

# Effects and mechanism of acid rain on plant chloroplast ATP synthase

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**Abstract** Acid rain can directly or indirectly affect plant physiological functions, especially photosynthesis. The enzyme ATP synthase is the key in photosynthetic energy conversion, and thus, it affects plant photosynthesis. To clarify the mechanism by which acid rain affects photosynthesis, we studied the effects of acid rain on plant growth, photosynthesis, chloroplast ATP synthase activity and gene expression, chloroplast ultrastructure, intracellular H<sup>+</sup> level, and water content of rice seedlings. Acid rain at pH 4.5 remained the chloroplast structure unchanged but increased the expression of six chloroplast ATP synthase subunits, promoted chloroplast ATP synthase activity, and increased photosynthesis and plant growth. Acid rain at pH 4.0 or less decreased leaf water content, destroyed chloroplast structure, inhibited the expression of six chloroplast ATP synthase subunits, decreased chloroplast ATP synthase activity, and reduced photosynthesis and plant growth. In conclusion, acid rain affected the chloroplast ultrastructure, chloroplast ATPase transcription and activity, and  $P_n$  by changing the acidity in the cells, and thus influencing the plant growth and development. Finally, the effects of simulated acid rain on the test indices were found to be dose-dependent.

**Keywords** Acid rain · Rice leaves · Chloroplast · ATP synthase · Gene expression

## Introduction

Acid rain is a major environmental problem that endangers Earth's ecosystems and threatens human survival and has thus received extensive attention worldwide (Abbasi et al. 2013). In particular, acid rain is a major problem in Europe, North America, and China (Abbasi et al. 2013). The total area that suffers from acid rain in Europe and North America is estimated to be about 1000 km<sup>2</sup> (Lajtha and Jones 2013). Based on nearly 20 years of continuous monitoring, the acidity of European rainwater has increased by 10 %; currently, the pH value of acid rain in Sweden, Denmark, Poland, Germany, Canada, and other European countries is usually between 4.2 and 4.5 (Lajtha and Jones 2013; Menz and Seip 2004). In 15 states of the USA, the pH value of acid rain is currently lower than 4.8 (Lajtha and Jones 2013; Menz and Seip 2004). In China, the area impacted by acid rain has reached two million square kilometers and is gradually increasing (Liu et al. 2011). An estimated 208 cities in China were polluted with acid rain in 2014, accounting for 44.3 % of the total cities in which rainfall was monitored in this year (Department EoCE 2015). Moreover, the cities in which the frequency of acid rain exceeded 25 or 75 % of total rainfall events accounted for 26.6 and 9.1 % of the total monitored cities, respectively (Department EoCE 2015). Moreover, the average pH value of rainfall in 44 of these cities was less than 5.6, and the pH of rainfall in these areas was approximately between 3.8 and 4.5 (Department EoCE 2015). Serious acid rain has had many effects in China (Department EoCE 2015), for example, the continuous acid rain pollution in the south of the

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Yangtze River has caused lake acidification, a reduction in fish numbers, and forest degradation (Zhao and Hou 2010).

In recent years, there have been many studies into the effects of acid rain on plants, which have shown that acid rain can cause direct or indirect harm to plant leaves (Imran et al. 2014; Macaulay and Enahoro 2015; Ramlall et al. 2015). A study showed that when the pH of acid rain is below 3.0, acid rain harms the plant cuticle, causing blade chlorosis (Macaulay and Enahoro 2015). When the pH of acid rain is below 1.0, acid rain causes necrotic spots on plant tissues, premature defoliation or premature aging, and other visible symptoms (Macaulay and Enahoro 2015). Moreover, many studies have shown that low-acidity acid rain improves seed germination, increases biomass accumulation, and promotes aboveground tissue growth of plants (Imran et al. 2014; Ramlall et al. 2015). With increasing acid rain acidity, the seed germination rate, plant survival rate, and plant biomass are reduced (Ramlall et al. 2015). In particular, highly acidic acid rain significantly inhibits plant growth and development (Ramlall et al. 2015).

It is well known that photosynthesis is the key metabolic basis for plant growth, development, and yield (Bernacchi et al. 2013). Photoreaction is an important component of photosynthesis, associated with light absorption, electron transfer, light phosphorylation, and other processes (Bernacchi et al. 2013). Numerous studies have shown that acid rain can affect plant photosynthesis (Hu et al. 2014; Wang et al. 2014; Wyrwicka and Skłodowska 2006). Wyrwicka and Skłodowska found that acid rain at pH 3.0 and pH 1.8 decreased the chlorophyll content of cucumber (*Cucumis sativus*) leaves by 25 and 46 %, respectively (Wyrwicka and Skłodowska 2006). Cao et al. showed that acid rain at pH 2.5 caused decreases in the chlorophyll content (30.71 %), net photosynthetic rate ( $P_n$ , 12.52 %), and transpiration rate (26.89 %) in rape leaves (Cao et al. 2010). Acid rain at pH 4.5 caused 2.17 and 2.41 % increases in the  $P_n$  and Hill reaction rate, respectively, in rice leaves; however, acid rain at less than pH 4.5 decreased the  $P_n$  and Hill reaction rate in the leaves (Hu et al. 2014; Wang et al. 2014). In particular, acid rain at pH 2.5 decreased the  $P_n$  and Hill reaction rate by 26.02 and 24.31 %, respectively (Hu et al. 2014; Wang et al. 2014). Moreover, acid rain at pH 2.5 also changes the chlorophyll fluorescence and damages the PSII reaction center, leading to electron transfer limitation, thereby inhibiting the absorption of light energy by plants (Liu et al. 2015). Furthermore, acid rain can reduce the integrity of chloroplast structures or destroy them entirely, and thus affect some chloroplast functions and photosynthesis (Wen et al. 2011). The aforementioned studies show that effects of acid rain on plant photosynthesis result from the changes in chloroplast composition and structure, and influence various

physiological processes, including light reactions and dark reactions.

Chloroplasts are important sites for plant photosynthesis (Bernacchi et al. 2013). The chloroplast coupling factor, adenosine triphosphate (ATP) synthase ( $CF_1$ – $CF_0$ ), is the key enzyme of photophosphorylation and an important factor in photosynthetic energy conversion, and thus, it is important for plant photosynthesis (Kartashov et al. 2015). Studies have shown that acid rain can affect the activity and transcription level of plasma membrane  $H^+$ -ATPase in rice (Liang et al. 2015; Zhang et al. 2015); however, the effects of acid rain on chloroplast ATPase have not been reported. In particular, when acid rain causes increased acidity of the cellular internal environment, the relationship between the integrity of chloroplast structures and the changes of chloroplast ATPase activity is currently unclear. Rice, as an important commercial crop in the agricultural planting structure, provides food for nearly three billion people around the world (Zhang et al. 2013). In China, the rice-growing area overlaps considerably with acid rain-affected areas (Gravois 1998). Therefore, in the present work, effects of simulated acid rain on the growth,  $P_n$  activity and gene expression of chloroplast ATP synthase, chloroplast ultrastructure and intracellular  $H^+$  level in rice were investigated. On this basis, the possible mechanisms behind the effects of acid rain on rice photosynthesis were analyzed using correlation analysis methods.

