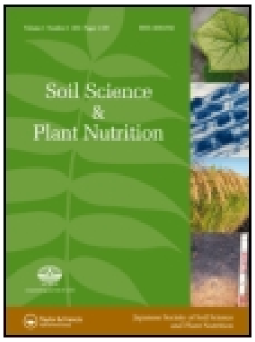

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Effects of Sulfur Nutrition on the Growth and Photosynthesis of Rice

Adoracion P. Resurreccion*,**, Amane Makino**, John Bennett*, and Tadahiko Mae**,[1]

**International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines; and **Department of Applied Plant Science, Graduate School of Agricultural Sciences, Tohoku University, Sendai, 981-8555 Japan*

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Rice plants (*Oryza sativa* L. cv. IR72) were grown hydroponically in 1.0 mM SO_4^{2-} for one week and transferred to 0, 0.01, 0.03, 0.1, 0.3, or 3 mM SO_4^{2-}. An increase in the sulfate concentration in the medium up to 0.03 mM resulted in a significant increase in the relative growth rate (RGR) due to the increase in the net assimilation rate (NAR). The leaf blade and leaf sheath showed a linear increase in the total S content at 0 to 0.1 mM SO_4^{2-} in the growth solution. Total S content in the roots continued to increase with increased S supply. Changes in the soluble S content followed the same pattern as the total S content but increased proportionally in the leaf sheath and roots. Total N content and N allocation to the leaves also decreased below 0.1 mM S. However, the decrease in the N content was not appreciable, compared with that of the S content. S-deficiency strongly decreased the leaf photosynthesis, which was caused by large decreases in the contents of chlorophyll and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) below 0.1 mM SO_4^{2-} in the growth solution.

Key Words: **growth, *Oryza sativa* L., photosynthesis, Rubisco, sulfur nutrition.**

Sulfur is an essential nutrient for plant growth and metabolism. Responses in crop growth and yield to the addition of S have been reported (Ergle and Eaton 1951; Rendig et al. 1976; Blair et al. 1979; Zhao et al. 1996, 1999). For optimal growth, sulfur requirement varies between 0.1 and 0.5% of the dry weight (Marschner 1995).

The shortage of the sulfur-containing amino acids cysteine and methionine in sulfur deficiency leads to chlorosis and inhibition of protein synthesis. Leaves of S-deficient plants have low chlorophyll contents (Dietz and Heilos 1990; Xu et al. 1996; Gilbert et al. 1997; Leoncini et al. 1997; Blake-Kalff et al. 1998). The decrease in the chlorophyll content caused by S deficiency is more evident in expanding young leaves than in old leaves (Dietz and Heilos 1990; Gilbert et al. 1997; Blake-Kalff et al. 1998). In sugar beets, the chlorophyll content decreases linearly with leaf S (Terry 1976).

Sulfur deficiency lowers ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) content and Rubisco enzyme activity (Gilbert et al. 1997; Sexton et al. 1997). In *Lemna minor*

[1] To whom correspondence should be addressed.

L., sulfur starvation led to the degradation of Rubisco even when the nitrate concentration (5 mM) was adequate (Ferreira and Teixeira 1992).

Reduced photosynthesis under sulfur-deficient conditions was observed in barley (Karmoker et al. 1991; Chen and Huerta 1997), rapeseed (Leoncini et al. 1997), spinach (Dietz and Heilos 1990), soybean (Sexton et al. 1997), and tomato (Xu et al. 1996). S deprivation decreased the CO_2 assimilation rates in all the leaves of young wheat seedlings (Gilbert et al. 1997). Photosynthetic carbon dioxide uptake decreased with a decrease in the total leaf S content from 2.5 to 0.5 mg g^{-1} dry weight in sugar beets (Terry 1976).

Although the requirements of higher plants for sulfur have long been recognized, interest has lagged behind that for other nutrients like N, P, and K (Zhao et al. 1999). The marked increase in crop yield results in increased nutrient removal from the soil; hence, it is predicted that sulfur deficiency will increase (Blair and Lefroy 1998; Hawkesford 2000). The use of fertilizers that do not contain sulfur and the decreasing deposition of sulfur from the atmosphere are also contributing to the incidence of sulfur deficiency.

