 nm by a spectrophotometer (JINGHRI, Shanghai, China).

Hormone content determination

Hormone contents in plants were extracted according to the method provided by Hou et al. (2008). The leaves of rice were quickly preserved in liquid nitrogen and kept in an ultra-low temperature freezer (−80 °C). Approximately 3 g of tissue was weighed and homogenized in small volumes of pre-cooled 80% methanol with trace amount of sliver diethyldithiocarbamate. The samples were extracted with 4–5 mL pre-cooled 80% methanol for 12 h at 4 °C. After centrifugation at 20,000×*g* for 20 min at 4 °C, the supernatant was collected and the residue re-extracted with 4–5 mL of pre-cooled 80% methanol. Following a further 12 h at 4 °C, the extract was centrifuged and the supernatants pooled. This process of extraction with 4–5 mL of pre-cooled 80% methanol was three times, and finally, all the supernatants pooled. The supernatants were concentrated to placing in a 40 °C water bath with Rotary Evaporator (RE-100, Bibby Sterlin LTD. Stone Stafford-shire, England). The organic phase was treated with 0.2–0.3 g of polyvinylpolypyrrolidone. After centrifugation again at 20,000×*g* for 15 min at 4 °C, the supernatant was collected and evaporated to dryness using a vacuum freeze-drying equipment. The dried samples containing ABA, IAA (indol-3-ylacetic acid), GA_3 (gibberellins), and ZT (zeatin) were re-dissolved with 10 mL methanol. Then, the endogenous hormone contents were determined using high-performance liquid chromatography (HPLC) according to Yang et al. (2014) with some modifications. The flow rate for all analyses was adjusted to 1 mL min^{-1}. Twenty microliters of samples was injected for HPLC analysis, and the detection wavelength was 254 nm. HPLC analysis was performed with an HP100 (Agilent, Palo Alto, CA, USA) coupled with a Diode array Detector. An Agilent ZORBAX SB-C_{18} column (5 μm, 4.6 mm × 250 mm) was used in analysis and the column temperature was set at 35 °C. Mobile phases were 100% methanol (A) and 0.6% acetic acid (B). The gradient elution performed as follows: 0–10 min, A 90% and B 10%; 10–20 min, A 50% and B 50%; after 20 min, A 10% and B 90%.

Statistical analysis

The significant differences between the different treatments were analyzed by one-way analysis of variance (ANOVA) using SPSS16.0. The error analysis for the parameters also used SPSS 16.0. All the data are presented as means ± standard deviation. Student's *t* tests were applied to determine the significance between different treatments ($P < 0.05$) (Ke et al. 2003).

Results

Effects of exogenous ABA on the growth of rice seedlings under SAR stress

Figure 1 shows the effect of exogenous ABA on the dry weight of the rice seedlings under SAR stress. After a 5-day exposure, SAR at pH 4.5 or 3.5 decreased dry weight by 24.5 and 38.9% compared with that of the control. A low concentration of ABA (10 μM) had no effect on dry weight whereas a high concentration of ABA (100 μM) decreased dry weight. The dry weight treated with the combination of SAR (pH 4.5 or 3.5) and 10 μM ABA was still lower than those of the control, but higher than those treated with the single SAR. The decrease in the dry weight treated with the combination of pH 3.5 SAR and 10 μM ABA was larger than those treated with the combination of pH 4.5 SAR and 10 μM ABA. However, the decrease in the dry weight of seedlings treated with the combination of SAR (pH 4.5 or 3.5) and 100 μM ABA was higher than those treated with the single SAR. After a 5-day recovery, the dry weight of leaves treated with the single SAR (pH 4.5 or 3.5) was still lower than those of the control. The dry weight of leaves treated with pH 4.5 SAR and 10 μM ABA was close to the control. The dry weight of leaves treated with pH 3.5 SAR and 10 μM ABA was higher than those treated with the single pH 3.5 SAR, although it was still lower than those of the control. However, the dry weight in rice seedlings treated with SAR (pH 4.5 or 3.5) and 100 μM ABA was even lower than those measured during the exposure period.