## Materials and methods

### Preparation of rice nutrient solution and simulated acid rain

Modified rice nutrient solution was prepared according to the ionic composition released by the International Rice Research Institute (IRRI) (Zhu et al. 2009). The full-strength modified nutrient solution had the following composition: 1.43 mM  $NH_4NO_3$ , 0.51 mM  $K_2SO_4$ , 1.00 mM  $CaCl_2$ , 1.64 mM  $MgSO_4$ , 9.47  $\mu$ M  $MnCl_2$ , 0.075  $\mu$ M  $(NH_4)_6Mo_7O_{24}$ , 19.00  $\mu$ M  $H_3BO_4$ , 0.15  $\mu$ M  $ZnSO_4$ , 0.16  $\mu$ M  $CuSO_4$ , and 36.00  $\mu$ M  $FeCl_3$ . Meanwhile,  $SiO_2$  was supplied as 1.67 mM with  $NaSiO_3 \cdot 9H_2O$  in nutrient solution. Then, 0.07 mM  $C_2H_4N_4$  was added as nitrification inhibitor to keep the concentration of  $NH_4^+$ -N. The nutrient solution pH was adjusted to 5.5 by using a pH meter (PHS-29 A, Shanghai Anting Scientific Instrument Factory, China).

Based on the ionic composition of rainwater sampled in the southeast of China (Xie et al. 2009), the control rain (pH 7.0) containing 0.83  $\mu$ M  $Ca^{2+}$ , 1.32  $\mu$ M  $Na^+$ , 0.15  $\mu$ M  $K^+$ , 5.34  $\mu$ M  $Mg^{2+}$ , 0.36  $\mu$ M  $SO_4^{2-}$ , 0.64  $\mu$ M  $NO_3^-$ , and 0.47  $\mu$ M  $Cl^-$  was prepared by adding the appropriate amounts of these ions to deionized water. The ionic composition and content of the control rain was nearly the same as rainwater

samples taken in the southeast of China. Simulated acid rain solutions at pH 4.5, 4.0, 3.5, 3.0, and 2.5 were prepared by adjusting the pH value of control rain with the mixture of concentrated H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> (1.1:1, v:v), and the pH value was measured using a pH meter (PHS-29A, Shanghai Anting Scientific Instrument Factory, China) (Kong et al. 2012).

### Plant culture and treatments

Rice seeds were surface sterilized in HgCl<sub>2</sub> (0.1 %) solution for 10 min and rinsed with deionized water several times. The sterilized seeds were placed in dishes under-laid with three layers of moistened gauze and germinated in the incubator for 2 days. Germinated seeds were sown in a sterilized sand bed. At the two leaf stage, healthy rice plants with similar growth status were transplanted into pots containing rice nutrient solution. Pots were placed in a greenhouse at 25 ± 3 °C, with a light intensity of 1000 μmol m<sup>-2</sup> s<sup>-1</sup>, a day/night cycle of 16/8 h, and a relative humidity of 70–80 %. Modified rice nutrient solution was used to irrigate the plants and to maintain the water content of the substrate at approximately 60 %. When rice seedlings reached the three leaf stage, healthy rice plants with similar growth status were selected to be sprayed with 300 mL simulated acid rain per pot and the control plants were sprayed with control rain every 3 days. The spray amount of simulated acid rain and control rain was calculated based on the precipitation and evaporation in the southeast of China (Kong et al. 2012). Moreover, the modified nutrient solution was supplied every 3 days. All treatments were performed in six replicates, and 1 mM KH<sub>2</sub>PO<sub>4</sub> was sprayed onto the foliage every other day to apply the required inorganic phosphate to the plants. At the tillering stage (35 days after the spraying of acid rain), the fresh leaves from the acid rain and control treatments were sampled for analyses.

### Determination of rice growth and P<sub>n</sub>

Growth indices include leaf area, dry weight of leaves, dry weight of aboveground organs (stems and leaves), and relative growth rate. Leaf area was measured using a handheld laser leaf area meter (CI-203, CID Bio-science, USA). Dry weights of leaves and aboveground organs for each plant were determined using a special-type electronic balance (EL303, Mettler Toledo, Switzerland, accuracy: 0.0001 g) after drying of the material at 80 °C in an oven for 12 h. The relative growth rate (RGR) was calculated based on the dry weight of leaves and stems with the following formula:  $RGR = (\ln W_2 - \ln W_1) / 35$ , where W<sub>1</sub> (g) and W<sub>2</sub> (g) represent the dry weight of leaves and stems before and after the acid rain and control treatment, respectively (Kingsbury et al. 1984).

The P<sub>n</sub> was measured by using a portable photosynthetic system (CIRAS-1, PP Systems International Ltd., UK) at 25 ± 5 °C, 320 μL L<sup>-1</sup> CO<sub>2</sub>, and 80 % relative humidity.

The photon flux density was 1000 μmol m<sup>-2</sup> s<sup>-1</sup>, which was provided by the tungsten-halogen lamp on the leaf chamber of CIRAS-1 photosynthesis system.

### Determination of Mg<sup>2+</sup>-ATPase and Ca<sup>2+</sup>-ATPase activities

Chloroplasts were exacted and isolated, and the Mg<sup>2+</sup>-ATPase activity was measured based on the previous method (Sun et al. 2012). The chloroplast suspension (0.1 mL) was incubated for 5 min at 20 °C in 0.8 mL reaction solution containing 62.5 mM Tris-HCl (pH 8.0), 25 mM NaCl, 6.25 mM MgCl<sub>2</sub>, 55 μM PMS, and 2.5 mM C<sub>4</sub>H<sub>10</sub>O<sub>2</sub>S<sub>2</sub> under white light (5000 μmol m<sup>-2</sup> s<sup>-1</sup>) and was activated for 5 min under white light (5000 μmol m<sup>-2</sup> s<sup>-1</sup>). Subsequently, 0.1 mL 5 mM ATP was added and incubated for 5 min at 37 °C, and then 0.2 mL 20 % trichloroacetic acid was added to terminate the reaction. The reaction mixture was centrifuged at 2000×g for 10 min. Sulfuric acid molybdate-ferrous sulfate solution (2.5 mL) was added to 0.5 mL of supernatant. After shaking, the OD values at 660 nm of the mixture were measured using a UV/Vis spectrophotometer (S24-752, Shanghai Jinghua Scientific Instrument Factory, China). The Mg<sup>2+</sup>-ATPase activity was calculated based on the standard curve of inorganic phosphorus.

The Ca<sup>2+</sup>-ATPase activity was measured as described in the previous method (Sun et al. 2012). Chloroplasts suspension (0.1 mL) was incubated for 10 min at 20 °C in 0.8 mL reaction solution containing 0.25 M Tris-HCl (pH 8.0), 20 mM EDTA, 10 mM ATP, and 2 mg mL<sup>-1</sup> trypsin. Subsequently, 0.1 mL of 5 mM ATP was added and incubated for 5 min at 37 °C, and finally, 0.1 mL 10 mg mL<sup>-1</sup> bovine serum albumin was added to terminate the reaction. The determination and calculation methods for Ca<sup>2+</sup>-ATPase activity was the same as Mg<sup>2+</sup>-ATPase activity.

### Transmission electron microscopy observation of chloroplast ultrastructure

Transmission electron microscopy (TEM) observation of chloroplast ultrastructure in rice leaves was performed based on previous method (Helliot et al. 2003). Fresh leaves were sliced into 1.5 × 2 mm sections. After fixation with 3.5 % glutaraldehyde for 24 h, leaf cells were post-fixed with 1 % osmic acid at 4 °C for 4 h and then were dehydrated with ethanol. Next, the samples were embedded in freshly prepared 100 % Epon-812 and polymerized at 80 °C for 24 h. For ultrastructural observations, the samples obtained were cut into ultrathin sections (~60 nm) on a LKB ultramicrotome. The ultrathin sections were placed upon 250-mesh grids and were post-stained with uranyl acetate and lead citrate. Finally, the TEM images were obtained with a TEM (H-600-A-2, Hitachi Ltd., Japan).

