

# Root Morphology and Growth Regulated by Mineral Nutrient Absorption in Rice Roots Exposed to Simulated Acid Rain

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**Abstract** Acid rain caused a severe loss on agricultural productivity, aggravating the challenge for achieving sustainable food production to feed the increasing globe population. To clarify the mechanism on adaptation of rice root to acid rain, we studied the root morphology and growth regulated by nutrient absorption under hydroponic conditions. Our results show that acid rain (pH 5.0 or 3.5) increased the density of root hair and root volume by increasing concentrations of  $K^+$ ,  $Na^+$ , and  $Ca^{2+}$  in rice roots, and the root dry weight was increased. However, strong acid rain (pH 2.5) decreased the root length, surface area, volume, and number of root tips by decreasing the concentrations of  $K^+$ ,  $Na^+$ , and  $Mg^{2+}$  in rice root, and fresh and dry weight were both decreased. After a 5-day recovery, the root morphology of rice seedlings treated with acid rain (pH 5.0 or 3.5) was recovered to the control levels, and the concentrations of  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  also had no difference from the control ( $p < 0.05$ ). However, the root growth treated with strong acid rain (pH 2.5) was still lower than the control because the inhibition on root

activity and hydrolytic activity of plasma membrane  $H^+$ -ATPase might have exceeded the self-regulating capacity of rice seedlings, and the absorption of mineral nutrient could not sustain the growth. Hence, we concluded that the adaption of root morphology of rice seedlings to acid rain was related to regulation of mineral nutrient absorption in rice root.

**Keywords** Acid rain · Rice · Root morphology · Mineral nutrient · Plasma membrane  $H^+$ -ATPase · Root activity

## 1 Introduction

Acid rain, as worldwide environmental pollute, causes a decrease in agricultural and forest production (Larssen et al. 2006; Meena 2013; Singh and Agrawal 2008). Previous studies show that acid rain has direct negative effects such as inhibiting plant growth, reducing photosynthetic rate, damaging cell membrane integrity, and destroying ultrastructure of chloroplast (Sun et al. 2011; Wen et al. 2011). Acid rain also can affect directly plant root by changing the pH value and other properties of soil. Root growth and development directly affect the growth of aboveground part by absorbing nutrient and water and synthesizing physiologically substances (Miller 2011). Huang et al. (2000) reported that root growth of soybean was inhibited by acid rain at pH 3.0, and even was stopped by acid rain at pH 2.0 for a 3-day exposure.

Root morphology (root length, surface area, volume and number of root tips) and biomass can be used to

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reflect root growth conditions because they are sensitive to environmental factors. Previous studies found that root morphology of some plant species was changed in response to abiotic stresses (Hallmark and Barber 1984; Halušková et al. 2009; Sui et al. 2007; Tong et al. 2012). For example, water stress causes the increases in the number of lateral root, average root length, and surface area, and a decrease in the diameter of root of maize to make an adaptation (Bo et al. 2008). Srivastav et al. (2009) reported that root length, number of secondary roots, and fresh and dry weight of mango roots are decreased by the increase in salinity levels. Actually, the changes in root morphology are related with concentrations of mineral nutrient in roots (Fan and Yang 2007; Nogueirol et al. 2016; Trubat et al. 2006). For example, Foehse and Jungk (1983) reported that root hair growth is correlated to internal P or N status of the plant. The transmembrane transport of most of nutrient elements ( $K^+$ ,  $Ca^{2+}$ ,  $Na^+$ ,  $Mg^{2+}$ , N, P, etc.) depends on plasma membrane  $H^+$ -ATPase. Plasma membrane  $H^+$ -ATPase can provide an energy source for transport of nutrient into the cell by extruding positive charges ( $H^+$ ) and thus forming a membrane potential (Zeng et al. 2015; Zhang et al. 2011). In addition, root activity is associated with the activity of dehydrogenase involved in regulating sugar (carbohydrate) and mineral absorption in plants (Onanuga et al. 2011). Hence, it should be important to reveal the adaptation mechanism on root morphology to acid rain stress based on mineral nutrient absorption in rice root, and the relevant information is rarely reported. The exposure of plants to acid rain is a discontinuous process where plants are first exposed to acid rain followed by a restoration period (Liang et al. 2015). Therefore, the exposure and recovery periods should be essential to simulate the situation when acid rain real happened.

This study aimed at (1) revealing adaptation of root morphology of rice seedlings under acid rain stress; the effect of root morphology on biomass production of rice root under acid rain stress; (2) clarifying response of  $K^+$ ,  $Ca^{2+}$ ,  $Na^+$ , and  $Mg^{2+}$  contents in rice roots to acid rain stress; the correlation between root morphology and the absorption of  $K^+$ ,  $Ca^{2+}$ ,  $Na^+$ , and  $Mg^{2+}$  under acid rain at different pH; and (3) studying response of root activity and hydrolytic activity of plasma membrane  $H^+$ -ATPase of rice seedlings to acid rain to clarify the relationship between the absorption of  $K^+$ ,  $Ca^{2+}$ ,  $Na^+$ , and  $Mg^{2+}$  and root activity and hydrolytic activity of plasma membrane  $H^+$ -ATPase. These results can be

helpful to know the mechanisms on the adaptation of rice root morphology to acid rain stress and can provide theoretical basis for finding effective ways to alleviate the damage to plants caused by acid rain stress.

