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Combination Effects of Sulfur Fertilizer and Rhizobium Inoculant on Photosynthesis Dynamics and Yield Components of Soybean

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Abstract: Sulfur (S) fertilization and rhizobium inoculation are important agronomic practices for improving soybean growth and yield. However, their combined effects on yield components and the resultant impacts on yield need further investigation. Our study aimed to verify the combined effects of S fertilizer and rhizobium inoculant on the yield components and seed yield of soybeans, as well as photosynthesis dynamics during the V5–R6 growth stages. A randomized block design incorporating two factors was employed for field experiments on soybean, involving the application of five rates of sulfur (0, 5.0, 9.8, 14.8, and 19.6 kg S ha^{-1}) with rhizobium inoculation or without. A reduction of 50% in mineral fertilizer according to farmer practice (FP) was applied alongside different rates of sulfur for field experiments on soybeans. The findings indicated that the application of sulfur fertilization significantly enhanced soybean yield by increasing the number of grains per plant (NG) and the number of pods per plant (NP). Maximum grain yield was reached under treatment with an S rate of 19.6 kg S ha^{-1}, which increased NG by 39% more than an S rate of 0 kg S ha^{-1}. Correlation analysis indicated that higher photosynthesis was closely associated with increased yield components. This study demonstrated that applying S fertilizers could improve soybean production by combining the appropriate sulfur concentration and rhizobia inoculation.

Keywords: soybean; sulfur fertilization; rhizobium inoculation; yield components; yield

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1. Introduction

Soybean [*Glycine max* (L.) *Merr.*] has been grown as an important oil and protein crop for thousands of years worldwide [1]. As a leguminous crop, soybeans can establish symbiotic associations with rhizobium bacteria to fix atmospheric nitrogen (N) for its growth through root nodule formation [2]. Sulfur (S) is a crucial nutrient for soybeans, influencing a range of physiological and metabolic processes such as photosynthesis, nitrogen fixation, and protein synthesis [3–5]. It makes some contributions to protein synthesis by orchestrating the folding and structural stability of a protein [6]. Some research showed the positive effect of sulfur on the yield of oil seed crops, such as groundnut [7], rapeseed [8], mustard [9], sesame [10], sunflower [11], and soybean [12,13]. Several field studies have demonstrated that the application of sulfur in conjunction with nitrogen in soybean fields has resulted in a significant increase in seed yield. Moreover, the application of S fertilizers and rhizobium inoculants is an important agronomic practice to improve legume crop productivity [14–16]. Previous studies have evaluated the effects of S fertilization or rhizobium inoculation on soybean yield and reported positive impacts [17,18]. Inoculation with Rhizobium resulted in significant increases in dry matter accumulation, nitrogen content, harvest index, and overall soybean yield [19]. It was shown that inoculation with Rhizobium spp. promoted soybean growth in comparison to non-inoculated plants. The impacts of inoculation had a significant on the number of pods and the dry weight of seeds [20]. Moderate S deficiency has been confirmed to reduce grain yield by affecting crop growth in the duration of seed fill. In general, the use of S fertilizer increases protein fractions in seeds and is essential in optimizing grain yield through proper S application and improving storage proteins in soybeans [21,22]. An investigation was conducted on soybeans to detect the performance of plants at different rates of sulfur under Rhizobium inoculation. The result revealed that 30 kg S/ha with rhizobium inoculation gave the maximum pod numbers per plant and grain yield. The interaction between Rhizobium and sulfur showed significant differences in pods per plant, yield, and oil content [23]. There is a paucity of information concerning the combined effects of S fertilization and rhizobium inoculation on photosynthesis and subsequent yield formation. Further research is needed to explore the interactive impact of these factors on leaf photosynthetic properties and their correlation with yield components.

Field studies should be conducted to evaluate different combination treatments of S fertilizer rates and rhizobium inoculum on the photosynthesis and yield parameters of soybeans. Correlation and regression analyses are needed to establish quantitative relationships between physiological parameters and yield components. Such results would help determine optimal combination rates of S fertilization and rhizobium inoculation for improving soybean productivity.

