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ORIGINAL PAPER

Effects of Silicon on the Growth, Photosynthesis and Chloroplast Ultrastructure of *Oryza sativa* L. Seedlings under Acid Rain Stress

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Abstract

Silicon (Si) is a beneficial element for plants and can increase plant resistance. In the present work, a hydroponic experiment was carried out to study the effects of Si on the growth and photosynthesis of rice (*Oryza sativa* L.) seedlings under simulated acid rain (SAR) stress. The growth, photosynthesis and chloroplast ultrastructure of rice seedlings treated with combined or single weak SAR (pH 4.0) and/or Si (1, 2 or 4 mM) were improved. Spraying with moderate or severe SAR (pH 3.0 or 2.0) significantly inhibited the growth and photosynthesis and severely damaged the chloroplast ultrastructure of rice seedlings. The incorporation of exogenous Si increased the growth and photosynthesis and improved the chloroplast ultrastructure of rice seedlings treated with moderate or severe SAR (pH 3.0 or 2.0). The 2.0 mM Si treatment had more significant promoting or alleviating effects than the 1 and 4 mM Si treatments. The stomatal conductance (G_s), chlorophyll content, maximum quantum efficiency of PSII photochemistry (F_v/F_m), actual photochemical quantum efficiency of PSII photochemistry (Y) and chloroplast ultrastructure were improved with the addition of Si to the SAR treatment, which indicated that the positive effect of Si on photosynthesis was partly associated with stomatal and non-stomatal factors. Thus, Si fertilization improves rice resistance to acid rain.

Keywords Silicon · Acid rain · *Oryza sativa* L. · Growth · Photosynthesis

1 Introduction

Acid rain is a by-product of the rapid development of modern industry, and damage to the ecological environment caused by acid rain has drawn considerable attention from scholars and policymakers across the world; thus, the prevention and control of the damage from acid rain have become important tasks, especially in Europe, North America and Asia [1–3]. Acid rain limits the growth and yield of crops when the pH level reaches a certain damage threshold, which results in enormous economic losses [4]. Therefore, exploring the injury mechanisms in plants and enhancing plant resistance to acid rain have become the subject of many scientific studies. Simulated acid rain (SAR) experiments have demonstrated that acid rain reduces the chloroplast content and net photosynthetic rate (P_n) of plants and damages the chloroplast ultrastructure in rice [5–7], soybean [8], maize [9], tomato [10], bamboo [11], and other plant species [12]. Since plant productivity and yield strongly depend on the P_n, plant productivity and yield under stress can be maintained by maintaining normal P_n [13].

Silicon (Si) is known to alleviate many plant stresses [14]. The Si content in different plant species is significantly different [15, 16], and studies have shown that the content ranges from 0.1 to 10.0 SiO_2 per dry weight or higher [17, 18]. Si promotes plant growth, improves chlorophyll content and photosynthesis, alleviates oxidative damage, especially in plants under stress, such as salt, heavy metal, drought and high temperature stress [19–24]. In accordance with these previous studies, we hypothesized that Si could potentially maintain photosynthesis to some extent and mitigate the deleterious effects of acid rain.

Rice (*Oryza sativa* L.) is the most consumed staple grain crop in southern China, which is one of the most severe acid rain regions in the world [25]. Rice, as an important food crop [21, 26], has been extensively studied as a model crop for its value in future potential applications. Moreover, rice is a

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heavy Si-accumulator, even when compared to many other Si-accumulators [15, 27]. A number of studies have investigated the influence of Si or acid rain on rice (Song et al. 2015; [5, 20]; Liang et al. 2015; [8]); however, the compound effects of Si and acid rain on rice need to be explored. Our previous study showed that under acid rain stress, Si could improve rice resistance by regulating mineral absorption, thus impacting on the activity of antioxidant enzymes and increasing the mechanical barriers in the leaf epidermis [2, 28, 29]. Therefore, in the present work, the effects of different concentrations of Si and acid rain on the growth, photosynthesis and chloroplast ultrastructure in the cells of rice seedlings were investigated.

2 Materials and Methods

2.1 Plant Culture and Treatments

Plant cultures were prepared according to our previous study [2, 28]. Rice seeds were sterilized with 1‰ $KMnO_4$ for 30 min and germinated in an incubator at 24 ± 2 °C for 6 days. On the sixth day, uniform rice seedlings were collected and transferred into turnover boxes (300 × 200 × 100 mm). The planting density was 48 holes, with 96 rice plants per box. With plant growth, 1/4-, 1/2- and full-strength the International Rice Research Institute (IRRI) nutrient solutions (pH 5.5) [30] were prepared according to Ju et al.'s [2] description, replaced successively every 9 days and renewed every 3 days, and water was added daily to maintain solution volume. The experiment was carried out in a greenhouse with relative humidity at 50% to 70% and a natural photoperiod of 14.5 L:9.5D in June and July in central and eastern China.