## 2 Materials and Methods

### 2.1 Plant Material and Culture Conditions

Seeds of rice “Huaidao 8” (*Oryza sativa*) (Wuxi Seed CO., Ltd., China) were surface disinfected with  $HgCl_2$  (0.1 %, w/v) for 10 min and washed three times with deionized water. After being soaked in distilled water for 12 h, the seeds were placed in a culture dish with three layers of filter paper and germinated in a standing-temperature cultivator at  $25 \pm 1$  °C. Then the germinated rice seeds were cultured in plastic box (6.88 L) filled with vermiculite for 25 days. When the first two leaves appeared, rice seedlings were cultivated in routine nutrition solution [150 mM  $(NH_4)_2SO_4$ , 24 mM  $KH_2PO_4$ , 42 mM  $K_2SO_4$ , 120 mM  $CaCl_2 \cdot H_2O$ , 120 mM  $MgSO_4 \cdot 7H_2O$ , 60 mM  $Na_2SiO_3 \cdot 9H_2O$ , 1.08 mM  $MnCl_2 \cdot 4H_2O$ , 2.4 mM  $H_3BO_3$ , 2.40 mM Fe(III)-EDTA, 46.82  $\mu M$   $Na_2MoO_4 \cdot 2H_2O$ , 92.39  $\mu M$   $ZnSO_4 \cdot 7H_2O$ , and 38.40  $\mu M$   $CuSO_4 \cdot 5H_2O$ , pH 5.5] according to the method provided by Zhu et al. (2009) in a growth chamber with a light intensity of  $300 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  photosynthetically active radiation, temperature of 25 °C/20 °C (14 h/10 h), and relative humidity of 70 %/80 % (day/night).

### 2.2 Simulated Acid Rain Treatment

Simulated acid rain (Saruhan et al.) 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), similar to the ratio of  $SO_4^{2-}/NO_3^-$  in ambient rain in South China (Jing et al. 2010). The solution was adjusted to pH 5.0, 3.5, and 2.5 with deionized water. Simulated acid rain (SAR) was sprayed at 24 h intervals on the leaves of rice seedlings (Liang et al. 2015). At the same time, the nutrient solution was adjusted to pH 5.0, 3.5, and 2.5. All treatments were done in triplicate. After a 5-day SAR treatment, half of the rice seedlings were collected for analysis. The rest rice seedlings were moved to be cultured under the control conditions for another 5-day, and then were collected for analysis.

### 2.3 Determination of Root Biomass

Fresh rice seedlings were collected and washed three times with distilled water, and were cleaned by filter paper. Biomass dry weight was determined after at 60 °C for 24 h (Rusak et al. 2009; Sun et al. 2013). All treatments were done in triplicate.

### 2.4 Scan and Determination of Root Morphology

The rice root was scanned by an automatism scanner (Perfection V700 Photo, Seiko Epson Corp, Japan). The root phenotype traits, including total root length, root surface area, root volume, and root tip number, were analyzed by WinRHIZO 2009a software (Version 2009a, Regent Instruments, Quebec, Canada). Three roots of rice seedlings were analyzed for each treatment group.

### 2.5 Mineral Element Concentrations Determination

The samples were prepared for mineral determination as previously described by Saruhan et al. (2015). Fresh plants were collected and washed three times with distilled water. The roots were dried in an oven and crushed into pieces of 1 mm in diameter. For each sample, 0.1 g crushed roots was then digested in 8 mL oxidizing solution (15 M HNO<sub>3</sub> and 9 M H<sub>2</sub>O<sub>2</sub>, *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. The content of K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> was determined using a flame atomic absorption spectrophotometer (3110, Perkin Elmer, Norwalk, CT, USA). All samples were analyzed in duplicate.

### 2.6 Plasma Membrane Isolation and Hydrolytic Activity of Plasma Membrane H<sup>+</sup>-ATPase

Plasma membrane of rice roots were isolated by two-phase partitioning according to Larsson et al. (1987) and Klobus and Buczek (1995). The plasma membrane obtained by this procedure was used to determine the hydrolytic activity of plasma membrane H<sup>+</sup>-ATPase. The concentration of the protein we extracted was measured according to the method described by Bradford (1976). To determine the purity for plasma membranes we obtained, we analyzed H<sup>+</sup>-ATPase hydrolytic activity of the membrane fraction in the presence and absence

of inhibitors (KNO<sub>3</sub> for tonoplast ATPases, Na<sub>2</sub>MoO<sub>4</sub> for nonspecific phosphatases, NaN<sub>3</sub> for mitochondrial membrane ATPases, and Na<sub>3</sub>VO<sub>4</sub> for plasma membrane ATPases) as described by Wakeel et al. (2010) (data were not shown).

Hydrolytic activity of plasma membrane H<sup>+</sup>-ATPase was determined by measuring the Pi amount after 30 min hydrolysis (Liang et al. 2015; Yan et al. 2002). The hydrolytic activity of plasma membrane was expressed in micromole per milligram per minute.

### 2.7 Determination of Root Activity

Root activity was determined by the triphenyltetrazolium chloride (TTC) reduction method (Sun et al. 2013). The rice roots (0.5 g) of each treatment group were washed with distilled water and placed in a plastic centrifuge tube (50 mL). Then, a 5 mL of 0.1 mM phosphate buffer solution (pH 7.0) and 5 mL of 0.4 % TTC were added to the tube. The reaction was performed at 37 °C for 2 h in the dark. Then, a 2 mL of 1 M H<sub>2</sub>SO<sub>4</sub> was added to the tube to stop the reaction. The root activity was expressed by the amount of triphenylformazan deoxidized by TTC.

