Sulfur Supplementation Enhanced the Growth and Photosynthesis of Lettuce in Hydroponic Production Using One-bag Complete Fertilizer

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Abstract. Sulfur (S) is an essential plant nutrient that regulates plant growth and metabolism. However, S is often absent from certain one-bag hydroponic fertilizers designed to provide a complete and balanced mixture of nutrients. We quantified the effects of S supplementation on the growth, morphology, and photosynthesis of lettuce grown in a deep-water culture hydroponic system. Two lettuce (Lactuca sativa) cultivars, green butterhead Rex and red oakleaf Rouxai, were grown using a prepackaged fertilizer specially formulated for reverse osmosis (RO) and other low-alkalinity water sources. The base nutrient solution was mixed using Jack’s FeED 12-4-16 fertilizer and RO water at a nitrogen concentration of 100 mg L⁻¹ (control). Three S supplementation treatments were implemented over a 4-week production period: 10 mg L⁻¹ supplemental S (provided using MgSO₄); 20 mg L⁻¹ supplemental S (MgSO₄); and a treatment using H₂SO₄ (instead of nitric acid) for pH adjustment. In both lettuce cultivars, shoot fresh and dry mass, total leaf area, leaf photosynthetic rate, total chlorophyll content, and leaf S concentration with all three S supplementation treatments increased significantly compared with those of the control. Notably, ‘Rouxai’ lettuce grown in the control treatment had intense red coloration with a 216.6% to 288.9% increase in the anthocyanin index. There were no statistical differences in any of the growth and morphological parameters among the three S supplementation treatments. Overall, we observed significantly enhanced lettuce growth and photosynthetic performance with S supplementation, resulting in a 144.0% to 215.9% increase in shoot fresh mass in the two cultivars compared with the control. Thus, we recommend that at least 10 mg L⁻¹ of S should be supplemented when growing lettuce hydroponically to ensure optimal plant growth, especially when S is absent or low in the fertilizer and water source.

Hydroponics is a technique used to grow plants in nutrient solution without mineral soil, often with the use of a soilless inert medium (Sharma et al. 2018). Hydroponic crop production is gaining popularity in the United States, with a growth rate of 1.8% each year over the past 5 years and an estimated revenue of $807.9 million in 2023 (US Specialized Industry Report 2023). Compared with conventional food crop production, hydroponic production has many advantages, such as high water and fertilizer use efficiency, easy environmental and pest control, and high crop yield and crop quality (Lee and Lee 2015). Because of these advantages, an increasing number of controlled environment agriculture productions are using hydroponic techniques. Hydroponic systems accounted for 53.7% of controlled environment agriculture production in the United States, as reported by the USDA-National Agriculture Statistics Service in 2019. Hydroponic systems can grow a wide range of crops, including tomatoes, cucumbers, peppers, leafy greens, and strawberries. Among these crops, tomatoes are the most cultivated, followed by lettuce (USDA-National Agriculture Statistics Service 2019).

Proper nutrient solution formulation and management are the cornerstones of successful hydroponic production and can have a significant impact on crop productivity and quality (Levine and Mattson 2021). Because the nutrient solution is the only source of mineral nutrients in a hydroponic system, it is of vital importance to provide all the essential mineral nutrients in optimal quantities when preparing a nutrient solution. Electrical conductivity (EC) of the nutrient solution is commonly measured in hydroponics as an indicator for the overall nutrient concentration. However, EC does not provide information regarding the concentrations of individual nutrients. It is not possible to determine whether a specific nutrient is absent or deficient solely by evaluating EC levels (Al Meselmani 2022). Thus, hydroponic growers must ensure that their nutrient solutions contain all required mineral elements, especially when pure water sources are used.

Commercial hydroponic growers often formulate nutrient solutions by blending fertilizer salts in-house or by using prepackaged complete fertilizers (Penn State Extension 2021; Walters et al. 2020). One-bag hydroponic fertilizers, designed to offer a complete and balanced blend of essential nutrients, are widely available on the market. These one-bag complete fertilizers are particularly popular among beginner hydroponic growers and hobbyists because of their ease of use (Penn State Extension 2021). A survey based on responses from 42 commercial hydroponic growers across 19 states in the United States found that 38% of growers used prepackaged complete fertilizers (Walters et al. 2020). However, a notable issue with one-bag complete fertilizers is that they may lack sulfur (S), an essential mineral nutrient.