Although rice is one of the three major crops in the world, studies on the effect of sulfur nutrition on rice have been very limited. Improvement in rice grain yield with sulfur fertilization has been documented (Blair et al. 1979). Critical S contents of rice plants as possible diagnostic indicators of S-deficiency have been reported (Islam and Ponnamperuma 1982). We conducted this physiological study on the effect of sulfur nutrition on rice because sulfur deficiency is becoming more widespread in Southeast Asia. We examined the effect of increasing levels of sulfur in the growth medium on early plant growth under high irradiance, photosynthetic rate, Rubisco content, chlorophyll content, and S allocation to different parts of the plant.

MATERIALS AND METHODS

Plant culture. Seeds of rice (*Oryza sativa* L. cv. IR72) were surface-sterilized, washed repeatedly with distilled water and germinated at 30°C for 72 h. The germinated seeds were put in demineralized water for 2 d and in the culture solution (described below) but with 1 mM K_2SO_4 plus $MgSO_4$ for 7 d. The seedlings were transferred to 2-L plastic pots containing the culture solution with 0, 0.01, 0.03, 0.1, 0.3, or 3 mM SO_4^{2-}. The culture solution with full SO_4^{2-} concentration contained NH_4NO_3 (1.4 mM), NaH_2PO_4 (0.3 mM), K_2SO_4 (0.5 mM), $CaCl_2$ (1.0 mM), and $MgSO_4$ (1.6 mM). The concentration of SO_4^{2-} was adjusted by substituting KCl for K_2SO_4 and $MgCl_2$ for $MgSO_4$. 3 mM SO_4^{2-} was prepared by the addition to the above solution of an appropriate volume of 0.09 M H_2SO_4.

Plants were grown in an indoor growth chamber under the following conditions: PPFD, 1,200 μmol m^{-2} s^{-1} at plant level; RH, 70%; light period, 12 h (6:00 A.M.–6:00 P.M.); day/night temperature, 29/24°C.

Plant growth analysis. Six plants per treatment were sampled on days 10 and 17. Leaf area was measured using a LI-COR 3100 area meter (Li-Cor, Lincoln, USA). Leaf blades, leaf sheaths, and roots were separated, dried at 80°C for 48 h, and weighed. Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) were calculated from the total dry weight and leaf area.

Gas exchange measurements. Leaf gas-exchange measurements on the second fully expanded leaf from the top of the main tiller were conducted using an IR gas analyzer (LI-6400, Li-Cor). External air was scrubbed of CO_2 and mixed with a supply of pure CO_2 to obtain a reference concentration of 400 μL L^{-1}. Flow rate was 500 μmol s^{-1}. Light intensity of 1,250 μmol m^{-2} s^{-1} was used. Relative humidity was 60–70%.

Rubisco and chlorophyll determination. The leaf blade was homogenized in 50 mM Na-phosphate (pH 7.0) containing 0.8% (v/v) 2-mercaptoethanol, 2 μM leupeptin, 0.01% (w/v) PMSF, and 5% (v/v) glycerol. The chlorophyll and Rubisco contents were determined on the leaf homogenate according to the method of Makino et al. (1994). Proteins in the leaf extract were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue R-250. The Rubisco bands were extracted with formamide and the amount determined spectrophotometrically (Makino et al. 1986). Purified Rubisco from rice leaves was used as standard.

Nitrogen and sulfur analysis. Ground oven-dried plant material was digested with K_2SO_4-Se and conc. H_2SO_4 according to the method of Varley (1966) and the amount of total N in the digest was determined colorimetrically as indo-phenol blue in a Technicon AutoAnalyzer II at 625 nm (Technicon AutoAnalyzer II Industrial Method No. 334-74W/B). Total S content was determined by digestion of the dry material with HNO_3 and $HClO_4$ (Johnson and Nishita 1952) and analysis using an ICAP 61-E Trace Analyzer (Thermo Jarell Ash, Franklin, USA) at wavelength 182.04 nm. Soluble S from dry plant material was extracted by stirring with water for 15 min in a boiling water bath. The extract was loaded directly into the spectrometer.