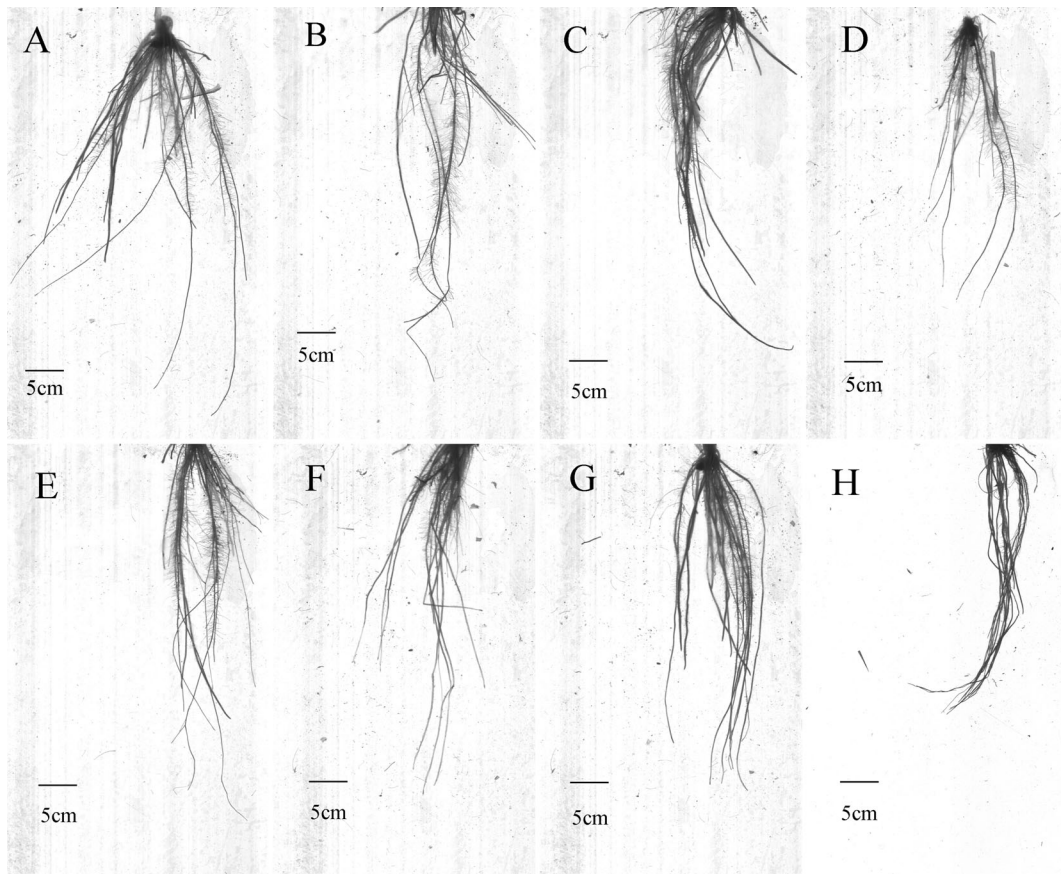
### 2.8 Statistical Analysis

Data were presented as mean ± standard deviation. The difference significance between different treatments was analyzed by the one-way analysis of variance (ANOVA) using SPSS 11.5 (*P* < 0.05).

## 3 Result

### 3.1 Effects of SAR on the Morphology and Growth of Rice Roots

After rice seedlings being treated with SAR at pH 5.0 for 5 days, the density of root hair was increased whereas the density of adventitious root was decreased, compared with those of the control (Fig. 1a, b). In addition, SAR at pH 5.0 increased the volume of rice roots (Table 1) and had no obvious effect on the length, surface area, and number of root tips (*P* < 0.05). After rice seedlings being treated with SAR at pH 3.5 for 5 days, the density of root hair was increased, and the adventitious roots were stronger than those of the control (Fig. 1a, c).



**Fig. 1** The change of morphology in rice roots treated with acid rain. During the stress period: **a** control; **b** pH 5.0 group; **c** pH 3.5 group; **d** pH 2.5 group; during the recovery period: **e** control; **f** pH 5.0 group; **g** pH 3.5 group; **h** pH 2.5 group

**Table 1** Effects of SAR on morphological parameters of rice roots. Data are expressed as mean values  $\pm$  SE from triple. In the same column, values with different lowercase are significantly

different at  $P < 0.05$  during stress period and values with different uppercase are significantly different at  $P < 0.05$  during recovery period

	Acid rain pH	Length (cm stock <sup>-1</sup> )	Surface area (cm <sup>2</sup> stock <sup>-1</sup> )	Volume (cm <sup>3</sup> stock <sup>-1</sup> )	Number of root tips
Stress period	5.5 (control)	155.60 $\pm$ 11.83a (100.0 %)	32.54 $\pm$ 0.98a (100.0 %)	0.528 $\pm$ 0.03c (100.0 %)	889 $\pm$ 5b (100.0 %)
	5.0	153.61 $\pm$ 10.84a (98.7 %)	33.72 $\pm$ 1.48a (103.6 %)	0.589 $\pm$ 0.01b (111.6 %)	962 $\pm$ 60b (108.2 %)
	3.5	131.95 $\pm$ 3.49b (84.8 %)	33.79 $\pm$ 1.13a (103.8 %)	0.692 $\pm$ 0.03a (131.1 %)	1082 $\pm$ 11a (121.7 %)
	2.5	131.30 $\pm$ 8.75b (84.4 %)	28.53 $\pm$ 1.72b (87.7 %)	0.449 $\pm$ 0.02d (85.0 %)	411 $\pm$ 26c (46.2 %)
Recovery period	5.5 (control)	165.92 $\pm$ 4.76A (100.0 %)	34.60 $\pm$ 2.83A (100.0 %)	0.508 $\pm$ 0.02A (100.0 %)	648 $\pm$ 44A (100.0 %)
	5.0	158.28 $\pm$ 5.64A (95.4 %)	31.92 $\pm$ 1.82A (92.3 %)	0.513 $\pm$ 0.05A (101.0 %)	631 $\pm$ 44A (97.4 %)
	3.5	170.87 $\pm$ 9.41A (103.0 %)	35.75 $\pm$ 2.17A (103.3 %)	0.524 $\pm$ 0.05A (103.1 %)	654 $\pm$ 17A (100.9 %)
	2.5	111.37 $\pm$ 3.87B (67.1 %)	24.24 $\pm$ 1.30B (70.1 %)	0.410 $\pm$ 0.04B (80.7 %)	555 $\pm$ 27B (85.7 %)