SAR (pH 2.0, 3.0, 4.0 and 6.5) and Si solutions (0, 1, 2 and 4 mM) were prepared according to previous work [2, 28]. The ionic composition in SAR was derived from rainfall data in central and eastern China [31, 32]. The pH was adjusted with a mix of H_2SO_4 and HNO_3, where the mole ratio of [SO_4^{2-}] to [NO_3^-] was 2.7:1 [33, 34]. The Si element was provided by $Na_2SiO_3 \cdot 9H_2O$. The experiment used a full factorial design in which the SAR and Si controls were pH 6.5 and 0 mM, respectively, and it included 16 total treatments with three replicates per treatment. Rice seedlings were treated with SAR and/or Si solutions according to previous work [2, 28]. Six-day-old seedlings were initiated to treat with Si, and 24-day-old seedlings were sprayed with SAR. After 7 days of SAR, the rice seedlings were collected for further analysis.

2.2 Determination of Dry Weight (DW)

Whole-plant rice seedlings were collected and dried to a constant weight at 80 °C in an oven, and then the DW was measured [8, 35].

2.3 Measurements of Chlorophyll Content

Rice leaves were cut into pieces and soaked in a solution containing 95% ethanol and 80% acetone (V:V = 1:2). Chlorophyll was extracted under dark conditions until the green colour disappeared from the leaf tissue. Absorbance of the supernatant was read at 663, 645 and 470 nm. Chlorophyll content was calculated using the corrected equation of Lichtenthaler [36].

2.4 Measurements of Photosynthesis and Chlorophyll Fluorescence Parameters

Photosynthesis parameters (photosynthetic rate (P_n), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i)) were measured from 9:00 to 11:00 a.m. using a portable photosynthetic system (GFS-3000, Heinz Walz Gmbh, Effeltrich, Germany). Measurements were performed on the second fully expanded leaves under conditions of 19.0–20.7 °C temperature, 60–75% ambient relative humidity, 453–512 ppm atmospheric CO_2 concentration, and 1000 μmolm^{-2} s^{-1} photosynthetically active photon flux density (PPFD) saturating light [6, 7].

According to the method described by Wegener and Vicherováet et al. [37], the chlorophyll fluorescence parameters (maximum quantum efficiency of PSII photochemistry (F_v/F_m) and actual photochemical quantum efficiency of PSII photochemistry (Y)) were measured on the second fully expanded leaves using a GFS-3000 instrument equipped with a fluorescence imaging unit (LED-Array/PAM module 3050-F, GFS-3000, Heinz Walz Gmbh, Effeltrich, Germany). The rice seedlings were dark-adapted overnight. Fluorescence induction was initiated with actinic light (100 μmolm^{-2} s^{-1}) and superimposed with 800 ms saturating pulses (10,000 μmol m^{-2} s^{-1}) at 20 s intervals.

2.5 Observation of Chloroplast Ultrastructure

Transmission electron microscopy (TEM) were used to observe the chloroplast ultrastructure of rice seedlings. Material selection and treatment referred to previous methods [29]. The middle sections of the 2nd set of leaves were cut into pieces, and then the leaf pieces were successively soaked with 4% glutaraldehyde and 2% osmic acid for fixation and then in an ethanol gradient for dehydration. Using an LKB-V ultramicrotome (Bromma, Stockholm, Sweden), the samples embedded in 100% Epon-812 were cut into ultrathin sections, which were then passed through 250 mesh grids and post-stained with uranyl acetate and lead citrate. Finally, a transmission electron microscope (JEM-2100, JEOL, Japan) was used to observe the chloroplast ultrastructure.

2.6 Statistical Analysis

In this experiment, the number of rice seedlings per treatment was 3 boxes×96 seedlings and 3 seedlings were randomly selected to measure index for per treatment. The significance of the differences among treatments and interactions between Si and SAR were analysed by one-way and two-way analyses of variance (ANOVAs) using SPSS 19.0. A correlation analysis was performed using Origin 8.0. The means were compared using Student's test at the 5% probability level ($P \leq 0.05$).

3 Results

3.1 Biomass Accumulation of Rice Seedlings Exposed to Si and SAR

The DW of rice seedlings exposed to1, 2 or 4 mM Si significantly increased compared with that of the control (CK), and under increasing Si concentrations, the DW gradually increased but then decreased. When the rice seedlings were exposed to pH 4.0 or 3.0 SAR, the DW did not clearly change, and in the pH 2.0 SAR treatment, the DW clearly decreased compared with that of the CK (Fig. 1). The incorporation of Si (1, 2 or 4 mM) into SAR (pH 4.0, 3.0 or 2.0) clearly increased the DW compared with the corresponding single SAR treatments. Under the same SAR pH, the DW gradually increased but then decreased with increasing Si concentrations. Under SAR stress, Si could increase the DW of rice seedlings and alleviate SAR stress. Moreover, the 2 mM Si treatment had a better effect than the 1 and 4 mM Si treatments. The interactive effect between Si and SAR on DW was obviously ($F = 55.027$, $p < 0.01$) (Fig. 1).