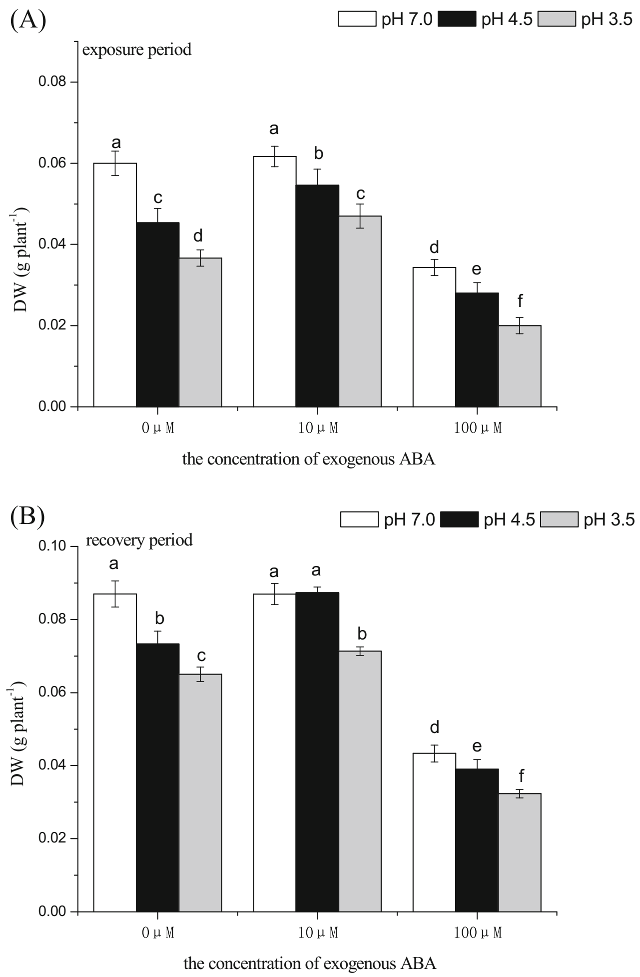

Fig. 1 Effects of exogenous ABA on the growth in rice seedlings under SAR stress during exposure and recovery periods. Significant difference at $P < 0.05$ was shown with *different letters*

Effects of exogenous ABA on the G_s and P_n of rice seedlings under SAR stress

The G_s and P_n in rice leaves exposed to SAR and exogenous ABA were shown in Fig. 2. SAR at pH 4.5 or 3.5 decreased the G_s and P_n by 31.0/32.3% and 41.0/43.1% compared with those of the control. A low concentration of ABA (10 μM) had no obvious effect on the G_s and P_n whereas a high concentration of ABA (100 μM) caused decreases in the G_s and P_n. When rice seedlings were treated with the combination of SAR and 10 μM ABA, the G_s and P_n were lower than those of the control, but higher than those treated with the single SAR. In addition, the decrease in the G_s and P_n of leaves treated with the combination of pH 3.5 SAR and 10 μM ABA was larger than those treated with the combination of pH 4.5 SAR and 10 μM ABA. Contrarily, the decrease in the G_s and P_n of leaves treated with the combination of SAR and 100 μM ABA was higher than those treated with the single SAR. After a 5-day recovery, the G_s and P_n in leaves treated with the single SAR were still lower than those of the control. The G_s and P_n in leaves treated with pH 4.5 SAR and 10 μM ABA were recovered at the control level. Moreover, the G_s and P_n in leaves treated with pH 3.5 SAR and 10 μM ABA were higher than those treated with the single pH 3.5 SAR although they were lower than those of the control. However, the G_s and P_n of leaves treated with the combination of SAR and 100 μM ABA were even lower than those measured during the exposure period.

Effects of exogenous ABA on nutrient contents of rice seedlings under SAR stress

Table 2 shows changes in nutrient element contents (K, Ca, Mg, nitrate, and phosphorus) in rice leaves treated with SAR and exogenous ABA measured during exposure and recovery periods. SAR at pH 4.5 or pH 3.5 decreased contents of K, Ca, Mg, nitrate and phosphorus compared with those of the control. A low concentration of ABA (10 μM) alone decreased K content and increased Ca content, but had no effects on Mg, nitrate, and phosphorus