In addition, SAR at pH 3.5 caused increases in the volume of rice root and number of root tips and a decrease in the root length, and had no obvious effect on the surface area of rice roots ( $P < 0.05$ ) (Table 1). After rice seedlings being treated with SAR at pH 2.5 for 5 days, the density of adventitious roots was decreased compared with that of the control. However, SAR at pH 2.5 did not affect the growth of root hair (Fig. 1a, d). As shown in Table 1, SAR at pH 2.5 decreased the length, surface area, volume, and number of root tips of rice roots compared with those of the control. After a 5-day recovery, the number of adventitious roots of rice seedlings treated with SAR at pH 5.0 was the similar with the control (Fig. 1e, f). At the same treatment, the length, surface area, volume, and number of root tips of rice roots were recovered to the control level (Table 1). For the group treated with SAR at pH 3.5, the density of root hair was lower whereas the adventitious roots were still stronger than those of the control (Fig. 1e, g). In addition, the length, surface area, volume, and number of root tips of rice seedlings treated with SAR at pH 3.5 were recovered to the level of the control (Table 1). For the group treated with SAR at pH 2.5, the density of root hair was lower than that of the control, and even lower than that of the stress period (Fig. 1e, h). In addition, the length, surface area, and volume of rice roots and the number of root tips treated with SAR at pH 2.5 were all still lower than those of the control, and the length, surface area, and volume of rice roots were even lower than those measured during the stress period (Table 1).

**Table 2** Effects of SAR on growth of rice roots. Data are expressed as mean values  $\pm$  SE from triple. In the same column, values with different lowercase are significantly different at

Acid rain pH	Fresh weight (g stock <sup>-1</sup> )		Dry weight (g stock <sup>-1</sup> )	
	Stress	Recovery	Stress	Recovery
5.5 control	0.360 $\pm$ 0.02a (100.0 %)	0.348 $\pm$ 0.01B (100.0 %)	0.054 $\pm$ 0.001b (100.0 %)	0.054 $\pm$ 0.003A (100.0 %)
5.0	0.381 $\pm$ 0.02a (105.8 %)	0.334 $\pm$ 0.01B (96.0 %)	0.057 $\pm$ 0.007b (105.6 %)	0.052 $\pm$ 0.004A (96.3 %)
3.5	0.387 $\pm$ 0.01a (107.5 %)	0.376 $\pm$ 0.01A (108.0 %)	0.060 $\pm$ 0.002a (111.1 %)	0.055 $\pm$ 0.006A (101.9 %)
2.5	0.281 $\pm$ 0.01b (78.1 %)	0.244 $\pm$ 0.02C (70.1 %)	0.045 $\pm$ 0.002c (83.3 %)	0.042 $\pm$ 0.003B (77.8 %)

### 3.2 Effects of SAR on the Biomass of Rice Roots

As shown in Table 2, SAR at pH 5.0 or 3.5 increased dry weight of rice roots compared with that of the control and had no obvious effect on the fresh weight of rice roots ( $P < 0.05$ ). However, SAR at pH 2.5 decreased both fresh and dry weight of rice roots compared with those of the control. After a 5-day recovery, the fresh and dry weight of rice roots treated with SAR at pH 5.0 was recovered to the levels of the control ( $P < 0.05$ ). For the group treated with SAR at pH 3.5, the fresh weight was higher than that of the control whereas the dry weight was recovered to the level of the control ( $P < 0.05$ ). For the group treated with SAR at pH 2.5, both fresh and dry weight of rice roots were lower than those of the control, and even lower than those measured during the stress period.

### 3.3 Effect of SAR on Concentrations of K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> in Rice Roots

Table 3 shows concentrations of mineral elements (K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>) in roots of rice seedlings treated with SAR at pH 5.0, 3.5, and 2.5 during stress and recovery periods. After a 5-day stress treatment, SAR at pH 5.0 increased concentrations of K<sup>+</sup> and Na<sup>+</sup> in rice roots compared with those of the control, and had no obvious effect on concentrations of Ca<sup>2+</sup> and Mg<sup>2+</sup> in rice roots ( $P < 0.05$ ). SAR at pH 3.5 caused an increase in the concentration of Ca<sup>2+</sup> and a decrease in the concentration of K<sup>+</sup> compared with those of the control. However, the

$P < 0.05$  during stress period and values with different uppercase are significantly different at  $P < 0.05$  during recovery period

**Table 3** Effect of SAR on concentrations of mineral elements in rice roots. Data are expressed as mean values  $\pm$  SE from triple. In the same column, values with different lowercase are significantlydifferent at  $P < 0.05$  during stress period and values with different uppercase are significantly different at  $P < 0.05$  during recovery period