RESULTS AND DISCUSSION

Plant growth

Total plant dry weight after 17 d of S treatment increased with the SO_4^{2-} concentration in the medium with a maximum increase observed at 0.03 mM (Table 1). Above 0.1 mM SO_4^{2-}, there was no additional increase in the dry weight. The decrease in the shoot-root dry weight ratio below 0.03 mM SO_4^{2-} showed that sulfur affected the growth of shoots more than that of roots as had been reported in other plants (Marschner 1995). The effect of sulfur on the leaf area was similar to that on the plant dry weight. The results indicated that 0.03 mM SO_4^{2-} in the medium was a critical concentration for normal rice plant growth under the experimental conditions. A concentration of S above 0.1 mM did not affect the growth further.

The growth rates after transfer to solutions with different levels of S nutrition were analyzed (Fig. 1). In the earlier part of the S treatment (0–10 d), plant growth was not affected by the amount of S in the medium, presumably due to the utilization of S which had been stored during the one-week pretreatment with 1.0 mM SO_4^{2-}. After 10–17 d of S

Table 1. Plant biomass and total leaf area of rice plants after 17 d of sulfate treatment.

Sulfate treatment (mM)	Shoot g plant^{-1}	Root g plant^{-1}	Shoot/root ratio	Leaf area cm^2 plant^{-1}
0	0.33±0.06	0.26±0.06	1.30±0.09	38.6±8.1
0.01	0.54±0.07	0.31±0.05	1.76±0.06	54.4±9.3
0.03	1.00±0.08	0.50±0.02	1.97±0.09	92.6±12.8
0.1	1.18±0.11	0.53±0.04	2.25±0.04	97.7±10.5
0.3	1.20±0.11	0.52±0.03	2.26±0.14	90.7±15.4
3	1.20±0.13	0.54±0.05	2.21±0.09	100.3±13.9

Shoot weight, root weight, and leaf area values are the arithmetic means±SD (n=6). Values for shoot/root ratios are the geometric means.

treatment, when the stored S had been used up, RGR increased with an increase in the sulfate concentration in the growth solution up to the optimum level, 0.03 mM SO_4^{2-}. Since LAR remained constant, this increase in RGR was attributed to the increase in NAR.

Sulfur and nitrogen allocation

The leaf blade and leaf sheath showed a linear increase in the total S content as the sulfate concentration in the culture solution was increased from 0 to 0.1 mM (Fig. 2). Above a 0.1 mM SO_4^{2-} concentration, there was a minimal increase in the total S content of the leaves and sheath. The total S content in the roots increased nearly linearly above 0.03 mM SO_4^{2-}.

Changes in the soluble S content with the increase in S supply followed the same trend

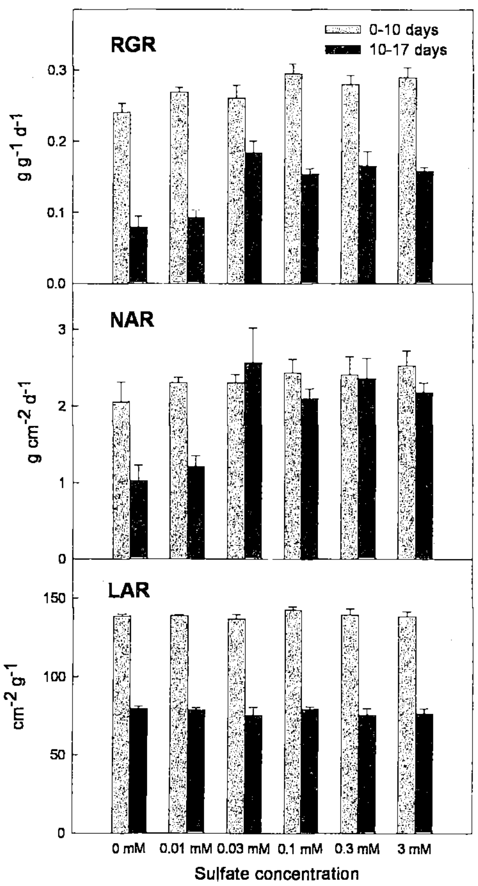

Fig. 1. Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) of IR72 plants between 0 to 10 d and between 10 and 17 d of growth in 0, 0.01, 0.03, 0.1, 0.3, and 3 mM SO_4^{2-}. RGR was calculated from the total plant dry weight, LAR from the ratio of total leaf area and plant dry weight, NAR from the ratio of RGR to LAR. The vertical bar on each column indicates the SD ($n=6$).