3.2 Photosynthetic Parameters of Rice Seedlings Exposed to Si and SAR

For the single 1 mM Si treatment, the P_n, G_s and C_i significantly increased compared with the CK. For the single 2 mM Si treatment, the P_n and G_s significantly increased while C_i was unchanged compared with the CK. When the concentration of Si increased to 4 mM, the P_n, G_s and C_i did not significantly differ from the CK. For the single pH 4.0 SAR treatment, the P_n and G_s significantly increased while C_i was unchanged compared with the CK. When the SAR pH values decreased to 3.0 (or 2.0), the P_n and G_s decreased and C_i significantly increased compared with the CK (Table 1).

Under the SAR pH 4.0 condition, the addition of 1 mM (2 mM) Si significantly increased the P_n and G_s, but C_i unchanged, with Si concentration increased to 4 mM, the P_n, G_s and C_i did not change relative to the single pH 4.0 SAR treatment. When the rice seedlings were exposed to 1 mM (2 mM) Si and the SAR pH 3.0 treatment, the P_n was unchanged, G_s increased significantly and C_i decreased significantly relative to the corresponding single SAR treatment. As the Si concentration increased to 4 mM, the P_n and G_s were unchanged but C_i decreased remarkably compared to the single pH 3.0 SAR treatment. Under the SAR pH 2.0 condition, the addition of 1 mM (2 and 4 mM) Si significantly increased the P_n and G_s

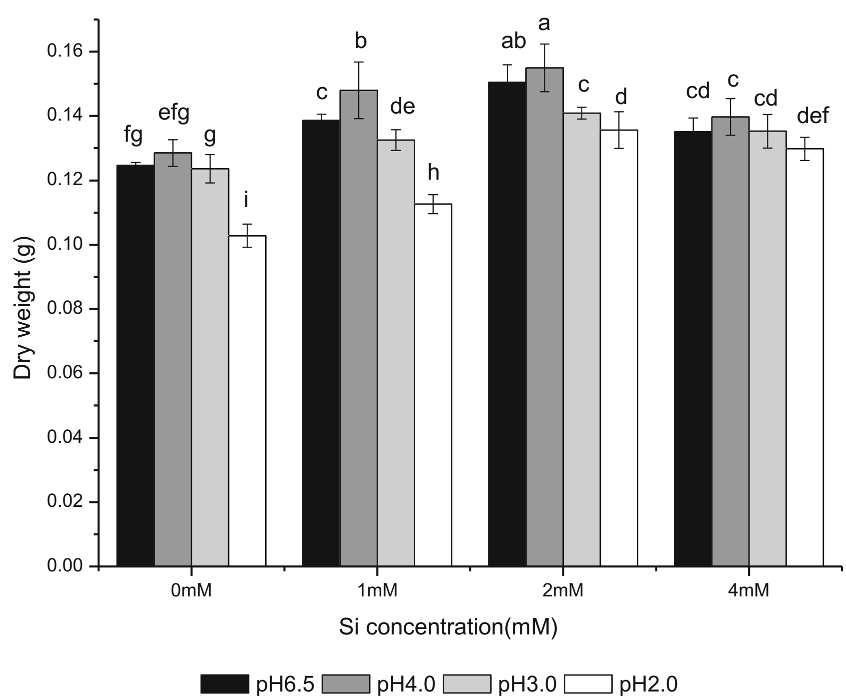

Fig. 1 Effects of Si and SAR on dry weight of rice seedlings. Significantly differences at $P < 0.05$ were showed with different letter. Vertical bars indicate the standard error

Table 1 Effects of Si and SAR on the P_n, G_s and C_i in rice seedlings

SAR (pH)	Si (mM)	P_n (μmol m^{-2} s^{-1})	G_s (μmol m^{-2} $s^{-1}H_2O_2$)	C_i (ppm)
6.5	0	17.671 ± 0. 845 fg (100.00)	191.504 ± 8.817 ef (100.00)	414.856 ± 8.242 h (100.00)
	1	18.754 ± 0.927 cde (106.13)	218.128 ± 6.934 ab (113.90)	431.780 ± 7.661 efg (104.08)
	2	18.787 ± 0.953 cd (106.32)	210.196 ± 7.953 bcd (109.76)	429.450 ± 7.930 fgh (103.52)
	4	17.833 ± 0.574 efg (100.92)	199.467 ± 7.856 de (104.16)	422.913 ± 9.587 gh (101.94)
4.0	0	19.934 ± 0.728 b (112.81)	211.940 ± 4.399 bc (110.67)	427.605 ± 9.884 gh (103.07)
	1	21.237 ± 0.581 a (120.18)	226.528 ± 8.917 a (118.29)	434.904 ± 8.396 efg (104.83)
	2	21.146 ± 0.725 a (119.66)	227.109 ± 6.518 a (118.59)	433.916 ± 5.172 efg (104.59)
	4	19.405 ± 0.416 bc (109.81)	213.378 ± 7.347 bc (111.42)	428.024 ± 11.654 gh (103.17)
3.0	0	16.855 ± 0.921gh (95.38)	177.090 ± 5.152 gh (92.47)	467.203 ± 11.075 bc (112.62)
	1	18.242 ± 0.520 defg (103.23)	190.010 ± 5.947 ef (99.22)	444.558 ± 7.628 def (107.16)
	2	18.534 ± 0.708 cdef (104.88)	203.457 ± 4.417 cd (106.24)	438.065 ± 6.443 efg (105.59)
	4	17.538 ± 0.609 g (99.25)	184.896 ± 7.130 fg (96.55)	446.431 ± 9.106 de (107.61)
2.0	0	9.915 ± 0.750 j (56.11)	157.603 ± 4.650 i (82.30)	506.211 ± 12.462 a (122.02)
	1	13.353 ± 0.757 I (75.56)	169.854 ± 4.767 h (88.69)	479.861 ± 5.672 b (115.67)
	2	15.849 ± 0.688 h (89.69)	183.814 ± 9.016 fg (95.98)	457.306 ± 13.063 cd (110.23)
	4	14.018 ± 0.755i (79.33)	172.173 ± 8.232 h (89.91)	468.554 ± 16.056 bc (112.94)
F		57.587	58.147	19.819
p		<0.001*	<0.001*	<0.001*