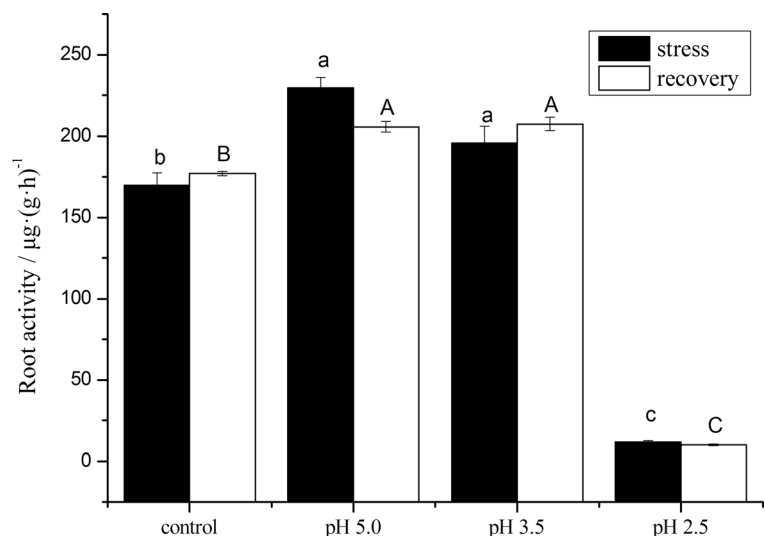
Acid rain pH	K <sup>+</sup> (mg·gDW <sup>-1</sup> )		Na <sup>+</sup> (mg·gDW <sup>-1</sup> )		Ca <sup>2+</sup> (mg·gDW <sup>-1</sup> )		Mg <sup>2+</sup> (mg·gDW <sup>-1</sup> )	
	Stress	Recovery	Stress	Recovery	Stress	Recovery	Stress	Recovery
7.0	45.13 $\pm$ 2.26b (100.0 %)	42.21 $\pm$ 2.31A (100.0 %)	31.34 $\pm$ 1.98b (100.0 %)	34.55 $\pm$ 2.36A (100.0 %)	13.50 $\pm$ 0.67c (100.0 %)	13.53 $\pm$ 1.12B (100.0 %)	1.42 $\pm$ 0.07a (100.0 %)	1.51 $\pm$ 0.04A (100.0 %)
5.0	50.25 $\pm$ 2.49a (111.4 %)	39.14 $\pm$ 2.56A (92.7 %)	39.75 $\pm$ 2.31a (126.8 %)	32.55 $\pm$ 2.19A (94.2 %)	13.63 $\pm$ 0.68c (101.0 %)	13.68 $\pm$ 1.21B (101.1 %)	1.43 $\pm$ 0.07a (100.7 %)	1.42 $\pm$ 0.03A (94.0 %)
3.5	40.25 $\pm$ 2.01c (89.2 %)	41.56 $\pm$ 2.51A (98.5 %)	30.65 $\pm$ 2.15b (97.8 %)	34.50 $\pm$ 2.41A (99.9 %)	16.38 $\pm$ 0.82b (121.3 %)	13.48 $\pm$ 1.14B (99.6 %)	1.49 $\pm$ 0.09a (104.9 %)	1.60 $\pm$ 0.03A (106.0 %)
2.5	23.00 $\pm$ 1.15d (51.0 %)	21.86 $\pm$ 1.96B (51.8 %)	26.11 $\pm$ 1.79c (83.3 %)	24.11 $\pm$ 1.16B (69.8 %)	22.88 $\pm$ 1.14a (169.5 %)	15.65 $\pm$ 1.12A (115.7 %)	0.94 $\pm$ 0.04c (66.2 %)	1.20 $\pm$ 0.02B (79.5 %)

concentrations of Na<sup>+</sup> and Mg<sup>2+</sup> in rice roots treated with SAR at pH 3.5 did not change ( $P < 0.05$ ). SAR at pH 2.5 decreased the concentration of K<sup>+</sup>, Na<sup>+</sup>, and Mg<sup>2+</sup> in rice roots compared with those of the control. However, the concentration of Ca<sup>2+</sup> in rice root being treated with SAR at pH 2.5 was increased by 69.5 % compared with that of the control. After a 5-day recovery, the concentration of K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> in rice roots treated with SAR at pH 5.0 and 3.5 was recovered to the control level ( $P < 0.05$ ). For the group treated with SAR at pH 2.5, concentrations of K<sup>+</sup>, Na<sup>+</sup>, and Mg<sup>2+</sup> in rice roots were lower than those of the control. In addition, the concentration of Na<sup>+</sup> was even lower than that measured during the stress period, but the Mg<sup>2+</sup>

concentration was higher than those measured during the stress period. Contrarily, the concentration of Ca<sup>2+</sup> in rice root treated with SAR at pH 2.5 was still higher than that of the control, but lower than that measured during the stress period.

### 3.4 Effects of SAR on Root Activity in Rice Roots

After a 5-day exposure, SAR at pH 5.0 or 3.5 increased root activity of rice seedlings compared with that of the control, whereas SAR at pH 2.5 decreased root activity of rice seedlings (Fig. 2). After a 5-day recovery, the root activity of rice roots treated with SAR at pH 5.0 or 3.5 was still higher than those of the control. In addition, the root

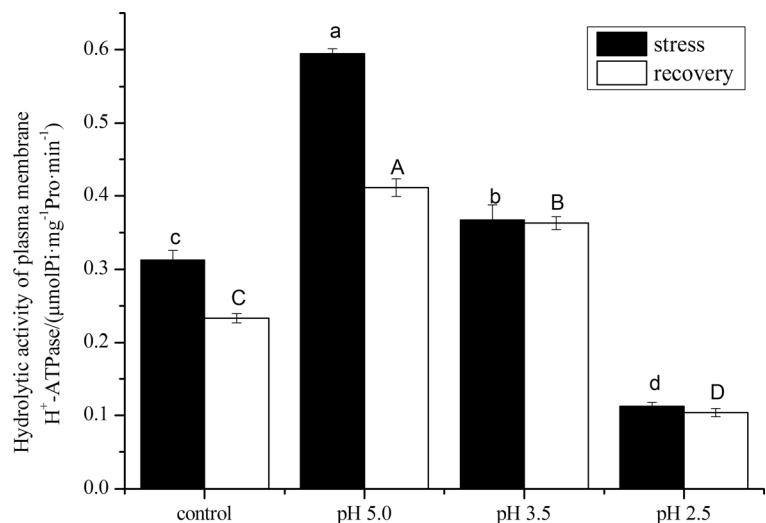
**Fig. 2** Effect of simulated acid rain on root activity in rice roots. Significant difference at  $P < 0.05$  during stress periods was shown with *different lowercase*, and during recovery period was shown with *uppercase*

activity of rice roots treated with SAR at pH 5.0 was lower than that measured during the stress period whereas the root activity of rice seedlings treated with SAR at pH 3.5 was higher. The root activity of rice seedlings treated with SAR at pH 2.5 was still lower than that of the control, and even lower than that measured during the stress period.