### Preparation of total RNA and real-time PCR

Real-time PCR analysis with specific primers for each gene was performed to evaluate the expression of three genes (subunit,  $\beta$  subunit, and  $\epsilon$  subunit) encoding the CF<sub>1</sub> of chloroplast ATP synthase and other three genes (I subunit, III subunit, and IV subunit) encoding the CF<sub>o</sub> of chloroplast ATP synthase. For the normalization of expression of each gene, the actin gene was used as the internal standard. All primers were designed on the basis of sequences published in GenBank by Primer Premier 5.0. Primer sequences of target gene and housekeeping genes are shown in Table 1.

Total RNA of rice leaves were isolated using a column extraction purification kit (SK8661 sangon) according to manufacturer's instructions and then reverse-transcribed into first-strand cDNA with a high-capacity cDNA reverse transcription kit (AMV first strand cDNA synthesis kit, Shanghai Biological Engineering Ltd., China) according to manufacturer's instructions. The cDNA was then used as the template for PCR amplification. Each 25  $\mu$ L reaction system contained 0.5  $\mu$ L template cDNA, 0.5  $\mu$ L primer F (10  $\mu$ M), 0.5  $\mu$ L primer R (10  $\mu$ M), 0.5  $\mu$ L dNTP (10 mM), 2.5  $\mu$ L Taq Buffer (10 $\times$ ), 2  $\mu$ L MgCl<sub>2</sub> (25 mM), 0.2  $\mu$ L Taq enzyme (5 U  $\mu$ L<sup>-1</sup>), and 18.3  $\mu$ L H<sub>2</sub>O. The PCR product was observed using 2 % agarose gel electrophoresis. After connection at 4 °C overnight, the PCR products were transformed with the step rapid competent cell preparation kit (SK9307), and then the plasmid DNA were extracted using plasmid extraction kit (SK8191). The OD value of the constructed plasmids was determined using a UV spectrophotometer (TU-1901, Beijing Purkinje General Instrument Ltd., China) at 260 nm and then converted into a copy number.

### Water content measurement

Leaf water content was calculated according to the following formula: leaf water content (%) = (W<sub>1</sub> - W<sub>2</sub>)/W<sub>1</sub> × 100 %, where W<sub>1</sub> is the fresh weight of leaves and W<sub>2</sub> is the dry weight of leaves determined after drying at 80 °C for 12 h.

### Protoplasts isolation and intracellular H<sup>+</sup> level measurement

Rice leaves were cut into 2-mm filaments and put into a test tube to which enzyme solution containing 2 % cellulase, 0.5 % macerase, 0.1 % (w/v) pectolyase (Y-23), 5 mmol L<sup>-1</sup> MES, and 0.45 mmol L<sup>-1</sup> mannitol was added. Tubes were shaken at 40 rpm and 30 °C in the dark for 3 h. Then, the reaction mixture was filtered through a 400 mesh plug. The filtrate was centrifuged at 1000 $\times$ g for 5 min. The supernatant was removed by pipette and the precipitate was washed twice with a solution containing 3 % sucrose (w/v) and 0.4 M mannitol. Protoplasts were passed through a 50- $\mu$ m pore-size

**Table 1** Primers used in real-time PCR and the size of amplified products. F and R represent the forward and reverse PCR primers

Gene name	Sequences of primers (5'-3')	Product size (bp)
Actin	F: TTATGGTTGGGATGGGACA R: AGCACGGCTTGAATAGCG	292
Atp-A	F: GAGGCTTACTTGGGTCGTGTT R: TGCTGTTTTGCCGGTTT	231
Atp-B	F: TCTTTCGTTTTGTTC AAGCAGG R: AATGTTGTAGCAGGAGCAGGGT	205
Atp-H	F: CCGTAGGCTTGTCTTCTATTGG R: TTCTGCTTCTGGCTGTCTCG	92
Atp-I	F: TCCCTGGGTCCCTTTATTG R: ATATGCCGCTGACGTGAGTAA	169
Atp-F	F: GCGGATGAGTATCGAATGAATG R: AAAACCCGTTGTCGGACCT	161
Atp-E	F: TTCTGTGGAGCGGTTTTGC R: CGGCTATTTCAAGTGCTGT	117

nylon mesh filter and then suspended in the sucrose and mannitol solution to maintain 5 × 10<sup>6</sup> protoplasts ml<sup>-1</sup>.

Isolated protoplasts were incubated with the pH-sensitive fluorescent dye [2', 7'-bis (carboxyethyl)-5(6)-carboxy, BCECF] at 37 °C for 60 min. Protoplasts were then washed with HEPES-buffered salt solution (pH 7.4) at 37 °C for 15 min to remove extracellular dye and attain complete de-esterification of the cytosolic dye. Radiometric measurement of BCECF fluorescence was conducted using a workstation (Intracellular Imaging Inc., Cincinnati, OH) consisting of a Nikon TSE 100 Ellipse inverted microscope with epifluorescence attachments. The wavelength of excited light was 500 nm, and the wavelength of emitted light was 530 nm. The fluorescence signal was adjusted to zero background for cells without dying, and then the intracellular H<sup>+</sup> level was expressed by the relative BCECF fluorescence (Undem et al. 2012).

### Statistical analysis

The data were analyzed by SPSS 17.0 and Origin 8. Student's *t* tests were used to determine the significance between different treatments (*p* < 0.05).

## Results

### Effects of simulated acid rain on rice growth and P<sub>n</sub> in rice leaves

Table 2 shows the effects of simulated acid rain on the growth and photosynthesis in rice seedlings. After treatment with simulated acid rain at pH 4.5, the dry weights of leaves, dry weights of aboveground organs, leaf areas, relative growth rates, and P<sub>n</sub> were higher than those in the control (pH 7.0).

**Table 2** Effects of simulated acid rain on the net photosynthetic rate ( $P_n$ ) and some growth indices in rice seedlings

pH	Dry weights of leaves (g)	Dry weights of leaves and stems (g)	Leaf area (cm <sup>2</sup> )	Relative growth rate (RGR)	Net photosynthetic rate ( $P_n$ )
7.0	0.159 ± 0.003b (100.00)	0.310 ± 0.009b (100.00)	23.219 ± 0.697b (100.00)	0.092 ± 0.002a (100.00)	13.37 ± 0.76ab (100.00)
4.5	0.171 ± 0.003a (107.55)	0.346 ± 0.121a (111.61)	26.015 ± 1.041a (112.04)	0.094 ± 0.002a (102.17)	14.03 ± 0.83a (104.99)
4.0	0.137 ± 0.003c (86.16)	0.268 ± 0.005c (86.45)	22.822 ± 0.685bc (98.29)	0.081 ± 0.002b (88.04)	12.83 ± 0.32b (96.01)
3.5	0.126 ± 0.003d (79.25)	0.241 ± 0.005d (77.74)	22.451 ± 0.263bc (96.69)	0.069 ± 0.001c (75.00)	12.47 ± 0.35b (93.27)
3.0	0.106 ± 0.002e (66.67)	0.203 ± 0.004e (65.48)	22.025 ± 0.551c (94.86)	0.057 ± 0.001d (61.96)	11.17 ± 0.35c (83.54)
2.5	0.102 ± 0.002e (64.15)	0.183 ± 0.003f (59.03)	17.704 ± 0.407d (76.25)	0.055 ± 0.001d (59.78)	10.23 ± 0.35c (76.56)