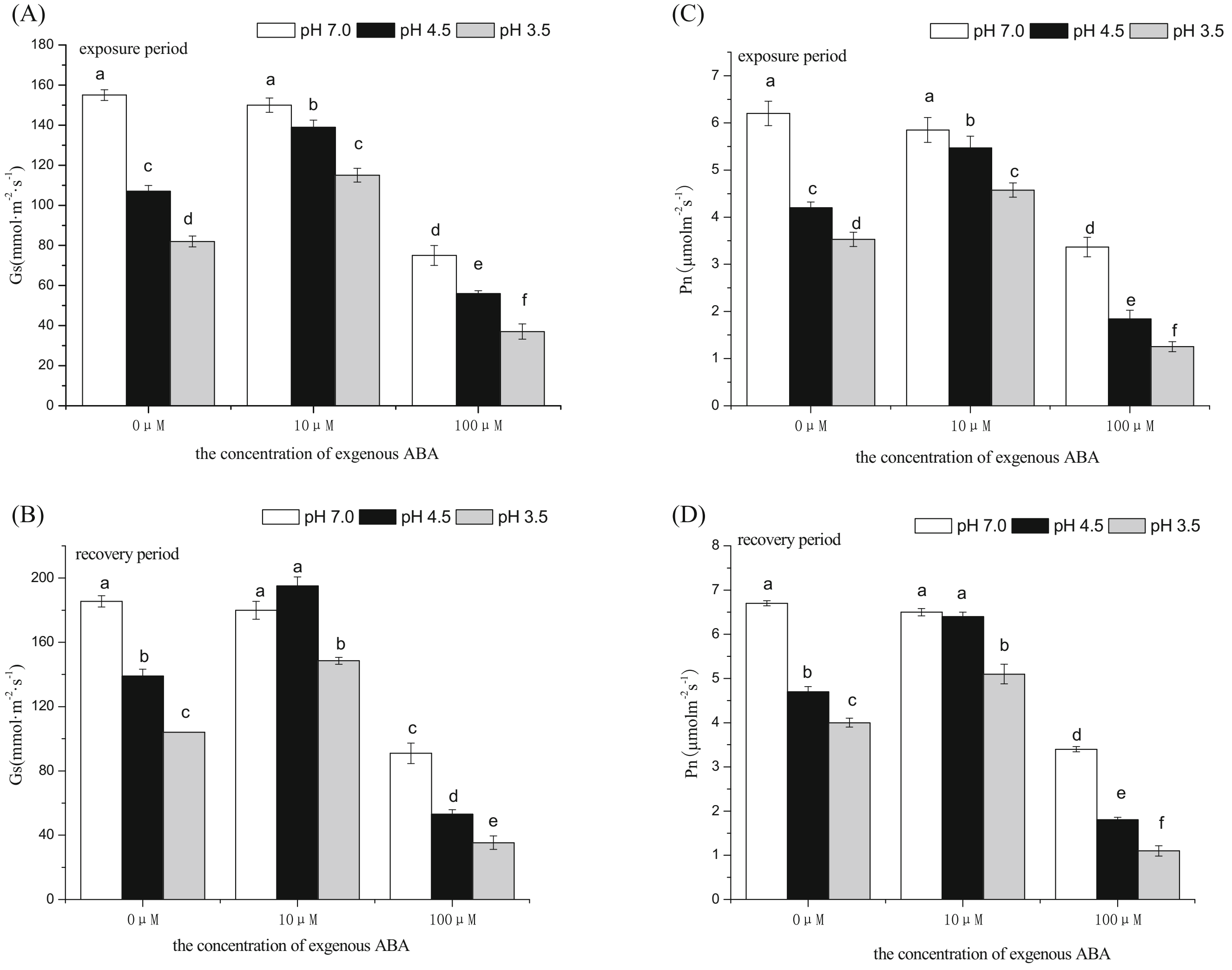

Fig. 2 Effects of exogenous ABA on the G_s and P_n in rice seedlings leaves under SAR stress during exposure and recovery periods. Significant difference at $P < 0.05$ was shown with *different letters*

contents. However, a high concentration of ABA (100 μM) decreased K, Mg, nitrate, and phosphorus contents and increased Ca content. When rice seedlings were treated with the combination of pH 4.5 SAR and 10 μM ABA, K and Mg contents were lower whereas Ca, nitrate, and phosphorus contents were higher compared with those of the control, and all were higher than those treated with pH 4.5 SAR. When rice seedlings were treated with the combination of pH 3.5 SAR and 10 μM ABA, contents of K, Mg, nitrate, and phosphorus in leaves were lower than those of the control whereas Ca content increased, and all of them were higher than those treated with the single pH 3.5 SAR. However, contents of K, Mg, nitrate and phosphorus in leaves treated with the combination of SAR and 100 μM ABA were lower than those treated with the single SAR, with the exception that Ca content in leaves was higher than those of the control. After a 5-day recovery, nutrient element contents in leaves treated with the single SAR were still lower than those of the control. Contents of K, Ca, Mg, nitrate, and phosphorus in leaves treated with pH 4.5 SAR and10 μM ABA were recovered at the control level, and better than those treated with pH 3.5 SAR and 10 μM ABA. Only the Ca content in leaves treated with pH 3.5 SAR and 10 μM ABA was higher than those of the control. However, contents of all nutrient elements in leaves treated with SAR and 100 μM ABA were still lower than those treated with the single SAR, and even lower than those measured during the exposure period.