### 3.5 Effects of SAR on Hydrolytic Activity of Plasma Membrane H<sup>+</sup>-ATPase in Rice Roots

After rice seedlings being treated with SAR for 5 days, SAR at pH 5.0 or 3.5 increased the hydrolytic activity of plasma membrane H<sup>+</sup>-ATPase in rice roots compared with that of the control whereas SAR at pH 2.5 decreased the hydrolytic activity of plasma membrane H<sup>+</sup>-ATPase (Fig. 3). After a 5-day recovery, the hydrolytic activity of plasma membrane H<sup>+</sup>-ATPase in rice roots treated with pH 5.0 SAR was still higher than that of the control, but lower than that measured during the stress period. For the group treated with SAR at pH 3.5, the hydrolytic activity of plasma membrane H<sup>+</sup>-ATPase in rice roots was still higher than that of the control, and also higher than that measured during the stress period. For the group treated with SAR at pH 2.5, the hydrolytic activity of plasma membrane H<sup>+</sup>-ATPase in rice roots was still lower than that of the control, and even lower than that measured during the stress period.

**Fig. 3** Effects of SAR on hydrolytic activity of plasma membrane H<sup>+</sup>-ATPase in rice roots. Significant difference at  $P < 0.05$  during stress periods was shown with *different lowercase*, and during recovery period was shown with *uppercase*



### 3.6 Correlation Analysis

The correlation coefficients between mineral nutrient content and root morphology are shown in Table 4. During the stress period, the positive correlation was significant between K<sup>+</sup> concentration and surface area of rice root. And we also found that the correlation between Mg<sup>2+</sup> concentration and root surface area and number of root tips was positive significantly. During the recovery period, the correlation was positive significantly between K<sup>+</sup>, Na<sup>+</sup>, and Mg<sup>2+</sup> concentrations and root length, surface area, volume, and number of root tips. And the correlation between Ca<sup>2+</sup> concentration and root length, surface area, volume, and number of root tips was negative significantly.

The correlation coefficients between mineral nutrient content and H<sup>+</sup>-ATPase activity and root activity are shown in Table 5. During the stress period, the positive correlation was significant between K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup> concentration and H<sup>+</sup>-ATPase activity. And we also found that the correlation between K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup> concentration and root activity was positive significantly. In addition, the correlation between Ca<sup>2+</sup> concentration and H<sup>+</sup>-ATPase activity and root activity was negative significantly. During the recovery period, the correlation was positive significantly between K<sup>+</sup> and Na<sup>+</sup> concentration and H<sup>+</sup>-ATPase activity. In addition, we also found the positive correlation between K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup> concentration and root activity. And the correlation between Ca<sup>2+</sup> concentration and H<sup>+</sup>-ATPase activity and root activity was negative significantly.

**Table 4** Relationships between mineral nutrient content and root morphology of rice root treated with SAR

	Mineral nutrient	Root length	Surface area	Volume	Number of root tips
Stress period	K <sup>+</sup>	0.784	0.910*	0.566	0.847
	Na <sup>+</sup>	0.696	0.734	0.413	0.620
	Ca <sup>2+</sup>	-0.808	-0.887	-0.528	-0.841
	Mg <sup>2+</sup>	0.491	0.984*	0.823	0.987*
Recovery period	K <sup>+</sup>	0.994**	0.979*	0.983*	0.994**
	Na <sup>+</sup>	0.997**	0.989*	0.979*	0.998**
	Ca <sup>2+</sup>	-0.994**	-0.973*	-0.993**	-0.991**
	Mg <sup>2+</sup>	0.968*	0.988*	0.933*	0.971*

\*\* Correlation is significant at the 0.01 level; \* correlation is significant at the 0.05 level

## 4 Discussion

### 4.1 Effects of SAR on the Morphology and Growth of Rice Roots

Plants can make an adaptation to environmental stress by changing its root morphology (Grossnickle 2005). Root morphology (root length, surface area, volume and number of root tips, etc.) can reflect the growth condition of plant root under environmental stresses (Fageria and Moreira 2011; Forino et al. 2012). In addition, biomass of plants is a prominent indicator to assess the healthy growth and productivity as related to environmental stresses (Makkonen and Helmisaari 2001). Our results show that SAR at pH 5.0 or 3.5 decreased the number of adventitious root (Fig. 1a–c). However, the density of root hair and the volume of adventitious root treated with SAR at pH 5.0 or 3.5 were increased (Table 1) to make an adaptation. Additionally, at the same treatment, the increase in dry weight of rice root indicates that the increased density of root hair and volume was beneficial for dry matter accumulation in rice root. Li et al. (2015) also found that maize increase their root hairs to improve the

acquisition of nutrients and water from the soil in response to nutrient deprivation. Strong acid (pH 2.5) inhibited rice root growth by decreasing the root length, surface area, volume, fresh and dry weight of rice root, and number of root tips. El-Mallakh et al. (2014) also found that simulated acid rain (pH 2.3–3.3) inhibited germination and growth of *Scindapsus aureus* root (root length and number of sprouted root). In our experiments, SAR at pH 5.0 or 3.5 increased the growth of root hair, and even that SAR at pH 2.5 did not affect root hair growth. The phenomena indicate that root hair as physiological activity interface function section of rice root can contribute to rice adaptation to acid rain stress.