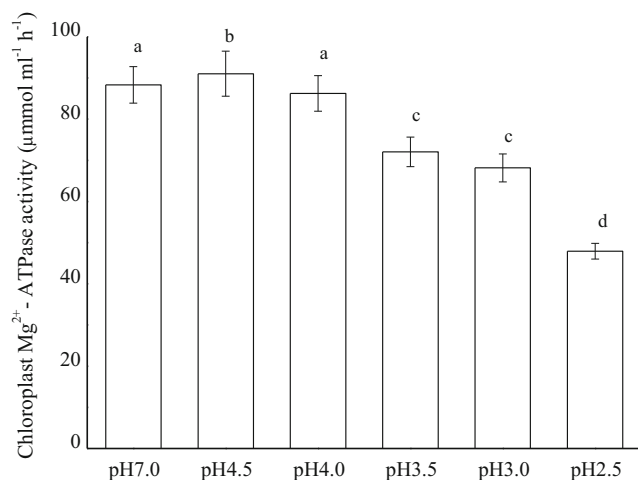
Values are means ± standard deviation errors,  $n = 6$

Significant differences at  $p < 0.05$  were showed with different letters in each column

The above indices in other treatment groups decreased, and the magnitude of this decrease increased with decreasing pH of the simulated acid rain.

### Effects of simulated acid rain on the activity and transcription level of chloroplast ATPase in rice leaves

Figure 1 shows that the chloroplast  $Mg^{2+}$ -ATPase activity in rice seedlings treated with simulated acid rain at pH 4.5 and pH 4.0 did not differ from that of the control. After rice seedlings were treated with simulated acid rain at pH 3.5, pH 3.0, or pH 2.5, the chloroplast  $Mg^{2+}$ -ATPase activity was lower than that of the control, and greater decreases were observed with the pH of the simulated acid rain decreased. In addition, the chloroplast  $Ca^{2+}$ -ATPase activity in all treatment groups treated with simulated acid rain decreased relative to the control. In addition, the inhibition effect became clearer as the pH of the simulated acid rain decreased (Fig. 2). Compared with the  $Mg^{2+}$ -ATPase activity, the chloroplast  $Ca^{2+}$ -ATPase activity was more sensitive to simulated acid rain (Fig. 2).

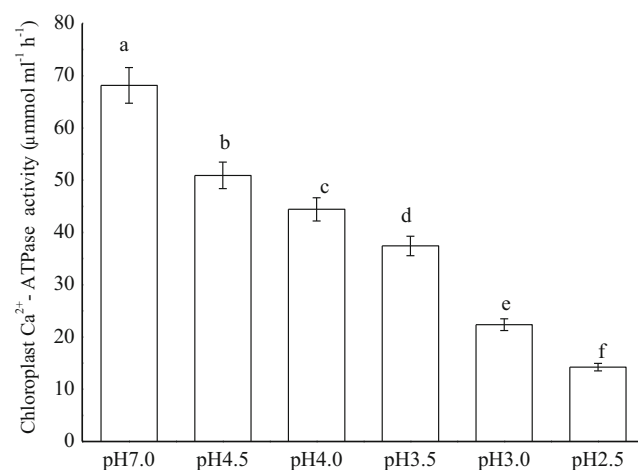


**Fig. 1** Effects of simulated acid rain on chloroplast  $Mg^{2+}$ -ATPase activity in rice seedlings. Significant differences at  $p < 0.05$  are shown with different letters,  $n = 6$

Table 3 shows that, compared with the control, the expression level of all subunits of chloroplast ATPase initially increased and then decreased as the pH of simulated acid rain decreased. The expression level of each subunit of chloroplast ATPase in plants treated with simulated acid rain decreased in the following order: pH 4.5:  $\beta$  subunit (*atp-B*) > IV subunit (*atp-I*) >  $\epsilon$  subunit (*atp-E*) >  $\alpha$  subunit (*atp-A*) > III subunit (*atp-H*) > I subunit (*atp-F*). pH 4.0: IV subunit (*atp-I*) > I subunit (*atp-F*) >  $\alpha$  subunit (*atp-A*) > III subunit (*atp-H*) >  $\epsilon$  subunit (*atp-E*) >  $\beta$  subunit (*atp-B*). pH 3.5:  $\beta$  subunit (*atp-B*) >  $\epsilon$  subunit (*atp-E*) > IV subunit (*atp-I*) > I subunit (*atp-F*) >  $\alpha$  subunit (*atp-A*) > III subunit (*atp-H*). pH 3.0:  $\epsilon$  subunit (*atp-E*) > III subunit (*atp-H*) >  $\beta$  subunit (*atp-B*) > I subunit (*atp-F*) >  $\alpha$  subunit (*atp-A*) > IV subunit (*atp-I*). pH 2.5: IV subunit (*atp-I*) > III subunit (*atp-H*) > I subunit (*atp-F*) >  $\epsilon$  subunit (*atp-E*) >  $\alpha$  subunit (*atp-A*) >  $\beta$  subunit (*atp-B*).

### Effects of simulated acid rain on chloroplast ultrastructure in rice leaves

Figure 3 shows that chloroplasts in the control were elliptical and arranged around the plasma membrane. The granum and



**Fig. 2** Effects of simulated acid rain on chloroplast  $Ca^{2+}$ -ATPase activity in rice seedlings. Significant differences at  $p < 0.05$  are shown with different letters,  $n = 6$

**Table 3** Effects of simulated acid rain on the expression level of chloroplast ATP synthase subunits in rice seedlings

pH	<i>atp-A</i>	<i>atp-B</i>	<i>atp-E</i>	<i>atp-F</i>	<i>atp-I</i>	<i>atp-H</i>
7.0	1.00 ± 0.00b (100.00)	1.00 ± 0.00cd (100.00)	1.00 ± 0.00bc (100.00)	1.00 ± 0.00cd (100.00)	1.00 ± 0.00cd (100.00)	1.00 ± 0.00c (100.00)
4.5	4.20 ± 1.74a (420.00)	6.84 ± 0.82a (684.00)	4.24 ± 1.13a (424.00)	2.98 ± 0.75a (298.00)	4.63 ± 0.45a (463.00)	3.60 ± 0.82a (360.00)
4.0	1.81 ± 0.44b (181.00)	1.45 ± 0.49c (145.00)	1.52 ± 0.61bc (152.00)	1.96 ± 0.44b (196.00)	2.01 ± 0.72b (201.00)	1.81 ± 0.59b (181.00)
3.5	1.28 ± 0.34b (128.00)	2.50 ± 0.37b (250.00)	1.89 ± 0.35b (189.00)	1.28 ± 0.34bc (128.00)	1.35 ± 0.32bc (135.00)	1.19 ± 0.24bc (119.00)
3.0	0.61 ± 0.17b (61.00)	0.68 ± 0.08d (68.00)	1.22 ± 0.27bc (122.00)	0.63 ± 0.10cd (63.00)	0.59 ± 0.11d (59.00)	0.71 ± 0.10c (71.00)
2.5	0.51 ± 0.21b (51.00)	0.50 ± 0.18d (50.00)	0.52 ± 0.23c (52.00)	0.54 ± 0.27d (54.00)	0.57 ± 0.25d (57.00)	0.56 ± 0.28c (56.00)

Values are means ± standard deviation errors, *n* = 6

Significant differences at *p* < 0.05 were showed with different letters in each column

stroma thylakoids were in an orderly arrangement and the chloroplast membrane was clear and intact. In simulated acid rain at pH 4.5 treatment group, the chloroplast structure remained unchanged. When the pH value of simulated acid rain decreased to 3.5, the thylakoids appeared slightly loose but were arranged in an orderly fashion, and the grana were clearly visible. Some thylakoids disintegrated when the rice was treated with simulated acid rain at pH 2.5.