Effects of exogenous ABA on endogenous hormone contents in rice seedlings under SAR stress

As shown in Table 3, SAR at pH 4.5 or 3.5 caused an increase in the ABA content and decreases in IAA, GA_3, and ZT contents in rice seedlings compared with those of

Table 2 Effects of exogenous ABA on nutrient contents in rice seedlings leaves under SAR stress

Treatment	K (mg g^{-1}DW)	Ca (mg g^{-1}DW)	Mg (mg g^{-1}DW)	Nitrate (μg g^{-1}FW)	Phosphorus (mg g^{-1}DW)
Exposure period					
Control	22.12 ± 0.66a (100.0)	9.38 ± 0.27e (100.0)	3.05 ± 0.09a (100.0)	4.21 ± 0.12b (100.0)	0.46 ± 0.09b (100.0)
4.5	17.84 ± 0.60c (80.7)	8.58 ± 0.49f (91.4)	2.48 ± 0.08c (81.3)	3.87 ± 0.11c (91.9)	0.40 ± 0.10c (86.9)
3.5	15.46 ± 0.46de (69.9)	7.58 ± 0.52 g (80.8)	2.13 ± 0.07d (69.8)	3.44 ± 0.10de (81.6)	0.36 ± 0.08d (78.3)
10	20.88 ± 0.72b (94.3)	10.40 ± 0.31d (110.9)	2.98 ± 0.09a (97.7)	4.26 ± 0.13b (101.1)	0.44 ± 0.05ab (96.5)
100	16.71 ± 0.57d (75.5)	10.95 ± 0.27c (116.7)	2.23 ± 0.07d (73.0)	3.26 ± 0.09e (77.5)	0.41 ± 0.09cd (88.8)
pH 4.5 + 10	20.39 ± 0.76b (92.1)	9.98 ± 0.24d (106.4)	2.83 ± 0.08b (92.6)	5.24 ± 0.16a (124.5)	0.50 ± 0.10a (107.7)
pH 3.5 + 10	18.24 ± 0.94c (82.5)	10.63 ± 0.37c (113.3)	2.63 ± 0.08bc (86.1)	3.91 ± 0.12c (92.8)	0.42 ± 0.04c (92.0)
pH 4.5 + 100	15.13 ± 0.52e (68.4)	11.80 ± 0.23b (125.8)	2.18 ± 0.07d (71.3)	3.55 ± 0.11d (84.3)	0.35 ± 0.03d (75.2)
pH 3.5 + 100	13.25 ± 0.46f (59.9)	12.43 ± 0.22a (132.5)	1.78 ± 0.06e (58.4)	2.75 ± 0.08f (65.3)	0.29 ± 0.05e (63.0)
Recovery period					
Control	23.23 ± 0.70a (100.0)	9.48 ± 0.28bc (100.0)	3.20 ± 0.10a (100.0)	4.47 ± 0.13a (100.0)	0.51 ± 0.09a (100.0)
4.5	21.06 ± 0.63b (90.7)	8.73 ± 0. 26d (92.0)	2.85 ± 0.08b (89.1)	4.12 ± 0.12b (92.1)	0.48 ± 0.10b (93.2)
3.5	17.49 ± 0.52c (75.3)	7.67 ± 0.49e (80.9)	2.58 ± 0.07c (80.5)	3.81 ± 0.11c (85.3)	0.42 ± 0.08c (82.3)
10	21.29 ± 0.73ab (91.6)	9.03 ± 0.27cd (95.2)	2.95 ± 0.09ab (92.2)	4.36 ± 0.16a (97.5)	0.48 ± 0.14ab (94.1)
100	16.80 ± 0.50c (72.3)	7.68 ± 0.26e (81.0)	2.35 ± 0.06d (73.4)	3.12 ± 0.12d (69.8)	0.40 ± 0.07c (78.4)
pH 4.5 + 10	24.05 ± 0.78a (103.5)	9.60 ± 0.30b (101.3)	3.33 ± 0.11a (103.9)	4.52 ± 0.16a (101.1)	0.53 ± 0.09a (104.3)
pH 3.5 + 10	20.72 ± 0.68b (89.2)	10.53 ± 0.37a (111.1)	2.83 ± 0.08b (88.3)	4.12 ± 0.13b (92.1)	0.47 ± 0.05b (92.1)
pH 4.5 + 100	15.72 ± 0.56d (67.7)	6.13 ± 0.23f (64.7)	2.18 ± 0.07d (68.0)	3.03 ± 0.11d (67.8)	0.34 ± 0.08d (66.7)
pH 3.5 + 100	12.05 ± 0.48e (48.1)	5.05 ± 0.12 g (53.3)	1.70 ± 0.07e (53.1)	2.26 ± 0.10e (50.6)	0.29 ± 0.06e (56.9)