Sulfur is a macronutrient essential for plant growth and metabolism (Shah et al. 2022). Deficiency in S during plant growth can significantly affect plant morphology and often leads to fewer and smaller leaves and reduced plant biomass (Bashir et al. 2020; Khalid et al. 2018; Resurreccion et al. 2001; Shah et al. 2022). Sulfur plays a critical role in the synthesis of amino acids (such as cysteine), enzymes, chlorophyll, proteins, and vitamins. Consequently, it is integral to many plant metabolic processes, including the regulation of enzyme activities, antioxidant metabolism (i.e., removal of reactive oxygen species through glutathione metabolism), photosynthesis, and respiration (Davidian and Kopriva 2010; Kopriva et al. 2019; Mukwevho et al. 2014; Tiwari and Gupta 2006). A deficiency of S in plants has been shown to reduce chlorophyll content and photosynthetic activities in various plant species (Astolfi et al. 2006; Bashir et al. 2020; Fatma et al. 2014; Lencioni et al. 1997; Lopez et al. 1996; Lunde et al. 2008; Sexton et al. 1997). Additionally, improvements in iron (Fe) translocation, phosphorous (P) and potassium (K) uptake, and salinity/toxicity tolerance have also been reported with an increased S supply in plants (Astolfi et al. 2018; Freitas et al. 2019; Wu et al. 2020). The effects of S supplementation on hydroponically grown crops have been moderately studied. For example, Chung et al. (2022) recently compared the effects of different S concentrations (64 mg L⁻¹ and 0.5 mg L⁻¹) on lettuce growth and metabolites using hydroponics and found increased shoot and root growth, chlorophyll and carotenoid contents, and metabolites such as amino acid and monosaccharides in lettuce treated with higher S concentrations. Furthermore, the
responses of different hydroponically grown lettuce cultivars to S supplementation were compared, with a red lettuce cultivar exhibiting greater tolerance to S deprivation compared with green cultivars (Abdalla et al. 2021). To date, limited studies have examined plant responses to S supplementation to the commercial one-bag complete fertilizers in hydroponic production. Because these fertilizers are generally marketed as “complete” fertilizers, many growers may be unaware of the S deficiency issue. Although S is likely to be present in water sources such as municipal water and well water, high salinity or alkalinity in these water sources can be found in many regions of the United States (Kauschal et al. 2018) and pose significant challenges to many commercial hydroponic operations. Growers often resort to using reverse osmosis (RO) water when they do not have access to good-quality water sources that meet the established water quality standards for hydroponic production (Van Os et al. 2016). In particular, some of the one-bag fertilizers are specially designed for use with pure water sources such as RO or deionized water.

The objective of this study was to investigate the responses of lettuce growth and quality to S supplementation in a hydroponic system. Specifically, this study aimed to quantify the effects of S supplementation to a commercial one-bag hydroponic fertilizer on lettuce growth and quality and identify the optimal level of S supplementation for lettuce production. The results from this study can help develop guidelines for S supplementation for hydroponic growers who use one-bag fertilizers that lack S.