The objectives of our study were to (i) quantify the effects of sulfur fertilization at different rates, specifically 0, 5.0, 9.8, 14.8, and 19.6 kg S ha^{-1}, in combination with farmer mineral fertilizer practice; (ii) evaluate the influence of sulfur fertilizer in combination with reducing the use of mineral fertilizer on yield and quality traits of soybean; and (iii) analyze the relationship between these physiological parameters and yield components under the different S rates with rhizobium inoculation. Understanding these dynamics will provide insight into optimal S fertilization rates for achieving high soybean productivity or quality.

2. Materials and Methods

2.1. Experimental Site

Field experiments were carried out during the soybean growing season in 2021 at the Agricultural Experiment Station of Heilongjiang Academy of Agriculture Sciences, in Minzhu Town, Harbin City, Heilongjiang Province, China (45.78° N, 126.65° E). The experimental site has a semi-humid continental monsoon climate with a mean annual rainfall of 500–600 mm, with most of the rainfall occurring from May to September. The experimental field is located in the black soil region, which is a soil type called the phaeozem. The average temperature in summer is 21.6 °C. Soil properties are shown in Table 1.

Table 1. Location and characteristics of experimental sites and the soil textures of fields.

Location/Site	Longitude	Latitude	Elevation (masl)	State	Previous Crop (s)	Soil Texture
Research Station of Heilongjiang Academy of Agricultural Sciences, Harbin	126.65° E	45.78° N	110	Heilongjiang Province, China	Maize	phaeozem soil
Soil properties						
pH	soil organic matter (g/kg)	AN, available nitrogen (mg/kg)	AP, available phosphorus (mg/kg)	AK, available potassium (mg/kg)	TN, Total N (g/kg)	
6.71	29.4	127.5	59.7	211	1.52	

2.2. Experimental Design and Treatments

The experimental arrangement is a split-plot design with three replicates, where the main plot comprised six fertilizer treatments applied with sulfur fertilizer in combination with reducing the amount of mineral fertilizer. Rhizobium inoculation (no inoculation (NI) and inoculation (I)) was the split plot. The experiment included five sulfur fertilizer application rates: 0, 5.0, 9.8, 14.8, and 19.6 kg of elemental sulfur per hectare (kg S ha^{-1}). The S fertilizer was applied in the form of Ammonium Thiosulfate (ATS) in the irrigation water at the specified rates for growing plants. The fertilization treatments were determined as follows: FP (farmer practice (FP), N–P_2O_5–K_2O: 14–18–16, 270 kg/hm^2); T1 (reduce the amount of FP of by 50% + 0 kg S ha^{-1}); T2 (reduce the amount of FP of by 50% + 5.0 kg S ha^{-1}); T3 (reduce the amount of FP of by 50% + 9.8 kg S ha^{-1}); T4 (reduce the amount of FP of by 50% + 14.8 kg S ha^{-1}); T5 (reduce the amount of FP of by 50% + 19.6 kg S ha^{-1}), and NF (no fertilizer for soybean) as a control. The nitrogen content was adjusted by urea according to ATS dosage to ensure the same level of nitrogen fertilizer in each treatment, as shown in Table 2. The rhizobia strain, SN7-2, was provided by the Department of Horticulture, Zhejiang University. The seeds were coated with a peat-based rhizobial inoculant before planting. Each plot was 6.5 m wide (10 rows, 65 cm row spacing), and each row was 20 m long. The soybean cultivar Heinong 84 was adopted according to the farmer's practice.

Table 2. Combination of the levels of mineral fertilizer and starter sulfur fertilizer application based on farmer practice (FP) in soybeans.

Treatments	Description	Mineral Fertilizer			Starter Sulfur Fertilizer (ATS)	
		N Rate (kg ha^{-1})	P_2O_5 Rate (kg ha^{-1})	K_2O Rate (kg ha^{-1})	N Rate (kg ha^{-1})	S Rate (kg ha^{-1})
NF	No fertilizer (NF)	0	0	0	0	0
FP	farmer practice (FP) (N–P_2O_5–K_2O: 14–18–16, 270 kg/hm^2)	37.8	48.6	43.2	0	0
T1	Reduce the amount of FP by 50% + 0 kg S ha^{-1}	18.8	24.3	21.6	0	0
T2	Reduce the amount of FP by 50% + 5.0 kg S ha^{-1}	16.6	24.3	21.6	2.2	5.0
T3	Reduce the amount of FP by 50% + 9.8 kg S ha^{-1}	14.2	24.3	21.6	4.6	9.8
T4	Reduce the amount of FP by 50% + 14.8 kg S ha^{-1}	12.0	24.3	21.6	6.8	14.8
T5	Reduce the amount of FP by 50% + 19.6 kg S ha^{-1}	9.8	24.3	21.6	9.0	19.6

All treatments include two split-plot designs of inoculation (I) and no inoculation (NI).