Values in parentheses are the percentage of treatment in control. In the same column, values with different letters are significantly different at $P < 0.05$

the control. A low concentration of ABA (10 μM) alone had no effects on contents of ABA, IAA, GA$_3$, and ZT whereas a high concentration of ABA (100 μM) alone caused an increase in ABA content and a decrease in contents of IAA, GA$_3$, and ZT in rice leaves compared with those of the control. When rice leaves were treated with the combination of SAR (pH 4.5 or 3.5) and 10 μM ABA, the ABA content in leaves was higher than those of the control, but lower than those treated with the single SAR, whereas contents of IAA, GA$_3$, and ZT were lower than those of the control, but higher than those treated with the single SAR. Compared to the single SAR and the control, the ABA content in leaves treated with SAR and 100 μM ABA was the highest, whereas contents of IAA, GA$_3$ and ZT in leaves were the lowest. After a 5-day recovery, the ABA content in leaves treated with pH 4.5 and 3.5 SAR was higher whereas contents of IAA, GA$_3$ and ZT were lower compared to the control. Differing from changes in leaves treated with the single pH 4.5 SAR, contents of ABA, IAA, GA$_3$, and ZT in leaves treated with pH 4.5 SAR and 10 μM ABA were recovered at the control level, better than those in leaves treated with pH 3.5 SAR and 10 μM ABA. However, the ABA content in leaves treated with SAR and 100 μM ABA was still higher than those of the control. In addition, contents of IAA, GA$_3$, and ZT in leaves treated with the combination of SAR and 100 μM ABA were still lower than those treated with the single SAR and even lower than those measured during the exposure period.

Discussion

Effects of exogenous ABA on the growth of rice seedlings under SAR stress

Biomass dry weight of plants is a prominent indicator to reflect the growth under environmental stresses. SAR at pH 4.5 or 3.5 caused a decrease in the dry weight of rice seedlings (Fig. 1a). However, the dry weight in rice seedlings treated with the combination of SAR and 10 μM ABA was higher than those treated with the single SAR. And the dry weight treated with the combination of pH 4.5 SAR and 10 μM ABA were higher than those treated with the combination pH 3.5 SAR and 10 μM ABA. These results indicate that exogenous ABA at the concentration of 10 μM can alleviate the inhibition caused by SAR on plant growth, and the regulating effect depended on the acidity of SAR. A similar result was also reported by Khadri et al. (2006) who found that the inhibition on dry weight of common beans treated with 100 mM NaCl stress can be alleviated by supplying exogenous 10 μM ABA. However, the dry weight in rice seedlings

Table 3 Effects of exogenous ABA on endogenous hormone contents in rice seedlings leaves under SAR stress