Materials and Methods

Plant materials and growing conditions. Two experiments were conducted to examine the responses of two lettuce cultivars to S supplementation treatments in a hydroponic system. In Expt. 1, green butterhead lettuce ‘Rex’ seeds (Johnny’s Selected Seeds, Winslow, ME, USA) were sown in rockwool cubes (AC 15500 water plugs; 200 plug cells per 25.4 cm × 50.8 cm sheet; Grodan, Milton, ON, Canada) on 2 Jan 2023. Before seeding, the rockwool cubes were soaked in a dilute nutrient solution containing (in mg L⁻¹) 50 nitrogen (N), 7.3 P, 55.4 K, 29.2 calcium (Ca), 8.5 magnesium (Mg), 0.085 boron (B), 0.085 copper (Cu), 0.625 Fe, 0.2 manganese (Mn), 0.005 molybdenum (Mo), and 0.15 zinc (Zn) that was prepared by mixing RO water and a water-soluble fertilizer (12N-1.75P-13.3K; Jack’s Nutrients FeED 12-4-16 RO) according to the manufacturer’s instructions. The nutrient solution in each reservoir was oxygenated using a circular air stone (diameter, 20 cm; Active Aqua AS3RD; Hydrofarm) and an air pump (60-W; Active Aqua AAPA70L; Hydrofarm). A quantum sensor (SQ-500; Apogee Instruments, Logan, UT) and a thermometer and relative humidity sensor (Model EIE08-SS; Apogee Instruments) were installed and connected to a data logger (CR1000X; Campbell Scientific, Logan, UT, USA) to measure photosynthetic photon flux density, air temperature, and relative humidity inside the greenhouse every 1 s. The photosynthetic photon flux density was calculated from air temperature and relative humidity measurements. Additionally, a total of three type-J thermocouples were connected to the data logger. One thermocouple was inserted in a randomly selected reservoir tank in each of the three treatment blocks (see Experimental design) to continuously monitor the nutrient solution temperature. The data logger calculated both hourly and daily averages of the data. The average daily light integral, air temperature, relative humidity, and vapor pressure deficit in the greenhouse were 9.4 ± 5.68 mol m⁻² d⁻¹ (mean ± SD), 18.6 ± 3.63 °C, 50.1 ± 12.97%, and 1.18 ± 0.411 kPa, respectively, in Expt. 1, and 14.6 ± 6.33 mol m⁻² d⁻¹, 23.0 ± 1.83 °C, 59.1 ± 18.18%, and 1.17 ± 0.438 kPa, respectively, in Expt. 2. The average nutrient solution temperatures were 17.8 ± 3.15 and 23.2 ± 1.95 °C, respectively, in Expts. 1 and 2.

S supplementation treatments. The commercial hydroponic fertilizer used in this study is designed and marketed as a complete fertilizer for crops grown in low-alkalinity water such as RO water, rainwater, and distilled water. The fertilizer provides most of the essential plants nutrients such as plant N, P, K, Ca, Mg, B, Cu, Fe, Mn, Mo, and Zn. However, it notably lacks S. To quantify the effects of this fertilizer on plant growth and the potential benefits of S supplementation, we grew plants in a base fertilizer solution containing (in mg L⁻¹) 100 N, 14.6 P, 110.8 K, 58.3 Ca, 17 Mg, 0.17 B, 0.17 Cu, 1.25 Fe, 0.4 Mn, 0.01 Mo, and 0.3 Zn (control), and in two treatments with S supplementation at 10 mg L⁻¹ S and 20 mg L⁻¹ S, respectively, by adding magnesium sulfate (MgSO₄) to the base fertilizer solution during the initial fill of nutrient solution. Nitric acid (HNO₃) was used for pH adjustment in those three treatments throughout the course of the study. Additionally, we included a fourth treatment in which plants received the same base nutrient solution as the control, but sulfuric acid (H₂SO₄) was used for pH adjustment in place of nitric acid throughout the study. H₂SO₄ added during pH adjustment served as a source of S in this treatment. EC and pH were measured using an EC/pH meter (HI9814; GroLine Waterproof Portable pH/EC/TDS meter Hanna Instruments, Smithfield, RI, USA) every 2 d.

The average EC values for the control treatment, 10 mg L⁻¹ S, and 20 mg L⁻¹ S supplemental treatments, and the treatment with H₂SO₄ for pH adjustment (averaged from measurements taken every 2 d among three replicates per treatment) were 1.22, 1.22, 1.30, and 1.33 dS m⁻¹, respectively, in Expt. 1, and 1.58, 1.54, 1.53, and 1.45 dS m⁻¹, respectively, in Expt. 2. The average pH values were 5.78, 5.64, 5.79, and 5.68, respectively, in Expt. 1, and 5.83, 5.90, 5.88, and 5.80, respectively, in Expt. 2.

Throughout the course of the study, the cumulative amounts of added N from pH adjustment using HNO₃ in the control treatment, 10 mg L⁻¹ S, and 20 mg L⁻¹ S supplementation treatment were 4.21, 4.44, and 4.58 g, respectively, in Expt. 1. This would have increased the N level by 42.1, 44.4, and 45.8 mg L⁻¹, respectively, if all the N had been added to a 100-L nutrient solution at once. In Expt. 1, the cumulative amounts of added N were 7.12, 7.64, and 7.62 g, respectively. This would have increased the N level by 71.2, 76.4, and 76.2 mg L⁻¹, respectively, if added to a 100-L nutrient solution at once.