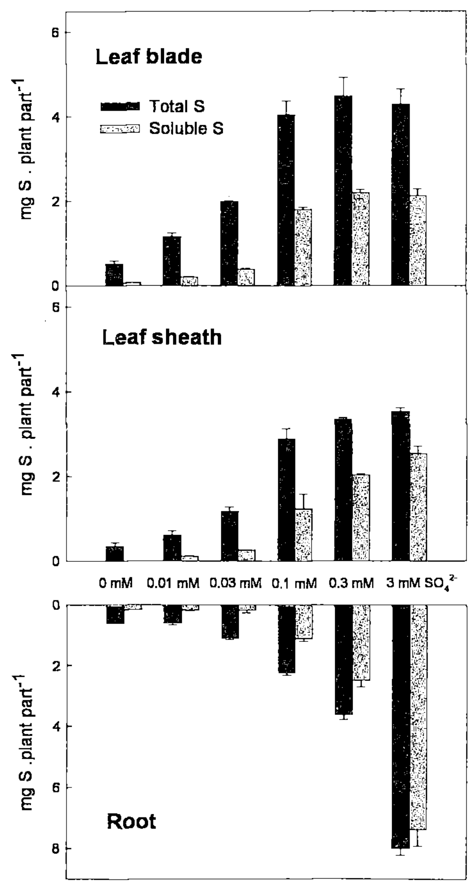

Fig. 2. Total S and soluble S contents in leaf blade, leaf sheath, and root of IR72 plants grown for 17 d in 0, 0.01, 0.03, 0.1, 0.3, and 3 mM SO_4^{2-}. The soluble S content in the leaf sheath in the 0 mM SO_4^{2-} treatment was below the detection limit. The vertical bar on each column indicates the SD ($n=3$).

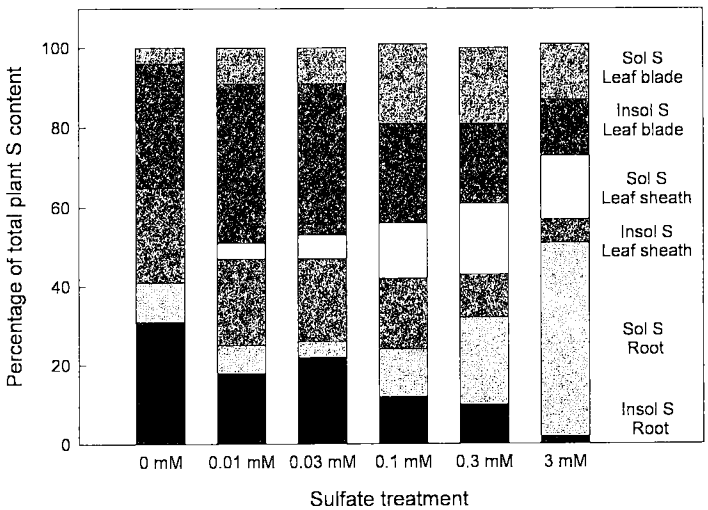

Fig. 3. Percentage value equivalent to the ratio of soluble S content/insoluble S content to whole plant total S in the leaf blade, leaf sheath, and root of IR72 plants after 17 d of growth in 0, 0.01, 0.03, 0.1, 0.3, and 3 mM SO_4^{2-}. The amount of insoluble S was obtained by the difference between the total S and the soluble S contents.