Treatment	ABA (μg g^{-1}FW)	IAA (μg g^{-1}FW)	GA$_3$ (μg g^{-1}FW)	ZT (μg g^{-1}FW)
Exposure period				
Control	0.84 ± 0.02f (100.0)	223.54 ± 6.71a (100.0)	552.86 ± 8.13a (100.0)	6.99 ± 0.21a (100.0)
4.5	1.06 ± 0.08d (126.1)	188.40 ± 5.65c (84.3)	454.07 ± 8.08c (82.1)	4.88 ± 0.15d (69.8)
3.5	1.09 ± 0.04c (132.1)	165.44 ± 4.96d (74.0)	412.09 ± 1.31e (74.5)	3.99 ± 0.05e (57.0)
10	0.86 ± 0.03ef (102.5)	220.41 ± 5.39a (98.6)	530.12 ± 5.10a (95.8)	7.00 ± 0.15a (100.1)
100	1.08 ± 0.02 cd (129.8)	173.87 ± 5.22c (77.8)	416.36 ± 5.05e (75.3)	5.18 ± 0.08cd (74.2)
pH 4.5 + 10	0.90 ± 0.04e (107.1)	199.32 ± 1.43b (89.2)	498.87 ± 6.06b (90.2)	6.51 ± 0.16b (93.1)
pH 3.5 + 10	1.01 ± 0.06d (120.2)	179.38 ± 0.45c (80.2)	447.30 ± 4.54d (80.9)	5.65 ± 0.10c (80.8)
pH 4.5 + 100	1.31 ± 0.02b (155.0)	161.10 ± 4.83d (72.1)	387.94 ± 5.06f (70.1)	4.12 ± 0.11e (58.9)
pH 3.5 + 100	1.40 ± 0.06a (166.2)	129.62 ± 3.10e (58.0)	332.29 ± 4.09 g (60.1)	3.42 ± 0.02f (48.9)
Recovery period				
Control	0.83 ± 0.03ef (100.0)	215.47 ± 7.64a (100.0)	539.39 ± 8.19a (100.0)	7.60 ± 0.23a (100.0)
4.5	0.99 ± 0.01c (120.3)	185.24 ± 2.12c (86.0)	491.37 ± 5.05bc (91.1)	6.31 ± 0.19c (83.0)
3.5	1.12 ± 0.02b (135.8)	173.02 ± 3.46d (80.3)	471.80 ± 4.06c (87.5)	5.87 ± 0.18d (77.2)
10	0.77 ± 0.03f (93.7)	199.15 ± 5.88a (92.4)	540.44 ± 5.13a (100.1)	8.86 ± 0.27a (116.5)
100	1.00 ± 0.04c (120.7)	162.83 ± 4.59d (75.6)	401.04 ± 4.03d (74.4)	5.18 ± 0.12d (68.1)
pH 4.5 + 10	0.85 ± 0.01e (103.2)	213.67 ± 6.41a (99.2)	529.97 ± 6.04a (98.3)	8.45 ± 0.26a (111.2)
pH 3.5 + 10	0.93 ± 0.06d (112.6)	186.59 ± 1.14bc (86.6)	503.31 ± 5.15b (93.2)	7.13 ± 0.05b (93.8)
pH 4.5 + 100	1.16 ± 0.06b (140.4)	134.44 ± 5.23e (62.4)	339.12 ± 3.08e (62.9)	4.20 ± 0.01e (55.3)
pH 3.5 + 100	1.22 ± 0.04a (148.3)	111.37 ± 4.84f (51.7)	306.15 ± 4.07f (56.8)	3.00 ± 0.01f (39.5)

Values in parentheses are the percentage of treatment in control. In the same column, values with different letters are significantly different at $P < 0.05$

treated with the combination of SAR and 100 μM ABA was obviously lower than those treated with the single SAR (Fig. 1a) indicates that the application of ABA at a higher concentration (100 μM) aggravated the SAR-induced inhibition on plant growth. After a 5-day recovery, the dry weight of the rice seedlings treated with the combination of pH 4.5 SAR and 10 μM ABA was recovered at the control levels. However, the decrease in the dry weight of the rice seedlings treated with SAR and 100 μM ABA was still lower than those treated with the single SAR, even lower than those measured during the exposure period. The phenomena indicate that the application of 10 μM ABA can promote the recovery of the rice seedling leaves following the withdrawal of SAR stress whereas the application of 100 μM ABA can hinder the recovery process.

Effects of exogenous ABA on the G_s and P_n of rice seedlings under SAR stress

Plant growth and development depend on synthesis and accumulation of organic substances that are produced by photosynthesis (Chia and He 1999, Li et al. 2014a). Therefore, the decrease in photosynthetic capacity of plants under environmental stresses is one of the key factors limiting plant growth (Kummerová et al. 2006). In the present work, we found that SAR at pH 4.5 or 3.5 caused inhibition on the G_s and P_n in leaves, and the decreased degree in rice treated with pH 4.5 SAR was lower (Fig. 2a, c). Wang et al. (2014b) also proved that stomatal conductance and photosynthetic rate were decreased with the increase in acidity of acid rain. With the application of 10 μM ABA, the G_s and P_n in leaves exposed to SAR were higher than those treated with the single SAR, and the alleviating effects of 10 μM ABA on the G_s and P_n under pH 4.5 SAR stress were better than those under pH 3.5 SAR stress. These results show that the application of 10 μM ABA can induce the stomatal opening to maintain CO_2 supply and then alleviate the decrease in photosynthetic rate caused by SAR. Teng et al. (2014) also found that the application of exogenous ABA (60 μM) alleviates P_n, $G_{s,}$ and transpiration rate in leaves under PEG stress and revealed that photosynthetic system II is repaired by the upregulation of genes related to chlorophyll and ABA biosynthesis. However, the G_s and P_n in leaves treated with the combination of SAR and 100 μM ABA were lower than those treated with the single SAR. It means that higher concentration of ABA aggravated the SAR-induced inhibition on the G_s by inducing the stomatal closure and then a heavier decrease in photosynthesis, leading to more loss in the dry weight. Hejnák and Kykalová (2009) also reported that foliar spraying with 100 μM ABA accelerated chlorophyll degradation in maize seedlings under water deficit conditions. After a 5-day recovery, the G_s and P_n in leaves treated with pH 4.5 SAR and 10 μM ABA was recovered at the control levels, indicating that 10 μM ABA promoted the recovery of the G_s and P_n. However, the decrease in the

G_s and P_n of leaves treated with SAR and 100 μM ABA was even lower than those measured during the exposure period, indicating that 100 μM ABA hindered the recovery process above.