Abbreviations: *SAR* simulated acid rain, *Si* silicon, P_n, photosynthetic rate, G_s stomatal conductance, C_i intercellular CO_2 concentration

Values are means ± standard deviation errors, $n = 3$. Values in the parentheses are the percentage of treatment in control

Significanty differences at $p < 0.05$ were showed with different letter in the same line

* Significance at 0.05 levels

but decreased the C_i remarkedly. Under the same pH SAR condition, as the Si concentration increased, the P_n and G_s increased first but then decreased while the opposite effect was observed for C_i (Table 1). This study showed that Si could alleviate SAR stress and the 2 mM Si treatment produced the best effect (Table 1).

The analysis showed an obvious interactive effect between Si and SAR on the P_n, G_s and C_i of rice seedlings. The results of the linear regression equations and correlation coefficients (*r*) indicated that DW was positively correlated with P_n and G_s but negatively correlated with C_i and the relationships were significant ($p < 0.01$) (Table 2).

Table 2 Relationship of P_n, G_s and C_i with DW of rice seedlings treated with Si and SAR

Linear regression equation	Correlation coefficient (*R*)
$P_n = 186.850DW-7.470$	0.691^{**}
$G_s = 1324.177DW + 19.526$	0.690^{**}
$C_i = -1281.244DW + 616.549$	-0.463^{**}

Abbreviations: *SAR* simulated acid rain, *Si*, silicon, P_n photosynthetic rate, G_s stomatal conductance, C_i, intercellular CO_2 concentration, *DW* dry weight

** Significant at the 0.01 level

3.3 Chlorophyll Content of Rice Seedlings Exposed to Si and SAR

The 1, 2 or 4 mM Si treatments increased the chlorophyll content significantly compared to the CK, and with an increase in the Si concentration, the chlorophyll content increased first and then decreased, and it reached the highest value in the 1 mM Si treatment. The SAR pH 4.0, 3.0 or 2.0 treatments reduced the chlorophyll content in rice seedlings, and with a decrease in the SAR pH, the chlorophyll content decreased gradually and reached a significant level at SAR with pH values of 3.0 and 2.0. Under SAR stress, the addition of Si increased the chlorophyll content, and with an increase of Si concentration, the chlorophyll content increased first and then decreased, and it reached the maximum in the 2.0 mM Si treatment (Fig. 2). The interactive effect between Si and SAR on chlorophyll content was obvious (Fig. 2), and the correlation analysis revealed that P_n was positively and significantly ($p < 0.01$) related to the chlorophyll content.

3.4 Chlorophyll Fluorescence Parameters of Rice Seedlings Exposed to Si and SAR

The addition of exogenous Si (1, 2 or 4 mM) increased the F_v/F_m and Y values in rice seedlings compared with the CK and