In the H₂SO₄ adjustment treatment, the cumulative amounts of added S were 4.33 g in Expt. 1 and 5.21 g in Expt. 2, which would have increased the S level by 43.3 and 52.1 mg L⁻¹, respectively, if the total amount of S was added to a 100-L nutrient solution at once.
Growth, pigmentation, and photosynthesis measurements. Lettuce shoot fresh mass (g/plant), shoot dry mass (g/plant), root dry mass (g/plant), and total leaf area (cm²/plant) were measured in both experiments. The shoot fresh mass, shoot dry mass, and root dry mass were calculated as the average of all 36 plants from each growing unit. At harvest, six representative plants from the middle of each growing unit were randomly selected to determine the total leaf area using a leaf area meter (LI-3000; LI-COR, Lincoln, NE, USA). The shoot dry mass and total root dry mass were measured after the shoots and roots were dried at 60 °C in a drying oven for 7 d. The ratio of shoot dry mass to fresh mass (shoot dry mass/fresh mass ratio) was calculated as the ratio of shoot dry mass to shoot fresh mass. The percentage of the root dry mass (%root dry mass) was calculated as root dry mass/(root dry mass + shoot dry mass) × 100. On the day of harvest, the leaf chlorophyll content (μmol·m⁻²) was estimated for six plants that were randomly selected from the middle of each growing unit by measuring the mature leaves with three averaged measurements per plant (MC-100 chlorophyll meter; Apogee Instruments, Logan, UT, USA).

To quantify the leaf anthocyanin content of the red oakleaf lettuce, measurements were performed for four randomly selected plants from each growing unit on the day of harvest. Five representative leaf disks were sampled from each plant using a cork borer with a diameter of 9.41 mm. These disks were submerged in 5 mL of extraction solution (5% 3M HCl + 15% H₂O + 80% C₂H₂O₅) (Gould et al. 2000) and placed in a refrigerator at 6 °C for 16 h. Subsequently, 3 mL of solution in each sample was transferred to a spectrophotometer vial (10-mm path length), and light absorbance of the extracted solution was measured at 530 nm and 653 nm using a spectrophotometer (GENESYS™ 180 ultra-violet-Visible Light; Thermo Fisher Scientific, Waltham, MA, USA). The anthocyanin index was determined using the equation described by Gould et al. (2000):

\[
\text{Anthocyanin index} = A_{530 \text{nm}} - A_{653 \text{nm}} \times 0.24
\]

Leaf photosynthesis measurements were conducted with a portable photosynthesis system equipped with a fluorometer (LI-6800; LI-COR Biosciences, Lincoln, NE, USA) 1 d before harvest. Light-response curves were conducted for plants grown in each growing unit, except for the 20 mg L⁻¹ S supplementation treatment because of time limitations. Recently mature lettuce leaves were exposed to eight light intensities in descending order, ranging from 1500 to 0 μmol m⁻² s⁻¹ (1500, 1250, 1000, 750, 500, 250, 50, 0 μmol m⁻² s⁻¹), provided by a built-in LED module of the fluorometer. The LED light was composed of 20% blue light and 80% red light. The temperature in the chamber was set at 25 °C, and the vapor pressure deficit was maintained at 1.0 kPa. The CO₂ concentration within the leaf cuvettes (C₀) was maintained at 400 μmol·m⁻² s⁻¹. Data were recorded after the leaves were provided with 3 min at each light level.

The photosynthetic “light response curve” data were fitted by nonlinear regression using SigmaPlot software (SigmaPlot 12.5; Systat Software Inc., Palo Alto, CA, USA). The following nonlinear exponential equation was fitted to photosynthetic light response data to estimate several photosynthetic parameters:

\[
A_n = A_{g, \text{max}} \times \left(1 - e^{-\alpha \frac{C_o}{C_d}}\right) - R_d
\]

where \(A_n\) is the net CO₂ assimilation rate, \(R_d\) is the dark respiration rate (expressed as a positive value), \(\alpha\) is the maximum quantum yield (calculated as the slope of the curve when photosynthetic photon flux density was zero), and \(A_{g, \text{max}}\) is the maximum gross photosynthetic rate.

Plant tissue analysis. Newly matured leaves of the harvested plants (30–50 g in fresh weight) were sampled from each experimental unit, oven-dried at 60 °C, and sent to a commercial laboratory at the Texas A&M University (https://soiltesting.tamu.edu) for a tissue elemental concentration analysis.