**Effects of simulated acid rain on the intracellular H<sup>+</sup> level and water content in rice leaves**

The intracellular H<sup>+</sup> fluorescence value was lower, which suggests that the intracellular concentration of H<sup>+</sup> was higher (Liang et al. 2015; Zhang et al. 2015). Figure 4 shows the water contents of rice leaves treated with simulated acid rain at different pH value. After treatment with simulated acid rain at pH 4.5, pH 4.0, pH 3.5, and pH 3.0, the leaf water content did not change relative to the control. When the pH value of simulated acid rain decreased to 2.5, the leaf water content was significantly lower than the control.

Figure 5 shows the effect of simulated acid rain on the intercellular H<sup>+</sup> level of rice leaves. The intracellular H<sup>+</sup> fluorescence value in all treatment groups treated with simulated acid rain decreased relative to the control, and the effect became more clear as the pH decreased.

**Correlation analysis**

Correlation analyses were performed among the different measured growth indices, *P<sub>n</sub>*, Mg<sup>2+</sup>-ATPase activity, Ca<sup>2+</sup>-ATPase activity, intracellular H<sup>+</sup> level, and water content, to determine how simulated acid rain affects the *P<sub>n</sub>* and how the change in the *P<sub>n</sub>* affects rice seedling growth. Table 4 shows that the growth indices were positively correlated with the *P<sub>n</sub>*

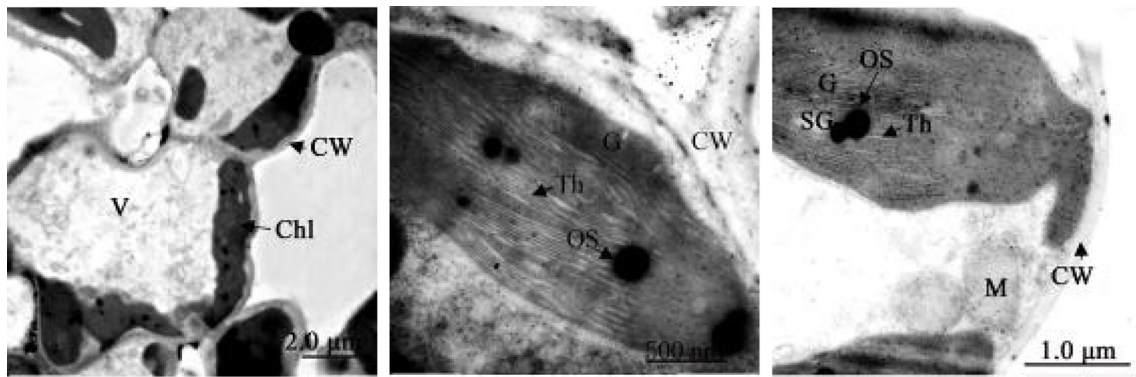
in rice seedlings. In addition, the *P<sub>n</sub>* was positively correlated with chloroplast Mg<sup>2+</sup>-ATPase activity and Ca<sup>2+</sup>-ATPase. The chloroplast Mg<sup>2+</sup>-ATPase and Ca<sup>2+</sup>-ATPase activities were positively correlated with water content and intracellular H<sup>+</sup> level in rice seedlings.

**Discussion**

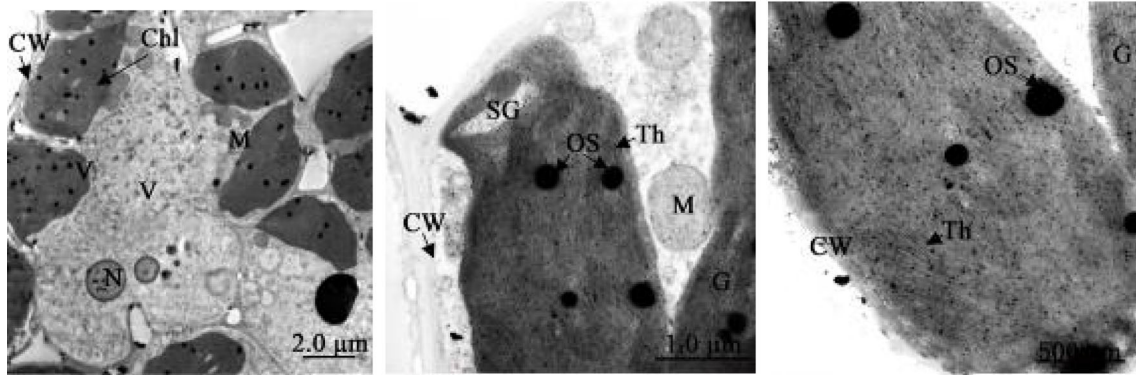
**Effects of simulated acid rain on rice growth and *P<sub>n</sub>* in rice leaves**

Acid rain can affect plant physiological functions directly or indirectly and has a particularly strong impact on photosynthesis (Liu et al. 2015). Under the stress of simulated acid rain, the biomass and the relative growth rate of rice first increased and then decreased as the pH value decreased (Table 2), and this finding was consistent with previous studies (Hu et al. 2014; Wang et al. 2014). The correlation results show that the effects of simulated acid rain on rice growth indices were closely associated with the changes in *P<sub>n</sub>* (Table 2, Table 4). Low-acidity simulated acid rain improved the *P<sub>n</sub>* of leaves and increased the accumulation of assimilation products, laying the material foundations for rice growth (Table 2, Table 4) (Meng et al. 2011). High-acidity simulated acid rain decreased the *P<sub>n</sub>* of leaves and reduced the accumulation of assimilation products, impeding rice growth (Table 2, Table 4). In addition, since plant growth is affected by photosynthesis and respiration, we speculate that the effects of weak acid rain on rice growth also occur for respiration processes, and their internal connections will be further investigated (Zhao 2014).

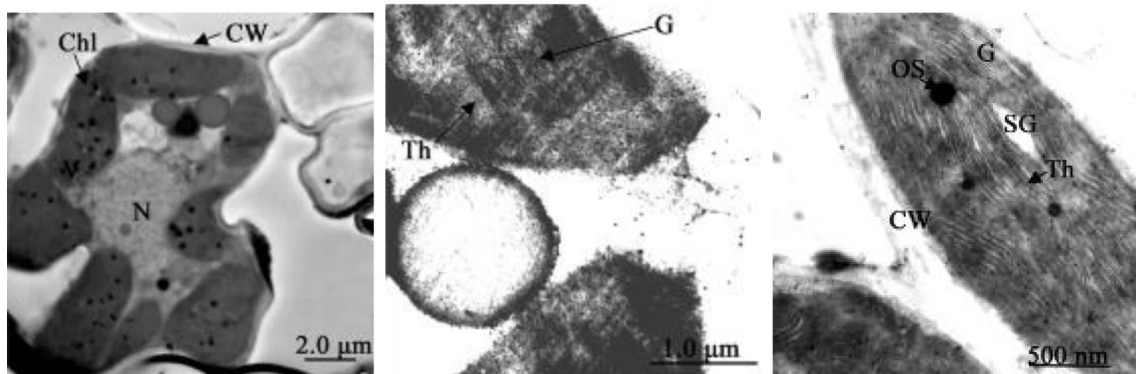
**Fig. 3** TEM images of chloroplast ultrastructure in rice seedlings treated with different pH values of acid rain. *Th* thylakoid, *OS* osmiophilic particles, *G* grana, *SG* starch grains, *CW* cell wall, *V* vacuole, *M* mitochondria, *N* nucleus, *Chl* chloroplast