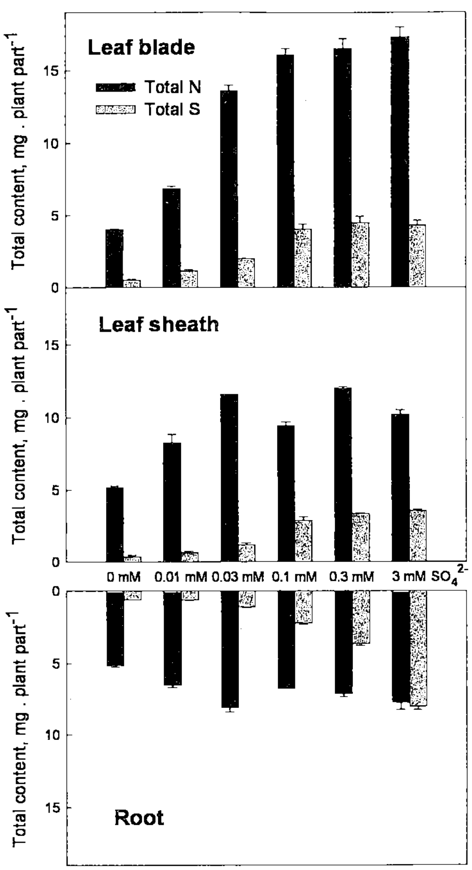

Fig. 4. Total N and total S contents in the leaf blade, leaf sheath, and root of IR72 plants grown for 17 d in 0, 0.01, 0.03, 0.1, 0.3, and 3 mM SO_4^{2-}. The vertical bar on each column indicates the SD (n=3).

as that of the total S content in all the plant parts. When the S concentration in the medium was high (0.1, 0.3, and 3 mM SO_4^{2-}), the amount of soluble S in the leaves was about 50% of the total S level. In the leaf sheath and roots, the proportion of soluble S to total S gradually increased with increasing SO_4^{2-} supply from 0.03 mM. In the plants grown in 3 mM SO_4^{2-}, about 70% of the total S content in the leaf sheath and more than 90% in the roots consisted of soluble S.

At the low sulfate supply of 0.01 and 0.03 mM, about half of the total plant sulfur was found in the leaf blades (Fig. 3). The other half was equally distributed in the leaf sheath and roots. The proportion of total S allocated to the leaves decreased from 44% (at 0.1 mM) to 27% (at 3 mM) while the root-S content increased from 24 to 50% of total S. Total sulfur allocation to the leaf sheath above a 0.1 mM SO_4^{2-} concentration decreased slightly compared to that of the leaf blade.

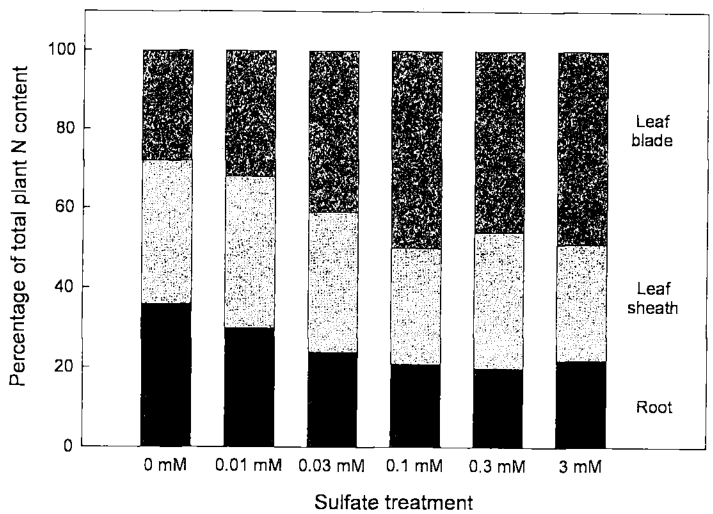

Fig. 5. Percentage value showing the ratio of plant part-N to whole plant-N in the leaf blade, leaf sheath, and root of IR72 plants after 17 d of growth in 0, 0.01, 0.03, 0.1, 0.3, and 3 mM SO_4^{2-}.

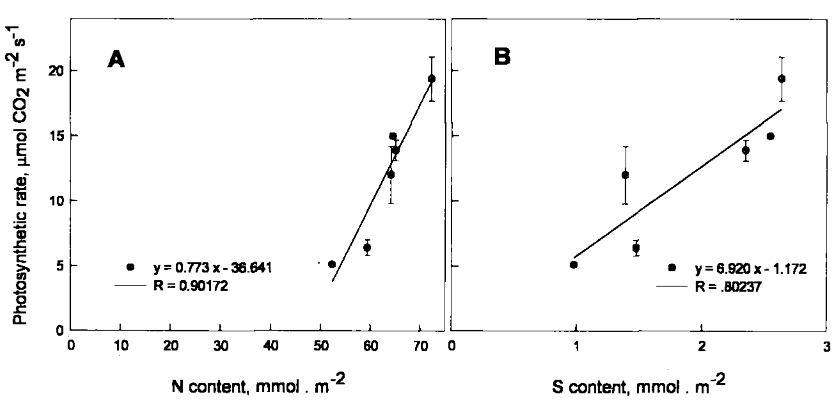

Fig. 6. Photosynthetic rate versus (A) N content and (B) S content of the second fully expanded leaf from the top of the main culm of IR72 plants grown in 0, 0.01, 0.03, 0.1, 0.3, and 3 mM SO_4^{2-}. Photosynthesis was measured at a PPFD of 1,250 μmol quanta m^{-2} s^{-1}. The vertical line across a point represents the SD ($n=3$).