Experimental design and statistical analysis. Two separate experiments were conducted to quantify the effects of S supplementation on the growth and quality of two lettuce cultivars, and both experiments used a randomized complete block design with three blocks and four treatments. Three greenhouse benches were used in each experiment, with each serving as a block containing four hydroponic growing units. Three S supplementation treatments and the control treatment were randomly assigned to the growing units within each block. An experimental unit consisted of a hydroponic growing unit with 36 plants. Data from each experiment were analyzed separately. Individual plants in each treatment were treated as subsamples and averaged before the statistical analysis. Data were analyzed using an analysis of variance (ANOVA) and SAS software (SAS 9.4; SAS Inc., Cary, NC, USA). Means were separated and compared among the treatments using Tukey’s honest significance difference test. A predetermined \(P\) value of 5% (\(P < 0.05\)) was considered statistically significant for all analyses.

Results

Plant growth responses. The shoot fresh mass and dry mass in all three of the S
supplementation treatments (i.e., 10 mg L\(^{-1}\) S provided by MgSO\(_4\), 20 mg L\(^{-1}\) S provided by MgSO\(_4\), and H\(_2\)SO\(_4\) for pH adjustment) were significantly higher than those in the control treatment for both lettuce ‘Rex’ (Fig. 1A and C) and ‘Rouxai’ (Fig. 1B and D). Specifically, the shoot fresh mass in the S supplementation treatments increased by 144.0% to 169.4% in ‘Rex’ and by 209.7% to 215.9% in ‘Rouxai’ compared with their respective controls. There were no significant differences among the three S supplementation treatments. In contrast, the ratio of the shoot dry mass to fresh mass was significantly higher in the control than in the S supplementation treatments in both lettuce cultivars. In lettuce ‘Rex’, the ratio of shoot dry mass to fresh mass decreased from 0.06 in the control to 0.04 in all three S supplementation treatments (Fig. 1E). Similarly, in lettuce ‘Rouxai’, the ratio of shoot dry mass to fresh mass decreased from 0.05 in the control to 0.029 to 0.03 in the S supplementation treatments (Fig. 1F). Both lettuce cultivars produced a higher root mass in the control treatment (Fig. 2A and B). The percentage of root dry mass was significantly reduced in the S supplementation treatments in lettuce ‘Rex’ (Fig. 2C); additionally, it was 5.4% to 5.9% in the S supplementation treatments in lettuce ‘Rouxai’ (Fig. 2D). The total leaf area showed a similar trend as those of the shoot fresh mass and dry mass. The total leaf area was 136.5% to 167.6% higher in the S supplementation treatments than in the control of both lettuce cultivars (Fig. 3A and B). The leaf mass per area was the highest in the control treatment and lowest in the S supplementation treatments in lettuce ‘Rex’ (Fig. 3C). However, there were no statistical significant differences in leaf mass per area in lettuce ‘Rouxai’ (\(P = 0.077\)) (Fig. 3D). Overall, lettuce plants grown in the S supplementation treatments exhibited increased shoot mass and total leaf area but decreased root mass compared with the control plants. However, no significant differences in biomass, leaf area, and other growth parameters were found among the 10 mg L\(^{-1}\) S MgSO\(_4\), 20 mg L\(^{-1}\) S MgSO\(_4\), and H\(_2\)SO\(_4\) pH adjustment treatments.

### Photosynthetic responses

The leaf net photosynthetic rate of lettuce ‘Rex’ was significantly higher in both the 10 mg L\(^{-1}\) supplemental S treatment and the treatment with H\(_2\)SO\(_4\) for pH adjustment compared with the control, and the photosynthetic rate was similar between these two S supplementation treatments (Fig. 4A). Photosynthetic responses in plants grown under 20 mg L\(^{-1}\) supplemental S treatment were not measured because of time constraints. The dark respiration rate was significantly higher in the 10 mg L\(^{-1}\) supplemental S treatment than that in the control, which had the lowest dark respiration rate (Fig. 4A magnified). Similar photosynthetic responses were observed in lettuce ‘Rouxai’ (Fig. 4B magnified).