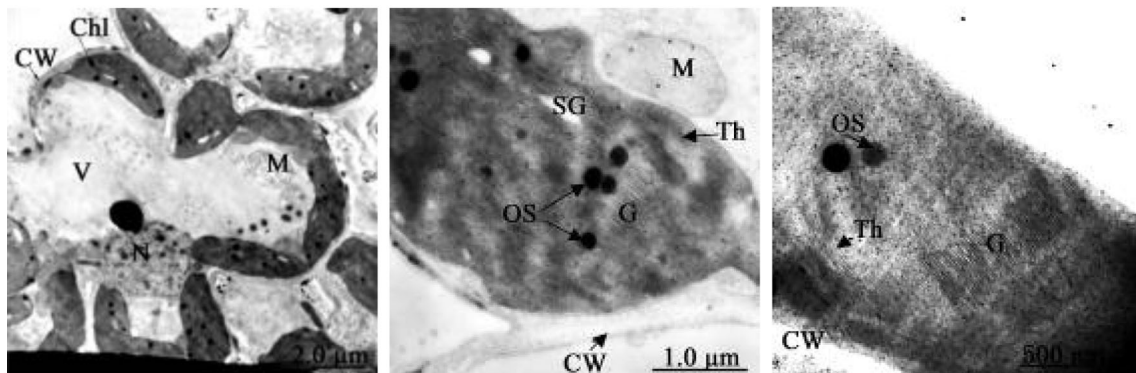
Control rain (pH 7.0)



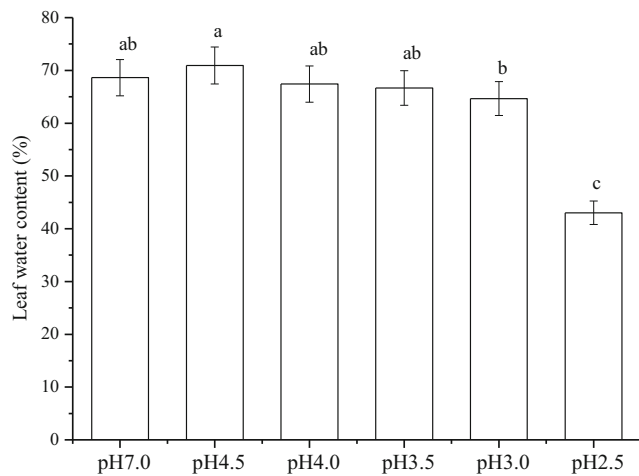
Simulated acid rain at pH 4.5



Simulated acid rain at pH 3.5



Simulated acid rain at pH 2.5



**Fig. 4** Effects of simulated acid rain on leaf water content in rice seedlings. Significant differences at  $p < 0.05$  are shown with different letters,  $n = 6$

Previous research shows that acid rain can influence the chlorophyll content, chlorophyll fluorescence, and Hill reaction activity of plants, which in turn affects the absorption of light by leaves, leading to a change of the  $P_n$  (Hu et al. 2014; Liu et al. 2015; Wang et al. 2014). In addition to the above factors, the change of the  $P_n$  might be related to the activity of chloroplast ATPase which determines the formation of photosynthesis assimilation (ATP).

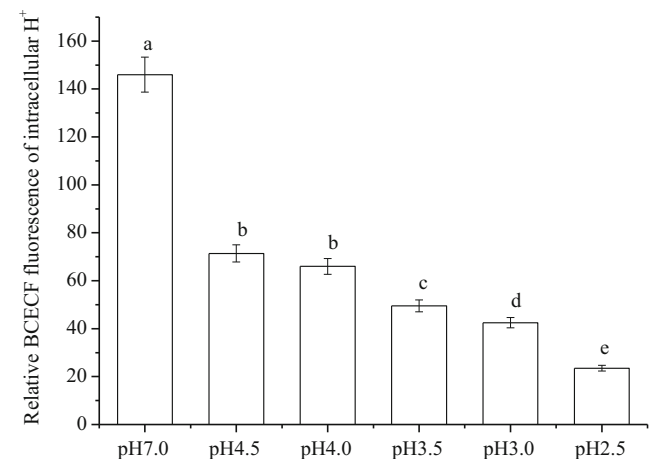
**Effects of simulated acid rain on the activity and transcription level of chloroplast ATPase in rice leaves**

Chloroplast ATP synthase ( $CF_1$ – $CF_0$ ) binds to the surface of thylakoid membranes in chloroplasts. It can combine with  $Mg^{2+}$  or  $Ca^{2+}$  to form  $Mg^{2+}$ -ATPase and  $Ca^{2+}$ -ATPase, catalyzing the ATP synthesis reaction (Kartashov et al. 2015; Kong et al. 2013; Konno et al. 2012; Walker 2013). The  $\epsilon$  subunit is an inhibitor of chloroplast ATP synthase and was dissociated from ATP synthase and product the activity to hydrolysis ATP, leading to a drop in plant endogenous ATP content (del Riego et al. 2006; Feniouk et al. 2006; Schmidt et al. 2007; Shirakihara et al. 2015). Low-acidity-simulated acid rain increased the expression levels of each subunit of chloroplast ATPase; in particular, the increase of  $\beta$  subunit expression level was clearer than that of the  $\epsilon$  subunit (Table 3). This effect increased  $Mg^{2+}$ -ATPase activity, ATP content, and RuBPase supply, promoting photosynthesis and increasing the  $P_n$  (Tezara et al. 1999). With the increase in the acidity of simulated acid rain, the increase in the expression level of the  $\epsilon$  subunit was clearer than that of the  $\beta$  subunit, and the inhibition of  $\epsilon$  subunit caused the observed decrease in the ATP synthase activity (Table 3, Figs. 1 and 2) (Feniouk et al. 2006; Shirakihara et al. 2015). High-acidity simulated acid rain decreased the expression levels and activity of

chloroplast ATPase (Table 3). This may have two main causes: first, the inhibition of  $\epsilon$  subunit (Feniouk et al. 2006; Shirakihara et al. 2015); and second, the expression levels of the I and IV subunits decreased, and these subunits play key assembly roles in chloroplast ATPase, leading to abnormal assembly of chloroplast ATPase in plants. This abnormal assembly caused the decrease in the synthesis amount of chloroplast ATPase, ATP content, and RuBPase supply, inhibiting photosynthesis and decreasing the  $P_n$  (Table 2) (Ghulam et al. 2012; Tezara et al. 1999). In addition, the change in acidity of the intracellular environment induced by acid rain stress also causes a change in chloroplast ATPase activity.