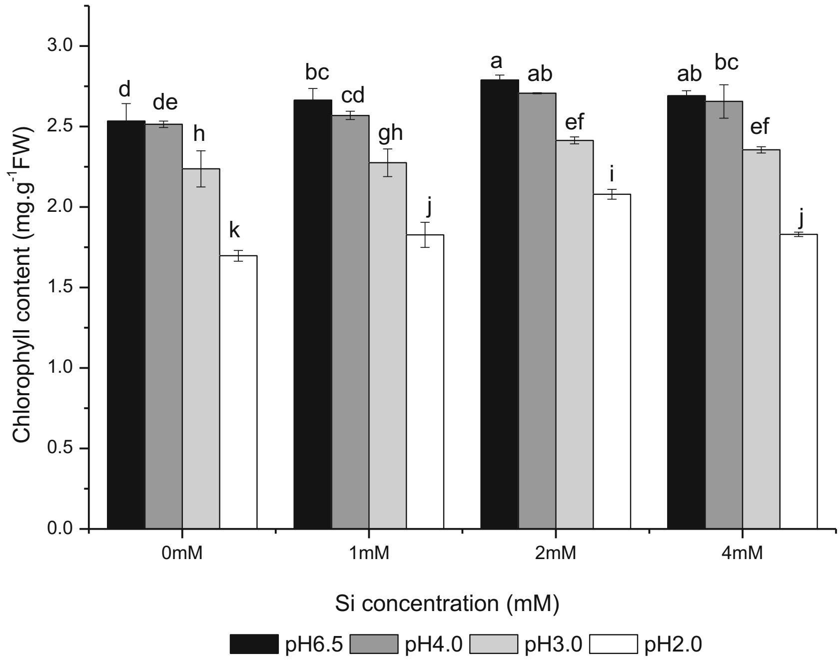

Fig. 2 Effects of Si and SAR on the chlorophyll content in rice seedlings. Significantly differences at $P < 0.05$ were showed with different letter. Vertical bars indicate the standard error

reached a significant level at 1 and 2 mM Si treatments. As the Si concentration increased, the F_v/F_m and Y increased first and then decreased. The SAR pH 4.0 treatment did not significantly increase the F_v/F_m and Y compared with the CK. As the pH of SAR decreased to 3.0 and 2.0, the F_v/F_m and Y decreased significantly compared with the CK. Under SAR stress, the addition of exogenous Si (1, 2 or 4 mM) increased the F_v/fm and Y compared with the corresponding single SAR treatment. With the increase of Si concentration, the F_v/F_m and Y increased first and then decreased, and it reached the maximum in the 1.0 and 2.0 mM Si treatments (Fig. 3). An obvious interactive effect was observed between Si and SAR on the F_v/F_m and Y. The correlation analysis revealed that P_n was positively and significantly ($p < 0.01$) related to the F_v/F_m and Y.

3.5 Chloroplast Structure of Rice Seedlings Exposed to Si and SAR

The chloroplasts of the control plants had an intact organized structure, numerous healthy grana an a thick matrix, and one or two enlarged starch granules had intact membranes (Fig. 4A). The starch granules in the chloroplasts of Si-treated rice seedlings became elongated at normal size, and the grana thylakoids, stroma thylakoids and lamellar structure of the thylakoid appeared healthier than that of the CK (Fig. 4B, C and D).

The structure of the chloroplasts after exposure to pH 4.0 SAR was intact, the thylakoid was regularly arranged, the lamellar structure of the thylakoid was clearer and thicker, and the starch granules became elongated at a normal size compared with that of the CK (Fig. 4E). The chloroplasts of rice seedlings exposed to 1 mM (2 or 4 mM) Si and SAR pH 4.0 were intact, the thylakoids of the grana, stroma and lamella were clearer and thicker compared with that of the CK as well as the corresponding single Si and SAR (Fig. 4F, G and H). The chloroplast structure of rice seedlings independently exposed to SAR pH 3.0 was swollen, the number of basal thylakoid layers decreased, the lamellar structure was loose, and the number of starch granules and osmium significantly increased compared with that of the CK, and the matrix in the chloroplast was thinner (Fig. 4I). The simultaneous exposure of Si and SAR pH 3.0 (Fig. 4J, K and L) regained the chloroplast structure, grana thylakoids and lamellar structure of the thylakoids to a certain extent, the number of starch granules and osmium significantly decreased relative to the single pH 3.0 SAR treatment, and the matrix in the chloroplast was thicker. For the pH 2.0 SAR treatment (Fig. 4M), the chloroplast membrane was broken, the matrix flowed outward, and the grana thylakoids and lamellar structure of the thylakoids were deformed and collapsed. Moreover, under the combined treatment with 1 mM Si and SAR pH 2.0 (Fig. 4N), the chloroplast structure remained intact but somewhat disorganized; the chloroplast membrane was uneven and wave-like; and the grana thylakoids and the lamellar structure of the thylakoids appeared to be damaged and difficult to discern. The chloroplasts of rice seedlings subjected to 2 mM Si and SAR pH 2.0 (Fig. 4O) remained intact despite some swelling and displayed a relatively clear grana and lamellar structure. The extent of damage of the chloroplast and thylakoids was less than that of the treatments with the single SAR pH 2.0 or the combined 1 mM Si and SAR pH 2.0. The chloroplasts in rice seedlings subjected to 4 mM Si and SAR pH 2.0 (Fig. 4P) narrowed to an abnormal shape. The inner structure of these chloroplasts was damaged and difficult to discern.