### Pigmentation and leaf mineral element concentrations

The total chlorophyll concentration was significantly increased in the S supplementation treatments compared with the control in both lettuce ‘Rex’ (Fig. 5A) and ‘Rouxai’ (Fig. 5B). However, there were no differences among the S supplementation treatments. In contrast, the anthocyanin index was significantly reduced in the S supplementation treatments in red oakleaf lettuce ‘Rouxai’ (Fig. 5C). The anthocyanin index was reduced by 69.9%, 68.4%, and 74.3% in plants grown in the 10 mg L\(^{-1}\) S MgSO\(_4\), 20 mg L\(^{-1}\) S MgSO\(_4\), and H\(_2\)SO\(_4\) pH adjustment treatments, respectively, compared with the control.

The leaf S concentration was significantly increased in the S supplementation treatments in both lettuce ‘Rex’ and ‘Rouxai’ (Fig. 6). Specifically, the S concentration was 0.785 mg g\(^{-1}\) in the control treatment, which was increased by 269.8%, 216.6%, and 187.3% in the 10 mg L\(^{-1}\) S MgSO\(_4\), 20 mg L\(^{-1}\) S MgSO\(_4\), and H\(_2\)SO\(_4\) pH adjustment treatments, respectively, in ‘Rex’ lettuce (Fig. 6A). In ‘Rouxai’ lettuce, the S concentration in the control was 0.746 mg g\(^{-1}\), and it was increased by 332.7%, 298.2%, and 308.0% in the same respective treatments (Fig. 6B).

In addition to S, the leaf N concentration differed among the four treatments. In lettuce ‘Rex’, the leaf N concentrations were 5.15% in the control treatment and 6.73%, 6.77%, and 6.68% in the 10 mg L\(^{-1}\) S MgSO\(_4\), 20 mg L\(^{-1}\) S MgSO\(_4\), and H\(_2\)SO\(_4\) pH adjustment treatments, respectively (Supplemental Table 1). In lettuce ‘Rouxai’, however, the N concentration was significantly higher in the control (5.8%) than in the 10 mg L\(^{-1}\) S MgSO\(_4\) (4.74%), 20 mg L\(^{-1}\) S MgSO\(_4\) (4.86%), and H\(_2\)SO\(_4\) pH adjustment treatments (4.94%), respectively (Supplemental Table 2). Other mineral elements, including P, K, Ca, Mg, Na, Zn, Fe, Cu, and B, showed no significant differences among the four treatments in either lettuce cultivar.

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**Fig. 2.** Root dry mass and percentage of root dry mass of lettuce ‘Rex’ (A, C) and lettuce ‘Rouxai’ (B, D) grown in the control treatment, 10 mg L\(^{-1}\) supplemental sulfur (S) (10 ppm S MgSO\(_4\)) treatment, 20 mg L\(^{-1}\) supplemental S (20 ppm S MgSO\(_4\)) treatment, and the treatment with H\(_2\)SO\(_4\) for pH adjustment (H\(_2\)SO\(_4\)) pH adjustment). Data represent the means ± SE of three independent replications (n = 3; individual plants in each treatment were treated as subsamples and averaged before the statistical analysis). Different letters indicate a significant difference (\(P \leq 0.05\), Tukey’s honest significance difference test) among the treatments.
Discussion

Lettuce biomass was enhanced by S supplementation. In this study, two lettuce cultivars, green butterhead Rex and red oakleaf Rouxai, were grown hydroponically using a nutrient solution mixed from one-bag hydroponic fertilizer and RO water. Three different S supplementation treatments (10 mg L⁻¹ S
MgSO₄, 20 mg L⁻¹ S MgSO₄, and H₂SO₄ pH adjustment) were applied, and the nutrient solution without S supplementation was set as a control. Biomass production of both cultivars was significantly increased with S supplementations compared with the control (Fig. 1). This is consistent with a previous study in which lettuce growth was promoted by the supplementation of S in a hydroponic production system (Abdalla et al. 2021). Sulfur is an essential plant macronutrient, and the deficiency of S can negatively affect crop growth (Hawkesford 2000; Kopriva et al. 2016; Resurreccion et al. 2001). Chung et al. (2022) found that hydroponically grown lettuce (‘Cheongchima’) treated with a high S concentration (2 mM or 64 mg L⁻¹) had a significantly higher fresh weight than that

Fig. 3. Leaf area (A, B) and leaf mass per area (C, D) of lettuce ‘Rex’ (A, C) and lettuce ‘Rouxai’ (B, D) grown in the control treatment, 10 mg L⁻¹ supplemental sulfur (S) (10 ppm S MgSO₄) treatment, 20 mg L⁻¹ supplemental S (20 ppm S MgSO₄) treatment, and the treatment with H₂SO₄ for pH adjustment (H₂SO₄ pH adjustment). Data represent the mean ± SE of three independent replications (n = 3; individual plants in each treatment were treated as subsamples and averaged before the statistical analysis). Different letters indicate a significant difference (P < 0.05, Tukey’s honest significance difference test) among the treatments.