**Effects of simulated acid rain on chloroplast ultrastructure in rice leaves**

The integrity and ordering of grana lamellae and stroma lamellae represent the structural foundation for normal photosynthesis in chloroplast (Zhang et al. 2014). After treatment with low-acidity simulated acid rain, chloroplast lamellae were densely arranged and there were no obvious changes to chloroplast structure (Fig. 3). However, chloroplast ATPase activity and expression levels and  $P_n$  increased to different degrees (Tables 2 and 3, Figs. 1 and 2). Results indicated that low-acidity simulated acid rain did not change the chloroplast structure but did affect photosynthesis function (Dias et al. 2010; Zhang et al. 2014). High-acidity-simulated acid rain damaged the chloroplast ultrastructure, decreased the quantities of grana and thylakoids (Fig. 3), disorganized grana lamellae, restricted chloroplast ATPase assembly, and reduced the synthesis of chloroplast ATPase. It is also possible that acid rain caused the excessive accumulation of reactive



**Fig. 5** Effects of simulated acid rain on intracellular  $H^+$  level labeled by BCECF fluorescence in rice seedlings. Significant differences at  $p < 0.05$  are shown with different letters,  $n = 6$



**Table 4** Relationships between growth indices,  $P_n$ ,  $Mg^{2+}$ -ATPase activity,  $Ca^{2+}$  ATPase activity, leaf water content, and intracellular  $H^+$  level in rice leaves

x	y	Linear regression equation	Correlation coefficient (r)
$P_n$	Dry weights of leaves	$y = 0.0161x - 0.0654$	0.861**
	Dry weights of leaves and stems	$y = 0.037x - 0.1981$	0.880**
	Leaf area	$y = 1.1454x + 4.4136$	0.790**
	RGR	$y = 0.0101x + 0.0499$	0.882**
$Ca^{2+}$ -ATPase activity	Dry weights of leaves	$y = 0.0013x + 0.0833$	0.892**
	Dry weights of leaves and stems	$y = 0.0028x + 0.1462$	0.888**
	Leaf area	$y = 0.1019x + 18.338$	0.728**
	RGR	$y = 0.0008x + 0.0421$	0.944**
	$P_n$	$y = 0.0621x + 9.8926$	0.817**
$Mg^{2+}$ -ATPase activity	Dry weights of leaves	$y = 0.0014x + 0.0271$	0.846**
	Dry weights of leaves and stems	$y = 0.0033x + 0.0052$	0.897**
	Leaf area	$y = 0.1479x + 11.188$	0.903**
	RGR	$y = 0.0009x + 0.0051$	0.905**
	$P_n$	$y = 0.076x + 6.6037$	0.855**
Intracellular $H^+$ level	$Mg^{2+}$ -ATPase activity	$y = 0.2768x + 57.224$	0.704**
	$Ca^{2+}$ -ATPase activity	$y = 0.4273x + 11.173$	0.929**
Leaf water content	$Mg^{2+}$ -ATPase activity	$y = 140.64x - 13.708$	0.892**
	$Ca^{2+}$ -ATPase activity	$y = 135.16x - 46.278$	0.733**

\*\*Significance at  $p < 0.01$

oxygen species (ROS) and induced the peroxidation of chloroplast membrane lipids, leading to the damage to chloroplast ultrastructure (Sena and Chandel 2012; Wen et al. 2011).

#### Effects of simulated acid rain on the intracellular $H^+$ level and water content in rice leaves

We next consider how simulated acid rain led to the ROS accumulation in mesophyll cells. We found that low-acidity simulated acid rain caused a small number of  $H^+$  ions to enter cells, causing slight acidification of the intracellular environment but without significant change in water content (Figs. 4 and 5). Results indicated that low-acidity-simulated acid rain did not affect the metabolic balance of the ROS and did not damage the plasma membrane or chloroplast structure (Chen et al. 2013). A small quantity of  $H^+$  entered mesophyll cells, which could increase the expression of the III subunit gene of chloroplast ATPase, which has the ability to transfer  $H^+$  (Table 3), and increase the  $H^+$  transfer function of chloroplast ATP synthase thus improving chloroplast  $Mg^{2+}$ -ATPase activity and  $P_n$  (Kish Trier et al. 2008). Interestingly, low-acidity-simulated acid rain decreased chloroplast  $Ca^{2+}$ -ATPase activity (Fig. 2). We speculate that the increase in intracellular  $H^+$  level caused the increase in intracellular  $Ca^{2+}$ , and the increased  $Ca^{2+}$  significantly inhibits  $Ca^{2+}$ -ATPase activity (Zhu et al. 2013). High-acidity simulated acid rain caused a large number of  $H^+$  to enter the cells, leading to serious acidification and loss of intracellular water (Figs. 4 and 5). These effects disrupted metabolism of ROS and increased the ROS accumulation in cell, leading to the observed change in plasma membrane permeability and the damage to chloroplast structure (Fig. 3) (Sena and Chandel 2012; Wen et al. 2011). The

gene expressions of all chloroplast ATP synthase subunits were significantly decreased (Table 3), indicating that when considerable  $H^+$  entered the cell, there was substantial inhibition of the activities of  $Ca^{2+}$ -ATPase and  $Mg^{2+}$ -ATPase and a decrease in the  $P_n$  (Table 4).

#### Conclusion

Low-acidity-simulated acid rain slightly increased the acidity within the plant leaf cells but did not affect chloroplast ultrastructure, increased the contents of  $\beta$  subunit that play a catalytic role, and increased chloroplast  $Mg^{2+}$ -ATPase activity. High-acidity simulated acid rain significantly increased the acidity in the cells, destroyed chloroplast ultrastructure, and increased the inhibition effect of the  $\epsilon$  subunit, causing the abnormal assembly of chloroplast ATPase and decreasing  $Mg^{2+}$ -ATPase activity. Moreover, unlike chloroplast  $Mg^{2+}$ -ATPase activity, acid rain significantly inhibited chloroplast  $Ca^{2+}$ -ATPase activity. In addition, acid rain affected the chloroplast ultrastructure, chloroplast ATPase transcription and activity, and  $P_n$  by changing the acidity in the cells and thus influencing the plant growth and development. Finally, the effects of simulated acid rain on the test indices were found to be dose-dependent.