The soluble S fraction consisted mostly of SO_4^{2-} based on turbidimetric analysis (AOAC Official Methods of Analysis 15th edition 1990). With increasing S supply and especially at higher SO_4^{2-} concentrations, a larger proportion of the soluble S remained in the roots. These results were not in agreement with the report showing that the uptake of SO_4^{2-} by the roots is down-regulated when the external S supply is sufficient, but increases in plants under S deficiency (Clarkson and Saker 1989; Hawkesford et al. 1993).

Figure 4 shows the total N and total S contents in the leaf blade, leaf sheath, and roots. Total N content of the all organs decreased with decreasing S concentration in the medium, and the decrease in the N content of the leaf blade was the most pronounced. Likewise, N allocation to the leaf blades decreased with decreasing S concentration in the medium (Fig. 5). There was a significant increase in the N content of the leaf sheath and roots when the amount of SO_4^{2-} (up to 0.03 mM) in the growth solution increased. However, SO_4^{2-} supply did not affect appreciably N allocation to the leaf sheath. The proportion of N in the roots

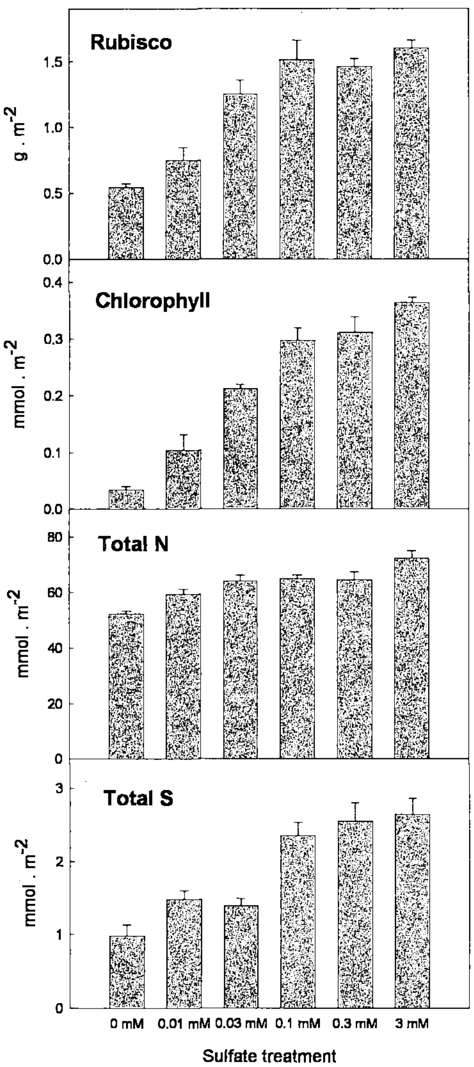

Fig. 7. Rubisco, chlorophyll, total N, and total S contents of the second fully expanded leaf from the top of the main culm of IR72 plants grown for 21 d in 0, 0.01, 0.03, 0.1, 0.3, and 3 mM SO_4^{2-}. The vertical bar on each column indicates the SD ($n=3$).

decreased as the S concentration in the medium increased to 0.1 mM. In the treatment above 0.1 mM S, N allocation to all the plant parts was almost constant. However, such effects on N were less conspicuous than those on the S content.

Although the plants were supplied with the same amount of N (2.8 mM), total plant N content varied. In the leaves, the most significant effect was observed from 0 to 0.03 mM SO_4^{2-} which is also the same concentration range where maximum increase of RGR was recorded. Calculation showed that the ratio of total N to insoluble S (difference of total S content and soluble S content) was almost constant, implying that the compounds were used for protein synthesis. In several crops grown under N-sufficient but S-deficient conditions, all the S in the plant was detected in the protein fraction (Stewart and Porter 1969). Total leaf N content was reported to be linearly related to the leaf S content in soybeans (Sexton et al. 1997). The present study also revealed the direct relationship of total N to total S in the plant under sulfur-limiting conditions (0–0.03 mM).