Fig. 4. Leaf net photosynthetic rate of lettuce ‘Rex’ (A) and lettuce ‘Rouxai’ (B) grown in the control treatment, 10 mg L⁻¹ supplemental sulfur (S) (10 ppm S MgSO₄) treatment, and the treatment with H₂SO₄ for pH adjustment (H₂SO₄ pH adjustment) under different photosynthetic photon flux densities (0 to 1500 μmol m⁻² s⁻¹). Data represent the mean ± SE of three independent replications (n = 3). Different letters indicate a significant difference (P < 0.05, Tukey’s honest significance difference test) among the treatments.
The ratio of shoot dry mass was significantly reduced in the S supplementation treatments compared with the control (Fig. 1E and F). The ratio of shoot dry mass to fresh mass was an indicator of the leaf water status, and a lower ratio represents a higher relative water content of the plant. Supplementation of S has been reported to increase the plant relative water content (Aziz et al. 2019; Namvar and Khandan 2015). Bouranis et al. (2003) similarly found an increased plant relative water content and reduced ratio of dry mass to fresh mass when S was added, which was consistent with our study.

In contrast to the lettuce shoot biomass, the root dry mass values of both lettuce cultivars were significantly higher in the control treatment than those in the S supplementation treatments (Fig. 2). The enhanced root growth might be a result of plant acclimation under S deficiency conditions to improve the ability of acquiring more nutrients from the nutrient solution, which is a response similar to that observed with N and P deficiencies (Hermans et al. 2006). The N-deficient and P-deficient plants were found to have a reduced shoot to root ratio, whereas this ratio was rarely altered in K-deficient and Mg-deficient plants (Hermans and Verbruggen 2005; Scheible et al. 2004). This indicated that the plant root growth response to different nutrient deficiencies is nutrient-specific. Hermans et al. (2006) further suggested that the reduction in the shoot-to-root ratio under N and P deficiencies was linked to alterations in the carbohydrate metabolism with the increased transport of sucrose from the shoot to the root. This could help explain the increases in root growth under S deficiency observed during our study. Additionally, plants adopt several metabolic pathways to adapt to S deficiency, including through the S metabolic pathway or interaction with the metabolic pathways of other nutrients (Kopriva et al. 2015; Lewandowska and Sirko 2008). For example, the breakdown of indole glucosinolates to form auxin in roots under S-deficient conditions, which results in physiological and morphological changes in roots, such as increased root length, root-to-shoot ratio, and enhanced root biomass to improve the S uptake (Falk et al. 2007; Parisa-Tanaka et al. 2020).

Sulfur supplementation increased the lettuce total leaf area in both lettuce cultivars (Fig. 3A and B). Sulfur deficiency often resulted in a reduced plant leaf area (Bouranis et al. 2003; Burke et al. 1986; Kastori et al. 2000; Riffat and Ahmad 2020), which was consistent with our findings (Fig. 3A and B). However, the leaf mass per area of lettuce ‘Rex’ was significantly reduced in the S supplementation treatments (Fig. 3C), indicating reduced leaf thickness and/or density. A similar trend in leaf mass per area was observed in lettuce ‘Rouxai’, although the data were not statistically significant (Fig. 3D). In general, changes in leaf mass per area caused by nutrient limitations are more of an alteration in the leaf thickness than in the thickness (Poorter et al. 2009). Therefore, the higher leaf mass per area of ‘Rex’ lettuce grown in the control treatment was most likely a result of increased leaf density caused by S deficiency.