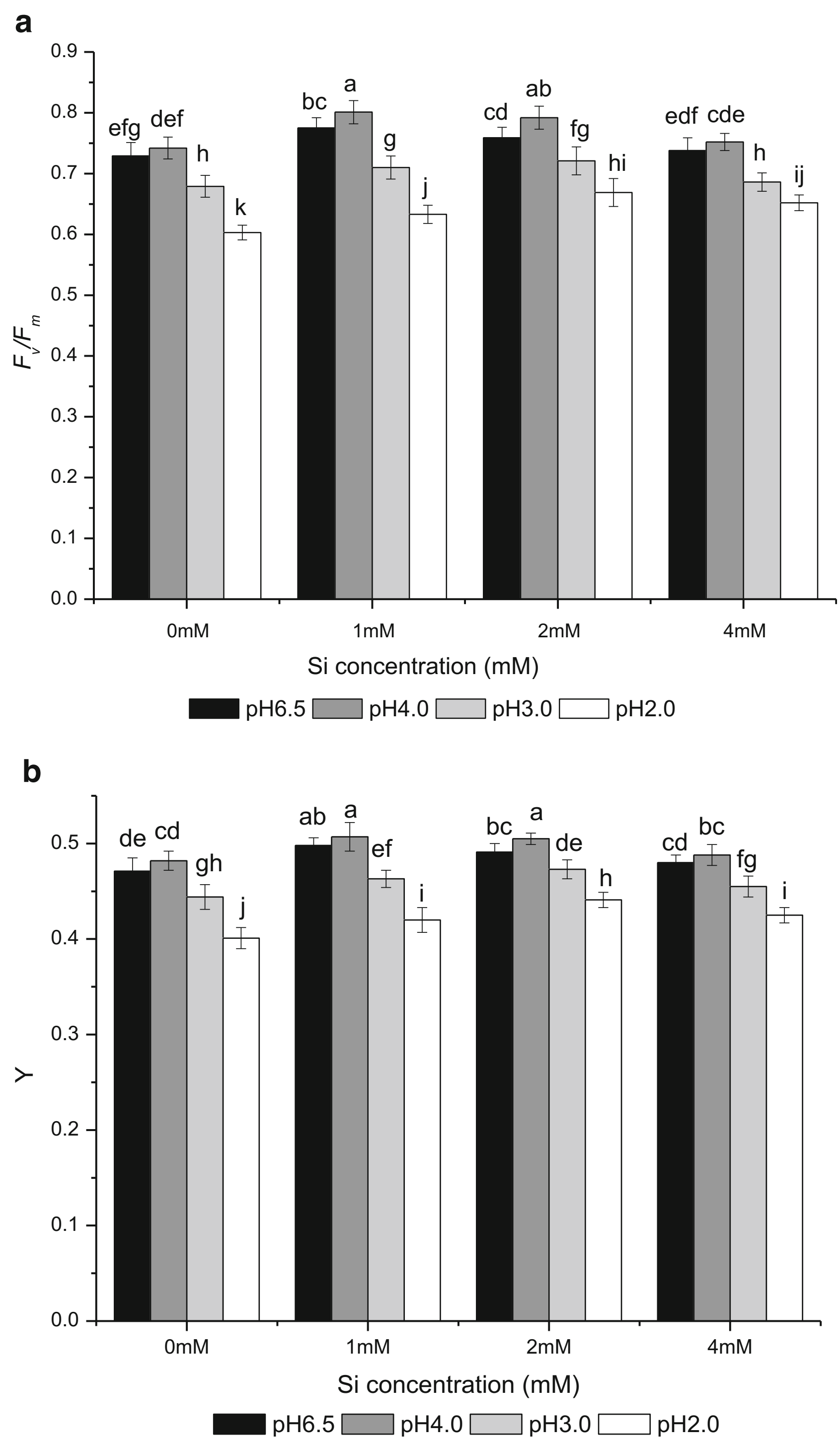

Fig. 3 Effects of Si and SAR on the maximum quantum efficiency of PSII photochemistry (F_v/F_m) (**a**), actual photochemical quantum of PSII photochemistry (Y) (**b**) in rice seedlings. Significantly differences at $P < 0.05$ were showed with different letter. Vertical bars indicate the standard error

4 Discussion

This study analysed the effects of acid rain and Si on the DW of rice seedlings, and the results showed that SAR spraying affected rice seedlings, with the light SAR promoting the growth of rice seedlings, which might be related to the absorption and utilization of the anions and anions in acid rain by plants as nutrient elements. However, the severe SAR (pH 2.0) significantly inhibited the growth of rice seedlings, which could be related to the physiological and molecular damage caused by severe acid rain, which limits plant growth. This conclusion has been accepted by many researchers [9, 38–40]. Under SAR stress, exogenous