Photosynthesis, Rubisco, and chlorophyll contents

Light-saturated photosynthesis was determined using the second fully expanded leaf from the top of the main culm after 21 d of sulfur treatment. A plot of the photosynthetic rate against the leaf N content showed the presence of a linear relationship with $R=0.90$ (Fig. 6A). It had been previously reported that the maximum rate of CO_2 assimilation per unit leaf area is almost proportional to the amount of total N per unit leaf area (Mae 1997). However, our results showed that the x-intercept of the regression line was considerably high. In contrast, the regression line between photosynthesis and the S content almost passed through the origin (Fig. 6B). This finding indicated that the decrease in the total N content could not account for the decrease in photosynthesis and that photosynthesis was an increasing function of the sulfate concentration in the medium. In young leaves of wheat, the effects of S deprivation on the CO_2 assimilation rates were detected before any decrease in the dry weight was observed (Gilbert et al. 1997). Reduced photosynthesis with lower leaf S content has also been observed in other crops (Terry 1976; Xu et al. 1996; Leoncini et al. 1997; Sexton et al. 1997).

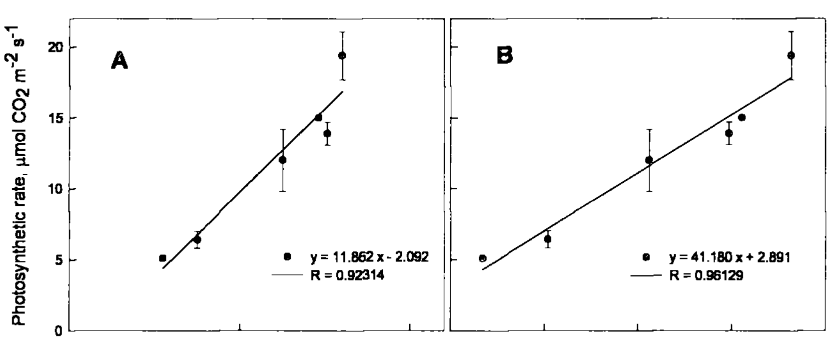

Fig. 8. Photosynthetic rate versus (A) Rubisco and (B) chlorophyll contents of the second fully expanded leaf from the top of the main culm of IR72 plants grown in 0, 0.01, 0.03, 0.1, 0.3, and 3 mM SO_4^{2-}. Photosynthesis was measured at a PPFD of 1,250 μmol quanta m^{-2} s^{-1}. The vertical line across a point represents the SD ($n=3$).

Although the total leaf N content decreased slightly with the decrease in the sulfate concentration in the medium, the Rubisco and chlorophyll contents of the second fully expanded leaf decreased largely below the 0.1 mM SO_4^{2-} treatment (Fig. 7). In addition, the decline in the chlorophyll content under sulfur deficiency was more remarkable than that of the Rubisco content. Leaf S content also declined with a decrease in the SO_4^{2-} concentration below 0.1 mM. The decrease in the chlorophyll and Rubisco contents observed with S deficiency has also been reported in rapeseed, spinach, and wheat (Dietz and Heilos 1990; Gilbert et al. 1997; Blake-Kalff et al. 1998).

Both Rubisco and chlorophyll contents showed a highly positive correlation with leaf photosynthesis (Rubisco, $R=0.92$; chlorophyll, $R=0.96$) (Fig. 8). The effect of the S content on photosynthesis was, thus, related to the effect of S supply on the Rubisco and chlorophyll contents, the two major components of the photosynthetic apparatus. The effect of the S content on the Rubisco content was expected because a functional molecule of Rubisco (with eight large subunits) contains a total of 120 to 168 cysteine and methionine amino acids (Miziorko and Lorimer 1988).

Therefore, it is concluded that S deficiency of rice occurring below 0.1 mM S supply led to large decreases in the chlorophyll and Rubisco contents, and consequently cause a decrease in photosynthesis and plant growth.

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