2.3. Field Agronomic Practices

Fall moldboard plowing was conducted to a depth of 30 cm and the field was cultivated to a depth of 10 cm before sowing in spring. All straw from the crop was returned to the field after harvesting. The soybean cultivar 'Heinong 84' was seeded at a rate of 60 kg ha^{-1} and the plant population was maintained at 220,000 ha^{-1} by hand thinning after one week of emergence. The planting date was 15 May. Herbicides were applied as needed to control vegetation before planting, and pre-emergence herbicides were spread soon after planting. Post-emergence herbicides were applied if insufficient treatment was given before emergence. The agronomic practices in this experiment were maintained uniformly for all plots.

2.4. Measurements and Sampling

The chlorophyll concentrations of the leaves were determined by a chlorophyll meter (SPAD-502 Plus, Konica Minolta, Tokyo, Japan). The leaf area index (LAI) was defined by a portable leaf area index analyzer (MIJ-LAI/P, Fukuoka, Japan). The net photosynthesis rate (Pn), intercellular CO_2 concentration (Ci), stomatal conductance (Gs), and transpiration rate (E) were measured using a portable photosynthesis system (Li-6400, LI–COR Inc., Lincoln, NE, USA) equipped with an LED leaf chamber. Physiological measurements were conducted on a sunny day between 10:00 a.m. to 1:00 p.m. under a CO_2 concentration of 400 umol mol^{-1} during the V5–R6 stages of soybean growth.

Ten replicate plants per plot enhance the representativeness of the measurements for the plot conditions. Plant height (PH) was determined by ten randomly chosen plants one week before harvesting in each plot. Number of nodes (NN), number of branches (NB), number of grains per plant (NG), number of pods per plant (NP), grain mass per plant (PW), and 100-seed Weight (HW) were also measured per plot. Four rows in the middle of the plot were harvested for yield measurement by a commercial plot harvester. Grains were dried to constant moisture and weighed as data to measure the biological yield of soybeans. Samples from each crop plot were analyzed for crude protein and oil using a near-infrared (NIR) analyzer (Perten, Springfield, IL, USA). The calibration curve was validated according to AOAC methods for unique samples.

2.5. Statistical Analyses

All the data were examined for a normal or homogeneous distribution. Shapiro–Wilk tests and Levene's tests were needed for ANOVA assumptions such as normality and homogeneity of variance. Data applied in figures and tables were computed independently for three replicates. The data were analyzed with IBM SPSS 19 software for Windows.

3. Results

3.1. Effects on Yield and Its Components

The ANOVA showed that S treatments and rhizobium inoculation had a significant effect on the soybean yield (Table 3). For yield components, a significant effect of S treatments on NB, NP, NG, PW, and HW was found. However, no significant effect of rhizobium inoculation on NN, NB, and NP was detected. NP, NG, PW, and HW showed significant differences in the treatments × inoculation interaction. Overall, soybean yields inoculated with rhizobium had an average higher yield value of 3.90 Mg ha^{-1} compared to those without inoculation. It has been shown that plants inoculated with rhizobium had higher average NG and PW values of 102.92 and 21.02 compared to uninoculated plants, but no significant differences for NN, NB, NP and HW, were detected. The S treatments significantly increased 100-seed Weight (HW) and demonstrated considerable differences among treatments. The highest PW and HW values of 21.37 and 21.04 were obtained under T5 treatment. Furthermore, applying 19.6 kg S ha^{-1} increased the NG by 39% more than when T1 was applied. The S treatments also changed the NB and NP among the different S rates applied (Table 3).