Effects of exogenous ABA on nutrient contents of rice seedlings under SAR stress

Nutrient elements are necessary for plant growth and development, and the deficiency of nutrient elements in plants are used as an indicator for revealing the environmental stress-induced damage to plants (Klimenko and Klimenko 2001, Maathuis 2009). In our experiments, contents of K, Ca, Mg, nitrate, and phosphorus in leaves treated with the single SAR were decreased (Table 2), indicating that acid rain led to nutrient deficiency, and the degree of deficiency depended on the acidity of acid rain. The decrease in K, Ca, and Mg was also found in other plant species under SAR stress (Zhou et al. 2003). Compared with those treated with the single SAR, the decrease in contents of nutrient elements in leaves under SAR stress was alleviated by exogenous 10 μM ABA (Table 2), indicating that ABA was involved in regulating nutrient element contents in rice seedlings under SAR stress. Usually, acid rain inhibits photosynthesis in plants partly because the SAR-induced increase in extracellular H^+ can replace the Mg^{2+} in Mg protoporphyrin IX, resulting in suppression of chlorophyll biosynthesis (Mohapatra and Tripathy 2007, Zhou and Huang 2002). Thus, the alleviating effects of 10 μM ABA on the decrease in Mg content in leaves under SAR stress can contribute to maintain photosynthetic capacity (Table 2) by alleviating SAR-induced inhibition on chlorophyll synthesis (Basak et al. 2012). In addition, the application of 10 μM ABA increased contents of nitrate and phosphorus in leaves treated with SAR to maintain protein or carbohydrate metabolisms, benefiting to accumulating dry matter in plants (Fig. 1). However, the application of 100 μM ABA aggravated the deleterious effects on nutrient element contents in leaves under SAR stress (Table 2). Although an increase in Ca is necessary for signal transduction, a continuous increase in Ca is lethal (Pan et al. 2008). The higher content of Ca could be one of the factors leading to the heavier decrease in growth of rice treated with SAR and 100 μM ABA. Hirschi (2004) also reported that a marked increase in Ca is harmful to cells because it leads to activation of particular Ca^{2+}-dependent enzymes, which have a potentially adverse effect. Even in some mammalian cells, Ca overload can also cause mitochondrial failure; if this becomes irreversible, it leads to cell death (Duchen 2000). On the other hand, the higher decrease in contents of K, Mg, nitrate, and phosphorus in leaves treated with SAR and 100 μM ABA destroyed ion homeostasis in cells and nutritional balance in leaves and then caused a heavy decrease in the dry weight (Fig. 1). After a 5-day recovery, nutrient element contents in leaves treated with pH 4.5 SAR and 10 μM ABA were recovered at the control level whereas those in leaves treated with the single pH 4.5 SAR were still worse than those of the control. This suggests that application of 10 μM ABA could alleviate the decrease in nutrient elements in leaves induced by acid rain, and promote selrestoration in rice seedlings during the recovery period. However, the decrease in nutrient element contents in leaves treated with SAR and 100 μM ABA was even worse than those measured during the exposure period. These phenomena indicate that the application of 100 μM ABA aggravated the SAR-induced nutrient deficiency, and hinder self-restoration in the rice seedling during the recovery period.

Effects of exogenous ABA on endogenous hormone contents of rice seedlings under SAR stress