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## References

- Abbasi T, Poomima P, Kannadasan T, Abbasi SA (2013) Acid rain: past, present, and future. *Int J Environ Eng* 97:229–272
- Bernacchi CJ, Bagley JE, Serbin SP, Ruiz Vera UM, Rosenthal DM, Vanloocke A (2013) Modelling C<sub>3</sub> photosynthesis from the chloroplast to the ecosystem. *Plant Cell Environ* 36:1641–1657
- Cao CX, Zhou Q, Han LL, Zhang P, Jiang HD (2010) Effects of simulated acid rain on oilseed rape (*Brassica napus*) physiological characteristics at flowering stage and yield. *J Appl Ecol* 21:2057–2062
- Chen J, Wang WH, Liu TW, Wu FH, Zheng HL (2013) Photosynthetic and antioxidant responses of liquidambar formosana and schima superba seedlings to sulfuric-rich and nitric-rich simulated acid rain. *Plant Physiol Biochem* 64:41–51
- Del Riego G, Casano LM, Martin M, Sabater B (2006) Multiple phosphorylation sites in the beta subunit of thylakoid ATP synthase. *Photosynth Res* 89:11–18
- Department EoCE (2015) China's environmental bulletin in 2014.37:1
- Dias BB, Leite ML, Farago PV, de Oliveira AV, Beruski GC (2010) Sulfur effect by simulated acid rain on morphophysiological parameters of the bean plant. *Acta Sci Agron* 32:433–439
- Feniouk BA, Suzuki T, Yoshida M (2006) The role of subunit epsilon in the catalysis and regulation of F<sub>0</sub>F<sub>1</sub>-ATP synthase. *Biochim Biophys Acta Bioenerg* 1757:326–338
- Ghulam MM, Zghidi Abouzid O, Lambert E, Lerbs-Mache S, Merendino L (2012) Transcriptional organization of the large and the small ATP synthase operons, atpL/H/F/a and atpB/E, in *Arabidopsis thaliana* chloroplasts. *Plant Mol Biol Report* 79:259–272
- Gravois KA (1998) Optimizing selection for rough rice yield, head rice, and total milled rice. *Euphytica* 101:151–156
- Helliot B, Swennen R, Poumay Y, Frison E, Lepoivre P, Panis B (2003) Ultrastructural changes associated with cryopreservation of banana (*Musa* spp.) highly proliferating meristems. *Plant Cell Rep* 21:690–698
- Hu H, Wang L, Liao C, Fan C, Zhou Q, Huang X (2014) Combined effects of lead and acid rain on photosynthesis in soybean seedlings. *Biol Trace Elem Res* 161:136–142
- Imran MA, Hussain S, Hussain M, Ch MN, Meo AA (2014) Effect of simulated acid rain (SAR) on some morphochemical aspects of mash (*Vigna mungo* L.). *Pak J Bot* 46:245–250
- Kartashov IM, Opanasenko VK, Malyan AN (2015) Effects of medium viscosity increasing agents on ATP synthesis in chloroplast thylakoids. *Biofizika* 60:481–486
- Kingsbury RW, Epstein E, Percy RW (1984) Physiological responses to salinity in selected lines of wheat. *Plant Physiol* 74:417–423
- Kish Trier E, Briere L, Dunn SD, Wilkens S (2008) The stator complex of the a<sub>(1)</sub>a<sub>(0)</sub>-ATP synthase-structural characterization of the E and H subunits. *J Mol Biol* 375:673–685
- Kong R, Zheng X, Huang W, Zhou L, Zhang G (2012) Ion composition and its sources of rainwater from shanghai urban and suburban areas. *Urban Environ Urban Ecol* 25:22–27
- Kong M, Wang F, Yang Z, Mi H (2013) ATPG is required for the accumulation and function of chloroplast ATP synthase in *Arabidopsis*. *Chin Sci Bull* 58:3224–3232
- Konno H, Nakane T, Yoshida M, Ueoka NH, Hara S, Hisabori T (2012) Thiol modulation of the chloroplast ATP synthase is dependent on the energization of thylakoid membranes. *Plant Cell Physiol* 53:626–634
- Lajtha K, Jones J (2013) Trends in cation, nitrogen, sulfate and hydrogen ion concentrations in precipitation in the United States and Europe from 1978 to 2010: a new look at an old problem. *Biogeochemistry* 116:303–334
- Liang CJ, Ge YQ, Su L, Bu JJ (2015) Response of plasma membrane H<sup>+</sup>-ATPase in rice (*Oryza sativa*) seedlings to simulated acid rain. *Environ Sci Pollut Res* 22:535–545
- Liu P, Xia F, Pan J, Chen Y, Peng H, Chen S (2011) Discuss on present situation and countermeasures for acid rain prevention and control in China. *Environ Sci Manag* 21:4–11
- Liu MH, Yi LT, Yu SQ, Yu F, Yin XM (2015) Chlorophyll fluorescence characteristics and the growth response of *Elaeocarpus glabripetalus* to simulated acid rain. *Photosynthetica* 53:23–28
- Macaulay BM, Enahoro GE (2015) Effects of simulated acid rain on the morphology, phenology and dry biomass of a local variety of maize (Suwan-1) in southwestern Nigeria. *Environ Monit Assess* 187:1–13
- Meng H, Dong DM, Wang J, Yang KN, Tian L, Sun W, Fang CS (2011) Effects of simulated acid rain on main nutritional indicators of three leafy vegetables. *Chem Res Chin Univ* 27:397–401
- Menz FC, Seip HM (2004) Acid rain in Europe and the United States: an update. *Environ Sci Pol* 7:253–265
- Ramlall C, Varghese B, Ramdhani S, Pammenter NW, Bhatt A, Berjak P, Sershen (2015) Effects of simulated acid rain on germination, seedling growth and oxidative metabolism of recalcitrant-seeded *Trichilia dregeana* grown in its natural seed bank. *Physiol Plant* 153:149–160
- Schmidt M, Luff M, Mollwo A, Kaminski M, Mittag M, Kreimer G (2007) Evidence for a specialized localization of the chloroplast ATP-synthase subunits alpha, beta, and gamma in the eyespot apparatus of *Chlamydomonas reinhardtii* (Chlorophyceae). *J Phycol* 43: 284–294
- Sena L, Chandel N (2012) Physiological roles of mitochondrial reactive oxygen species. *Mol Cell* 48:158–167
- Shirakihara Y, Shiratori A, Tanikawa H, Nakasako M, Yoshida M, Suzuki T (2015) Structure of a thermophilic F-1-ATPase inhibited by an epsilon-subunit: deeper insight into the epsilon-inhibition mechanism. *FEBS J* 282:2895–2913
- Sun Z, Wang L, Chen M, Wang L, Liang C, Zhou Q, Huang X (2012) Interactive effects of cadmium and acid rain on photosynthetic light reaction in soybean seedlings. *Ecotoxicol Environ Saf* 79:62–68
- Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW (1999) Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* 401:914–917
- Udem C, Rios EJ, Maylor J, Shimoda LA (2012) Endothelin-1 augments Na<sup>+</sup>/H<sup>+</sup> exchange activity in murine pulmonary arterial smooth muscle cells via rho kinase. *PLoS One* 7:1602–1603
- Walker JE (2013) The ATP synthase: the understood, the uncertain and the unknown. *Biochem Soc Trans* 41:1–16
- Wang LH, Wang W, Zhou Q, Huang XH (2014) Combined effects of lanthanum (III) chloride and acid rain on photosynthetic parameters in rice. *Chemosphere* 112:355–361
- Wen KJ, Liang CJ, Wang LH, Hu G, Zhou Q (2011) Combined effects of lanthanum and acid rain on growth, photosynthesis and chloroplast ultrastructure in soybean seedlings. *Chemosphere* 84:601–608
- Wyrwicka A, Skłodowska M (2006) Influence of repeated acid rain treatment on antioxidative enzyme activities and on lipid peroxidation in cucumber leaves. *Environ Exp Bot* 56:198–204
- Xie Z, Du Y, Zeng Y, Li Y, Yan M, Jiao S (2009) Effects of precipitation variation on severe acid rain in southern China. *J Geogr Sci* 19:489–501
- Zhang H, Chen TT, Liu LJ, Wang ZQ, Yang JC, Zhang JH (2013) Performance in grain yield and physiological traits of rice in the Yangtze river basin of China during the last 60 yr. *Integr Agric* 12:57–66
- Zhang J, Jiang XD, Li TL, Cao XJ (2014) Photosynthesis and ultrastructure of photosynthetic apparatus in tomato leaves under elevated temperature. *Photosynthetica* 52:430–436

- Zhang YK, Zhu DF, Zhang YP, Chen HZ, Xiang J, Lin XQ (2015) Low pH-induced changes of antioxidant enzyme and ATPase activities in the roots of rice (*Oryza sativa* L.) seedlings. PLoS One 10:2–6
- Zhao Z (2014) Effects of simulated acid rain on photosynthetic physiology characteristics of *Ipomoea cairica* seedling. Ecotoxicol Environ Sci 19:13–21
- Zhao Y, Hou Q (2010) Characteristics of the acid rain variation in China during 1993–2006 and associated causes. Acta Meteorol Sin 24:239–250
- Zhu Y, Di T, Xu G, Chen X, Zeng H, Yan F, Shen Q (2009) Adaptation of plasma membrane H<sup>+</sup>-ATPase of rice roots to low pH as related to ammonium nutrition. Plant Cell Environ 32:1428–1440
- Zhu X, Feng Y, Liang G, Liu N, Zhu JK (2013) Aequorin-based luminescence imaging reveals stimulus and tissue-specific Ca<sup>2+</sup> dynamics in Arabidopsis plants. Mol Plant 6:444–455