After a 5-day recovery, the length, surface area, volume, and number of root tips of rice roots treated with SAR at pH 5.0 or 3.5 were recovered to the control level ( $P < 0.05$ ) (Table 1), showing that the effect of SAR at pH 5.0 or 3.5 on root morphology can be recovered withdrawing acid rain stress. Additionally, dry weight of rice root treated with (SAR pH 5.0 or 3.5) was also recovered to the control level ( $P < 0.05$ ) (Table 2). The phenomena indicate that root morphology played a role in the root

**Table 5** Relationships between nutrient content and H<sup>+</sup>-ATPase activity and root activity in rice roots treated with SAR

		K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>
Stress period	H <sup>+</sup> -ATPase	0.903*	0.974**	-0.807*	0.768*
	Root activity	0.958**	0.824*	-0.925**	0.962**
Recovery period	H <sup>+</sup> -ATPase	0.768*	0.749*	-0.813*	0.696*
	Root activity	0.967**	0.958**	-0.983**	0.897*

\*\* Correlation is significant at the 0.01 level; \* correlation is significant at the 0.05 level



biomass production. Lequeue and Draye (2014) also found that the biomass of tomato was related to root system morphology under nitrogen deficiency. For the group treated with SAR at pH 2.5, the root length, surface area, and volume of rice root were lower than those of the control, and even lower than those measured during the stress period. In addition, the inhibition caused by pH 2.5 SAR on growth of root hair was heavier than that measured during the stress period. Results show that the change of root morphology caused by strong acid (pH 2.5) cannot be recovered after a 5-day recovery. Therefore, the biomass (fresh and dry weight) of rice root was decreased compared with those of the control.

#### 4.2 Effect of SAR on Concentrations of Mineral Elements in Rice Roots

Mineral elements such as  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  are necessary for plant growth (Remko et al. 2008). In addition,  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  are transported by the same protein (Morgan et al. 2014). Root can absorb nutrients to maintain growth and resistance to environmental stresses (Maathuis 2009). In our experiments, weak acid rain (pH 5.0) increased  $Na^+$  and  $K^+$  concentrations in rice root, and had no effect on the concentrations of  $Ca^{2+}$  and  $Mg^{2+}$ . That could be because excess  $H^+$  caused by weak acid can activate some enzymes to regulate osmotic potential for enhancing the uptake of nutrient substance (Mo et al. 2011; Page and Di 2006). Combined with the increase in density of root hair and volume of adventitious root of rice seedlings treated with SAR at pH 5.0, we inferred that  $K^+$  and  $Na^+$  can stimulate the growth of root hair and adventitious root. Wu et al. (2009) reported that plant cell growth can be stimulated by increasing osmotic pressure resulting from ion potential. In our experiment, the increase in  $K^+$  and  $Na^+$  concentration increased ion potential and stimulated the growth of root hair and adventitious root. For the group treated with SAR at pH 3.5, the  $K^+$  concentration in rice root was decreased whereas  $Ca^{2+}$  concentration in rice root was increased. The increase in  $Ca^{2+}$  concentration was because the plants need  $Ca^{2+}$  to alleviate the inhibition on root cell growth caused by acid rain (pH 3.5) (Ali et al. 2002). The decrease in  $K^+$  resulted from the competition between  $Ca^{2+}$  and  $K^+$ . According to our results, the increased  $Ca^{2+}$  concentration was

beneficial for the growth of root hair and adventitious roots (Fig. 1 and Table 3) because  $Ca^{2+}$  plays an important role in stabilizing cell wall, promoting cell elongation, and transferring the stress signal in plants (Liu et al. 2011; Munoz et al. 2014; Zhang et al. 2014). Strong acid (pH 2.5) decreased the concentration of  $K^+$ ,  $Na^+$ , and  $Mg^{2+}$  in rice root. Combined with the root growth of rice seedlings treated with SAR at pH 2.5, the deficiency of  $K^+$ ,  $Na^+$ , and  $Mg^{2+}$  inhibited the growth of rice root. Ding and Xu (2011) reported that  $K^+$  and  $Mg^{2+}$  are important activators or regulators of many key enzymes in plant physiological processes and the deficiency of  $K^+$  and  $Mg^{2+}$  inhibit the growth of plant root. However, the increase in  $Ca^{2+}$  concentration indicated that strong acid (pH 2.5) is beneficial for the accumulation of  $Ca^{2+}$  in rice roots. Sun et al. (2013) also reported that acid rain could increase the  $Ca^{2+}$  concentration in soybean root. However, the location of  $Ca^{2+}$  in rice root exposed to SAR at pH 2.5 need to be further studied. According to relationship analysis (Table 4), during stress period, the root surface area was regulated by concentration of  $K^+$  and  $Mg^{2+}$  in rice root, and the number of root tips was regulated by the  $Mg^{2+}$  concentration under acid rain stress.

After a 5-day recovery, the  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  concentration in rice root treated with SAR at pH 5.0 or 3.5 was recovered to the level of the control, indicating that rice seedlings can endure acid rain stress (pH 5.0 or 3.5). Combined with root growth and morphology of rice seedlings treated with SAR at pH 5.0 or 3.5, we found that the ionic homeostasis of  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  was beneficial to maintaining the normal growth of rice root. For the group treated with SAR at pH 2.5, the  $Ca^{2+}$  concentration was still higher than that of the control whereas the concentration of  $K^+$ ,  $Na^+$ , and  $Mg^{2+}$  was still lower than that of the control, but higher than those of the stress period. The phenomenon indicated that increase in  $Ca^{2+}$  was beneficial to alleviating the inhibition on  $K^+$ ,  $Na^+$ , and  $Mg^{2+}$  caused by strong acid (pH 2.5). Cramer et al. (1987) also found  $Ca^{2+}$  has a protect function to the maintenance of  $K^+/Na^+$  selectivity under salt stress. Additionally, the biomass of rice root treated with SAR at pH 2.5 cannot be recovered after a 5-day recovery was resulted from the deficiency of  $K^+$ ,  $Na^+$ , and  $Mg^{2+}$ . According to relationship analysis (Table 4), during the recovery period, the root length, surface area, volume, and number of root tips of rice

seedlings were regulated by concentration of  $K^+$ ,  $Na^+$ , and  $Mg^{2+}$  in rice root.