In addition, sulfur treatments considerably affected the seed yield when we compared the average value among treatments, as shown in the column with lowercase letters (Figure 1). Applying S substantially increased the soybean yield under treatments T2, T3, T4, and T5 when compared to T1. On average, under treatments T5 and T4, the yield of soybeans increased by 22% and 15% in comparison with T1, respectively. The soybean yield increased with S fertilization, which was significantly higher than NF. The maximum yield of soybeans was obtained under T5 (reduce the amount of FP by 50% + 19.6 kg S ha^{-1}). Compared with the FP (farmer practice), yield under the T5 treatment produced a 4.3% higher seed yield. Furthermore, when the average value between inoculation and non-inoculation was compared, as shown in the differently colored columns (Figure 1), the yield of soybean was found to largely depend on the interaction of S treatments × inoculation. The statistically significant effect of seed inoculation on the yield of soybean was found under the T5 treatment. The result showed that the yield increased with rhizobium inoculation by 22.2% when compared to uninoculated seeds in this treatment. However, no statistical significance was found for the inoculation effect on the yield of soybeans under FP and T2 treatment (Figure 1).

Table 3. Effect of sulfur treatments and rhizobium inoculation on yield, plant height (PH), number of node (NN), number of branch (NB), number of pods (NP), number of grains per plant (NG), grain mass per plant (PW), and 100-seed weight (HW).

ANOVA	Yield	PH	NN	NB	NP	NG	PW	HW
Treatments	0.000 ***	0.005 **	0.001 **	0.000 ***	0.000 ***	0.000 ***	0.000 ***	0.000 ***
Inoculation	0.000 ***	0.001 **	0.647	0.334	0.052	0.000 ***	0.000 ***	0.004 **
Treatments × inoculation	0.000 ***	0.000 ***	0.025 *	0.000 ***	0.000 ***	0.000 ***	0.001 **	0.000 ***
NF	2.78 a	82.33 ab	18.94 ab	1.44 a	43.67 a	96.94 bc	17.75 a	17.32 a
FP	3.93 c	84.17 ab	20.00 b	2.17 b	57.78 c	107.33 c	20.21 b	19.45 b
T1	3.36 b	83.03 ab	18.61 ab	1.06 a	40.94 a	76.11 a	17.68 a	20.31 c
T2	3.77 c	87.44 b	17.61 a	1.22 a	41.33 a	97.67 bc	20.56 b	20.70 c
T3	4.01 c	80.61 a	18.33 a	2.22 b	56.67 bc	98.28 bc	21.25 b	19.22 b
T4	3.86 c	84.31 ab	18.22 a	1.33 a	45.61 a	94.17 b	20.33 b	20.71 c
T5	4.10 c	85.97 b	18.83 ab	1.50 a	47.44 ab	106.28 c	21.37 b	21.04 c
Inoculation	3.90	85.62 **	18.59	1.62	49.29	102.92 ***	21.02 ***	20.02
No Inoculation	3.47 ***	82.34	18.71	1.51	45.98	90.44	18.73	19.62

Note: *, **, *** represent differences between groups according to Tukey's test, $p < 0.05$ level, $p < 0.01$ level and $p < 0.001$ level, respectively. Letters (a, b, or c) represent differences between groups according to Tukey's test, $p < 0.05$ level.