ABA, IAA, GA_3, and ZT are pivotal endogenous hormones regulating plant growth and development, and changes in their concentrations affect cell growth (Zhao et al. 2012). SAR at pH 4.5 or 3.5 elevated the ABA content in rice leaves, being consistent with the accumulation of ABA in plants under abiotic stresses usually. ABA, as a signal molecule, is involved in plant response to abiotic stresses such as water deficit and salinity (Ghanaatiyan and Sadeghi 2015, Milosavljević et al. 2012). In addition, the decrease in IAA, GA_3, and ZT in leaves treated with the single pH 4.5 or 3.5 SAR (Table 3) also could be one of the factors inhibiting plant growth (Fig. 1) because IAA, GA_3, and ZT can regulate plant growth by participating in cell division, expansion and differentiation (David and Machteld 2001, Rademacher 2000, Woodward and Bartel 2005). We found that the ABA content in leaves treated with the combination of SAR and 10 μM ABA was higher than those of the control, but lower than those treated with the single SAR. The increase in ABA in leaves could be induced by exogenous ABA, and be transported by phloem to roots to enhance root tolerance to acid rain stress (Ikegami et al. 2009), thus lowered ABA in leaves compared to the single SAR. In addition, Ca has been identified as a possible mediator of ABA-induced stimulus–response coupling (Netting 2000). Therefore, we inferred that exogenous ABA induced the Ca content in leaves to regulate the stomatal conductance (Table 2), which might lead to the decrease in the G_s (Fig. 2a), prevent acid rain injury to the cell. Consequently, the application of exogenous ABA can regulate endogenous ABA in leaves to enhance rice tolerance to acid rain stress. Meanwhile, the alleviating effects of exogenous ABA on IAA, GA_3, and ZT in leaves under SAR stress (Table 3) also contribute to enhance rice tolerance because the higher IAA can stimulate the acquirement of plants to nutrition and the translocation of nutrient elements by enhancing cell elongation and division (López et al. 2007). These findings indicate that the application of 10 μM ABA can trigger the signaling network in rice response to pH 4.5 or 3.5 SAR by regulating endogenous ABA and other phytohormones (IAA, GA_3, and ZT) as well. When rice seedlings were treated with the combination of SAR and 100 μM ABA, the ABA content was increased whereas contents of IAA, GA_3, and ZT were decreased compared with the single SAR (Table 3). The excess accumulation of endogenous ABA caused by the application of 100 μM ABA can induce the stomata closure (decrease in G_s) (Fig. 2a) to block CO_2 entering into cells (Li et al. 2015), thus the P_n was decreased (Fig. 2c). The decrease in IAA, GA_3, and ZT contents in leaves treated with SAR and 100 μM ABA inhibited cell growth and division (Fahad et al. 2015) and then led to the decrease in the dry weight of rice (Fig. 1). After a 5-day recovery, the ABA content in leaves treated with SAR and 10 μM ABA was lower than those treated with the single SAR whereas contents of IAA, GA_3, and ZT were higher. In addition, endogenous hormones in leaves treated with pH 4.5 SAR and 10 μM ABA were recovered at the control level, indicating that 10 μM ABA can promote the self-restoration by regulating endogenous hormones in leaves during the recovery period, and the recovery degree was dependent on the acidity of acid rain. Contrarily, contents of IAA, GA_3, and ZT in leaves treated with SAR and 100 μM ABA were still the lowest compared to the single SAR, and even lower than those measured during the exposure period. It indicates that the application of 100 μM ABA not only aggravated the SAR-induced inhibition on endogenous hormones in leaves, but also hindered the self-restoration of rice seedlings. Meanwhile, the higher increase in the ABA content in leaves treated with SAR and 100 μM ABA still induced the stomatal closure, causing the heavier decrease in the P_n and dry weight (Fig. 2b, d) in rice seedlings.

Conclusions

SAR at pH 4.5 or 3.5 exerted deleterious effect on growth of rice by decreasing contents of nutrient element, IAA, GA_3, and ZT, as well as increasing the ABA content in leaves. The application of 10 μM ABA can alleviate SAR-induced inhibition on growth by maintaining the G_s and P_n contents of nutrient elements (K, Mg, nitrate, and phosphorus) and the balance of endogenous hormones in leaves under SAR stress. In addition, the application of 10 μM ABA could stimulate the Ca content as signaling molecules under SAR stress. However, the application of 100 μM ABA aggravated the deleterious effect on growth of rice seedlings caused by SAR through increasing deficiency of nutrients and disturbing the balance of hormones. After a 5-day recovery, exogenous 10 μM ABA can promote self-restoration process in rice whereas 100 μM ABA can hinder the restoration. In addition, the more treatments (an intermediate treatment of ABA between 10 and 100 μM) could also be informative to reveal the improving effect of exogenous ABA on the tolerance of rice seedlings to acid rain stress.

These results could enrich our understanding on the role of exogenous ABA in regulating the adaptation of plants to acid rain and provide a new direction to find ways for alleviating the damage to plants cause by acid rain. Based on the information got under the laboratory conditions, the regulating effect of exogenous ABA on tolerance of plants to acid rain needs to be identified by different crop species in field experiments before its application in agriculture.

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