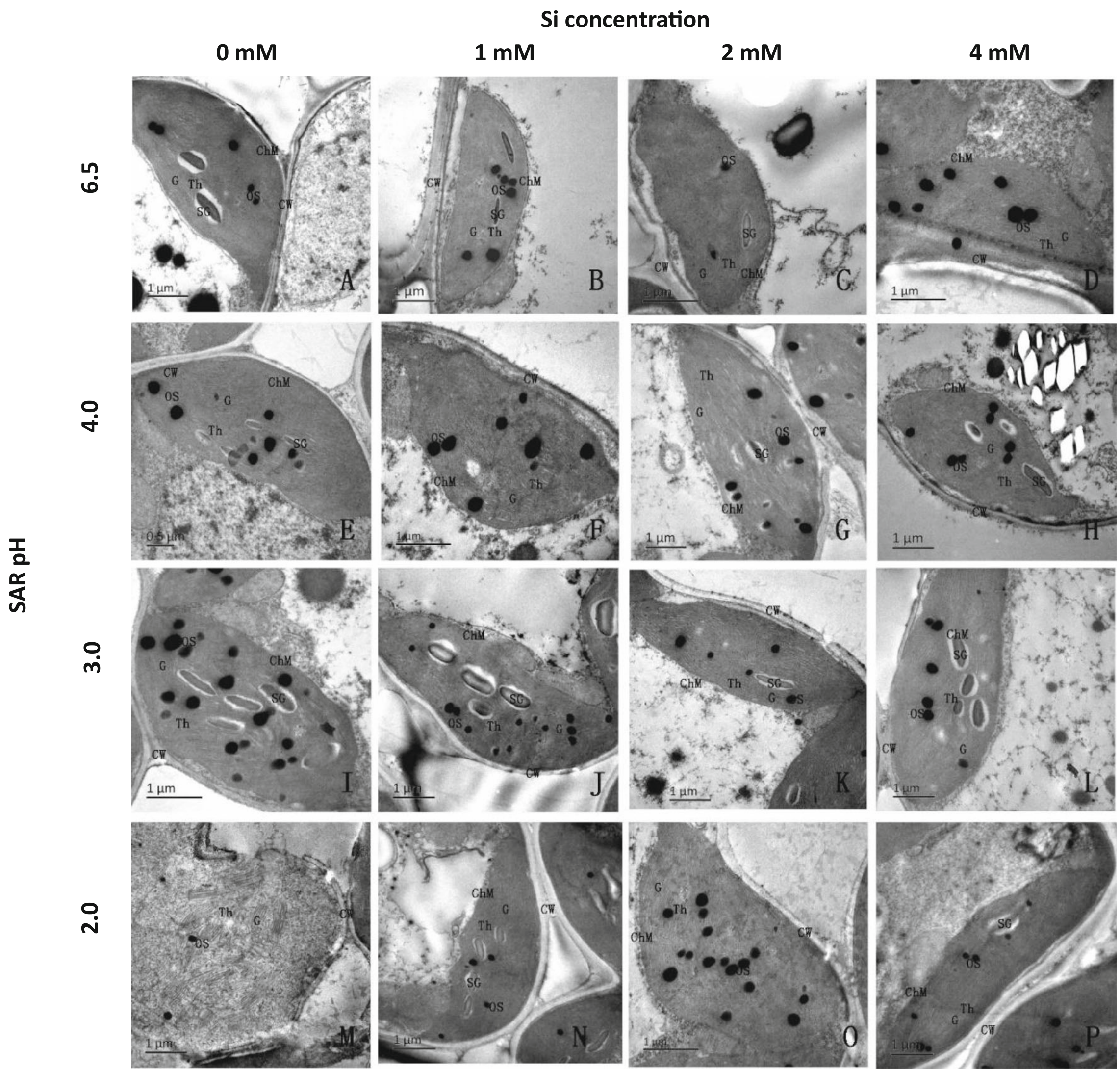

Fig. 4 TEM of the chloroplast structure in rice seedlings under different treatments

Si addition, especially a moderate concentration of Si (2 mM), promoted rice growth (Fig. 1), which indicated that Si could improve the resistance of rice to acid rain. This finding might be related to the ability of Si to protect the photosynthetic system, improve plant stress resistance and increase the DW under environmental stress [41–43]. Moreover, this study showed that exogenous Si increased the P_n under SAR stress, and the correlation analysis showed that DW is positively correlated with P_n (Table 2). This result suggests that under acid rain stress, the addition of exogenous Si helped regulate the photochemical process and promote photosynthesis, which were among the important strategies for increasing the resistance of rice seedlings.

The correlation analysis showed that P_n was significantly related to G_s and C_i (Table 3). Stomatal factors affect P_n [44], and the correlation indicated that exogenous Si could regulate stomatal factors to affect the P_n of rice seedlings under acid rain stress. In the present work, when Si and SAR acted on rice seedlings, the changes in P_n and the stomatal and non-stomatal factors were dependent on the Si concentration and the SAR pH value. The single treatments or combination treatments with Si (1, 2 or 4 mM) and SAR pH 4.0 increased the G_s and C_i in rice leaves; thus, stomatal factors played an important role and ultimately promoted photosynthesis, which might be related to the ability of Si and nutrient elements in the SAR treatments to promote the morphogenesis of tissues and organs in leaves. However, the SAR pH 3.0 and SAR pH 2.0 treatments decreased the G_s but increased the C_i; moreover, non-stomatal factors played an important role and ultimately inhibited photosynthesis, which might be related to SAR-induced injury to the PSII system,

Table 3 Relationship of the CC, F_v/F_m,Y, G_s and C_i with the P_n of rice seedlings treated with Si and SAR