Chlorophyll and anthocyanins content. The S supplementation significantly affected both chlorophyll and anthocyanin contents in lettuce. The total chlorophyll content was significantly increased by the addition of S in nutrient solution in both lettuce cultivars (Fig. 5A and B). Sulfur deprivation has been reported to reduce the chlorophyll content in plants (Bouranis et al. 2003; Burke et al. 1986; Chung et al. 2022; Namvar and Khandan 2015; Resurreccion et al. 2001). A direct cause of the reduced chlorophyll content might be the deficiency in the biosynthesis of the Fe–S cluster (essential for chlorophyll biosynthesis) under S-deficient conditions.
(Hu et al. 2017). Interestingly, in red oakleaf lettuce ‘Rouxai’, a significantly higher anthocyanin content was observed in the control treatment than in the S supplementation treatments (Fig. 5C). A previous study found that S deficiency could activate the expression of phenylpropanoid and flavonoid pathway genes. The accumulation of anthocyanins under S-deficient conditions was probably a mechanism to protect the plant from stress conditions such as high light because plants become more sensitive to high light stress under S-deficient conditions (Nikiforova et al. 2003). Consistent with our findings, Gao et al. (2016), Lunde et al. (2008), and Zhou et al. (2013) also found an increased anthocyanin level in S-deficient plants.

Photosynthesis was improved by S supplementation. Reduced photosynthesis under S deficiency have been reported by many studies (Ahmad et al. 2005; Kastori et al. 2000; Resurreccion et al. 2001; Waraich et al. 2022). Similarly, we found that the leaf photosynthetic rate increased significantly in the S supplementation treatments compared with the control (Fig. 4). Additionally, the light response curve indicated that the maximum photosynthetic rate of lettuce was significantly higher in the S supplementation treatments than in the control (Fig. 4). Sulfur plays a key role in photosynthesis and the electron transport system (Shah et al. 2022). The deficiency of S leads to lower contents of chlorophyll and carboxylation enzyme Rubisco, which are key factors directly involved in the photosynthetic processes (Akoumouche et al. 2019; Khan et al. 2015; Masood et al. 2012). Particularly, Lunde et al. (2008) found that the chlorophyll content and Rubisco content reduced by two-fold and six-fold, respectively, in S-deficient rice plants. A lower Rubisco content can generally cause a reduced photosynthetic rate. Sulfur deficiency was also found to reduce the photosystem I activity and electron transport capacity of photosystem I (Lunde et al. 2008), which resulted in a limited cyclic electron transport rate; furthermore, a 25% to 30% higher nonphotochemical quenching was found in S-deficient plants that mainly originated from high ΔpH caused by fewer electrons leaving the thylakoid lumen. Overall, the lower photosynthetic rate of the lettuce plants grown in the control treatment was probably a result of the reduced capacity to harvest and use light energy because of reductions in chlorophyll, Rubisco content, and photosystem I activity. Additionally, we observed that the dark respiration rate increased in the S supplementation treatments compared with the control (Fig. 4 magnified), which was consistent with previous findings that S deficiency led to a reduction in the dark respiration rate in bean plants and sugar beet (Juszczuk and Oastweswiska 2011; Kastori et al. 2000; Sexton et al. 1997).

Optimizing nutrient formulation for hydroponic lettuce production. In hydroponics, the nutrient solution is the sole source of S. A lack of S in the hydroponic fertilizer was found to significantly reduce lettuce growth in our study. Because S deficiency appears to be a prevalent issue in one-bag complete fertilizers, it is necessary for fertilizer manufacturers to recognize the need for S supplementation and provide comprehensive fertilizer application instructions, especially if pure water sources like RO and deionized water are used. Based on our study, we recommend that S should be supplemented at a concentration of 10 mg L⁻¹ for hydroponic lettuce production. However, additional studies may be needed for specific crops with different S requirements such as cruciferous and allium vegetables (kale, arugula, and onions).

Conclusions
During this study, we supplemented S to nutrient solution mixed from a one-bag complete water-soluble fertilizer and RO water in hydroponic lettuce production. Our results indicated that supplementing S significantly improved biomass and photosynthetic responses in lettuce ‘Rex’ and ‘Rouxai’, and that 10 mg L⁻¹ of S supplementation is adequate for enhancing lettuce growth. We recommend that hydroponic growers should use one-bag complete fertilizers, and that growers using pure water sources should supplement S if it is lacking. Considering that some of the one-bag fertilizers are marketed as “complete” sources for all essential mineral nutrients, we strongly recommend that fertilizer manufacturers should include comprehensive fertilizer application instructions including guidelines for S supplementation as appropriate.

References Cited


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