#### 4.3 Effects of SAR on Root Activity and Activity of Hydrolytic Activity of Plasma Membrane $H^+$ -ATPase in Rice Roots

The nutrient absorption was regulated by plasma membrane  $H^+$ -ATPase as well as root activity (Osaki et al. 1997; Palmgren 2001). Root activity is often used to evaluate the ability of roots to absorb water and mineral elements (Islam et al. 2007). Plasma membrane  $H^+$ -ATPase is the key enzyme to provide energy for transporting nutrition into cells because it can extrude positive charges ( $H^+$ ) and thus forms a membrane potential (negative on the inside) (Alvarez-Pizarro et al. 2014; Palmgren 2001; Zhu et al. 2009). In our experiments, SAR at pH 5.0 or 3.5 increased root activity and hydrolytic activity of plasma membrane  $H^+$ -ATPase in rice roots after a 5-day exposure. The increase in root activity and hydrolytic activity of plasma membrane  $H^+$ -ATPase accelerated the absorption of  $Na^+$ ,  $K^+$ , and  $Ca^{2+}$  (Table 3). Strong acid (pH 2.5) decreased the hydrolytic activity of plasma membrane  $H^+$ -ATPase and root activity in rice roots after a 5-day exposure. Our previous studies (Liang et al. 2015; Sun et al. 2013) also reported that strong acid rain (pH 2.5) decreased hydrolytic activity of plasma membrane  $H^+$ -ATPase in rice leaves, and acid rain (pH 3.0) decreased the root activity of soybean. The decrease in root activity and hydrolytic activity of plasma membrane  $H^+$ -ATPase inhibited the absorption of  $Na^+$ ,  $K^+$ , and  $Mg^{2+}$ . Therefore, the concentrations of  $Na^+$ ,  $K^+$ , and  $Mg^{2+}$  in rice root were decreased under SAR at pH 2.5 (Table 2). However, the concentration of  $Ca^{2+}$  in rice root treated with SAR at pH 2.5 was increased, indicating the absorption of  $Ca^{2+}$  did not only rely on root activity and the hydrolytic activity of plasma membrane  $H^+$ -ATPase. It was reported that  $Ca^{2+}$  can enter into plant cell through the depolarization activated calcium channels (DACC) and the channel can be activated by the inflow of some cations such as  $H^+$ ,  $K^+$ , etc. (White and Broadley 2003).

After a 5-day recovery, the hydrolytic activity of plasma membrane  $H^+$ -ATPase and root activity in rice roots were still higher than those of the control, benefiting to maintain normal absorption of  $Na^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  (Table 2). For the group treated with SAR at pH 2.5, the root activity and the hydrolytic activity of plasma membrane  $H^+$ -ATPase were still lower than

those of the control, indicating the inhibition on root activity and hydrolytic activity of plasma membrane  $H^+$ -ATPase exceeded the self-regulating capacity of rice seedlings. Moreover, the decrease in root activity and the hydrolytic activity of plasma membrane  $H^+$ -ATPase led to the decrease in concentrations of  $Na^+$ ,  $K^+$ , and  $Mg^{2+}$  compared to the control (Table 3). According to our relationships analysis shown in Table 5,  $K^+$ ,  $Na^+$ , and  $Mg^{2+}$  concentrations in rice root were positively correlated with plasma membrane  $H^+$ -ATPase activity and root activity in rice roots during stress and recovery periods. The phenomenon indicates that the absorption of  $K^+$ ,  $Na^+$ , and  $Mg^{2+}$  was regulated by  $H^+$ -ATPase activity and root activity under acid rain stress. However, the  $Ca^{2+}$  concentration in rice root was negatively correlated with plasma membrane  $H^+$ -ATPase activity and root activity, indicating the concentration of  $Ca^{2+}$  in rice root under acid rain stress did not only rely on plasma membrane  $H^+$ -ATPase activity and root activity. The source and location of  $Ca^{2+}$  need to be further studied.

## 5 Conclusion

Acid rain (pH 5.0 or 3.5) was beneficial to the growth of root hair and the accumulation of dry mass in rice root by increasing the absorption of  $K^+$ ,  $Na^+$ , and  $Ca^{2+}$ , whereas strong acid (pH 2.5) inhibited root growth of rice seedlings by decreasing the absorption of  $K^+$ ,  $Na^+$ , and  $Mg^{2+}$ . Meanwhile, the absorption of  $K^+$ ,  $Na^+$ , and  $Mg^{2+}$  except  $Ca^{2+}$  was regulated by the change in root activity and hydrolytic activity of plasma membrane  $H^+$ -ATPase treated with acid rain. After a 5-day recovery, the ionic homeostasis of  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  was beneficial to maintaining the normal morphology and growth of rice roots treated with acid rain (pH 5.0 or 3.5). However, strong acid (pH 2.5) caused irrecoverable inhibition on root growth because the root activity and hydrolytic activity of plasma membrane  $H^+$ -ATPase may have exceeded the self-regulating capacity of rice seedlings, and the absorption of mineral nutrient cannot sustain the growth. These results will help us understand the adaptation of root morphology to acid rain stress and provide directions for finding effective ways to alleviate such damage.

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