Linear regression equation	Correlation coefficient (*R*)
$CC = 0.104\ P_n + 0.553$	0.775**
$F_v/F_m = 0.018\ P_n + 0.399$	0.872**
$Y = 0.010\ P_n + 0.288$	0.895**
$G_s = 6.556\ P_n + 81.728$	0.844**
$C_i = -7.091\ P_n + 569.408$	−0.732**

Abbreviations: *SAR* simulated acid rain, *Si* silicon, P_n photosynthetic rate, G_s stomatal conductance, C_i intercellular CO_2 concentration, F_v/F_m, maximum quantum efficiency of PSII photochemistry, Y, actual photochemical quantum efficiency of PSII photochemistry, *CC* chlorophyll content

** Significant at the 0.01 level

chloroplast structure and thylakoids [45]. The incorporation of Si (1, 2or 4 mM) with SAR pH 3.0 or 2.0 alleviated the damage of SAR, increased the G_s, decreased the C_i and promoted photosynthesis in rice seedlings.

The correlation analysis showed that P_n was positively correlated with the chlorophyll content, F_v/F_m and Y in rice seedlings exposed to Si and SAR (Tables 2, 3), which indicated that exogenous Si could increase P_n by improving non-stomatal factors (chlorophyll content, F_v/F_m and Y) in rice seedlings under acid rain stress. In the present study, the single or combined treatments with Si (1, 2or 4 mM) or SAR (pH 4.0) increased the P_n, which might be related to the ability of Si and nutrient elements in the SAR treatments to promote plant growth and development and improve chloroplast electron transport activity in leaves, including the absorption of light energy and the conversion of light energy, which resulted in improved photosynthetic processes. Under the moderate or severe SAR (pH 3.0 or 2.0) stress, the decrease in P_n might be due to the inhibition of both the absorption of light energy and the conversion of light energy (Fig. 3). The addition of Si (1, 2 or 4 mM) to the SAR pH 3.0 or 2.0 treatments increased the chlorophyll content, F_v/F_m and Y (Figs. 2, 3), which led to increased photosynthesis (Table 1) compared to the corresponding single SAR treatment. This finding indicates that exogenous Si application can maintain a higher photosynthetic efficiency in plants under environmental stress [42, 43], which may be related to the improved absorption of light energy and conversion of light energy capacity to a certain extent.

Chloroplasts, as non-stomatal factors, are the sites of photosynthesis in plant cells [13, 46]. This study showed that when rice seedlings were treated with Si and SAR, the P_n was closely related to the damage and development of the chloroplast structure. When rice seedlings were exposed to the single Si and SAR (pH 4.0), the P_n increased, which might have been related to the ability of the Si and nutrient elements in the SAR treatments to promote the development of the chloroplast structure. The grana thylakoids and lamellar structure led to an increase in chlorophyll content and improvement of the absorption of light energy and conversion of light energy capacity (Fig. 3). Moderate and severe SAR damaged the chloroplast structure (Fig. 4I), which led to a decrease in the photosynthetic and the chlorophyll fluorescence parameters. Hu et al. [5] reported similar results. Under moderate and severe SAR stress, the Si addition improved the structure of chloroplasts and thylakoids, which led to an increase of the P_n and DW, which might have been related to the ability of the relatively complete chloroplast structure to maintain a high absorption of light energy and conversion of light energy capacity.

The starch content in the chloroplasts was obviously different in the leaves of the rice seedlings treated with Si and SAR. When the rice seedlings were exposed to the single Si and SAR (pH 4.0), the volume and number of starch grains decreased, which might be related to the ability of the Si and nutrient elements in the SAR treatments to promote the growth of rice seedlings by metabolizing a large number of photosynthetic products, thereby reducing the accumulation of starch in the chloroplasts. When rice was exposed to moderate SAR, the volume and number of starch grains were markedly increased; this finding could be related to acid rain's interference with the physiological metabolism of rice and damage to the cell and tissue structure of rice seedlings, which blocks photosynthate transport. When the SAR pH increased to 2.0, the starch grains disappeared, which might be related to the inhibition of photosynthesis [47, 48]. The addition of Si to moderate or severe SAR was significantly improved the accumulation of starch in chloroplasts, which could be because the exogenous Si improved the chloroplasts structures, increased the P_n, and promoted photosynthate transport.

In this study, we mainly focused on the crop seedling stage under hydroponic conditions, However, as basic research, this study provides good theoretical guidance for applied research. In short, acid rain affected the growth of rice seedlings. The incorporation of exogenous Si increased the P_n, G_s, chlorophyll content, F_v/F_m and Y; decreased the C_i; and improved the chloroplast ultrastructure. The photosynthesis effects were due to the changes in stomatic (G_s and C_i) and non-stomatic (chlorophyll content, F_v/F_m, Y, and chloroplast ultrastructure) factors, which are dominant depending on the concentration of Si and the pH of acid rain. The moderate concentration of Si was the most effective.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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