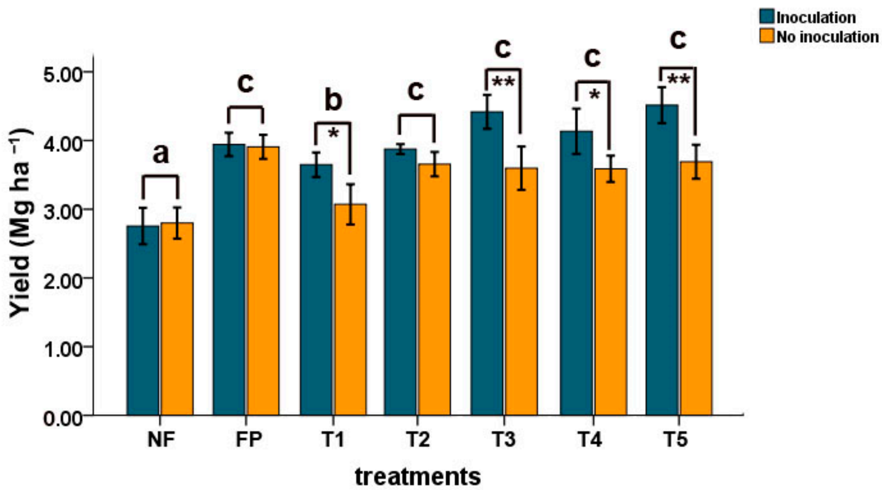

Figure 1. Yield of soybean under sulfur treatments supplemented with rhizobium inoculation and no inoculation. All of the results are expressed as mean values ± SE, with at least three independent replications. Different letters above the column composition indicate significant differences at $p \leq 0.05$. a, b, and c represent the significant difference groups in yield among various treatments. * indicates $p < 0.05$, ** indicates $p < 0.01$. Here, NF = control, no fertilizer for soybean, FP = farmer practice, N–P_2O_5–K_2O: 14–18–16, 270 kg/hm^2, T1 = reduce the amount of FP of by 50% + 0 kg S ha^{-1}, T2 = reduce the amount of FP of by 50% + 5.0 kg S ha^{-1}, T3 = reduce the amount of FP of by 50% + 9.8 kg S ha^{-1}, T4 = reduce the amount of FP of by 50% + 14.8 kg S ha^{-1}, and T5 = reduce the amount of FP of by 50% + 19.6 kg S ha^{-1}.

The associations between soybean yield and NP are presented in Figure 2. Soybean yield and NP in the validation dataset (n = 504) ranged from 2.06 to 5.13 Mg ha^{-1} and from

28 to 92 pods (Figure 2A). The yield was positively correlated with NP as the yield increased with the growth of more pods per plant. In addition, the yield showed a significant correlation with the advancement of the inoculation of rhizobium. Furthermore, NPs generally corresponding to the yield under different fertilization treatments were further explored. The T3 treatment showed greater yield variation with more NP than the other treatments generally. The yield based on T4 averaged 2.94 kg ha^{-1}, with NP ranging from 41 to 91 pods (Figure 2B).

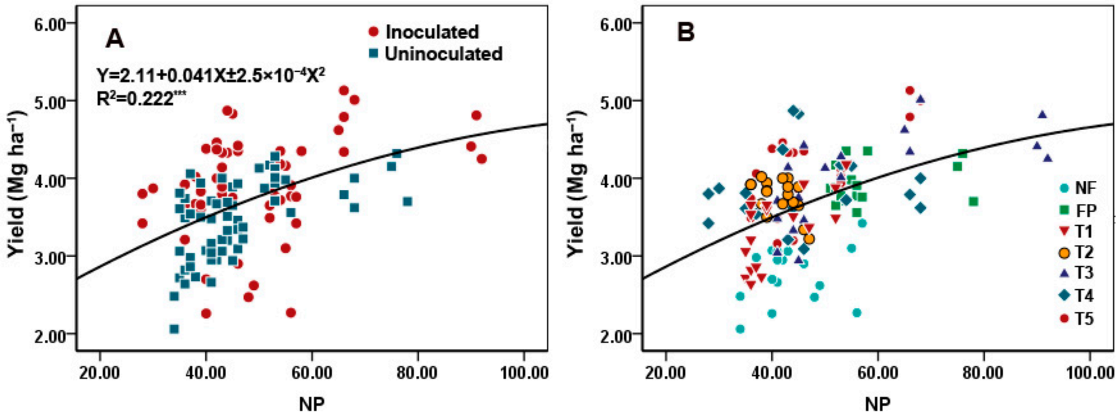

Figure 2. Relationship between yield and number of pods per plant (NP). Parameters of the fitted curve (solid line) and coefficient of determination (r^2) are also shown in (**A**). *** indicates $p < 0.001$. Soybean yield distribution between the rhizobia inoculation and no inoculation are shown (**A**) among the sulfur treatments are shown (**B**).

A significant positive correlation between the yield and the number of grains per plant (NG) was also found in our experiment (Figure 3). Both the yield of soybean and the number of grains per plant in the inoculation treatment were obviously higher than that of the non-inoculation treatment (Figure 3A). It also indicated that rhizobia inoculation could increase yield by increasing the grain number per plant. For fertilization treatment, the T3 treatment showed a higher yield than that of other treatments, but the distribution of grain number per plant was more concentrated, ranging from 84 to 114 grains per plant. The yield and grain number per plant of the NF treatment were significantly lower than those of other treatments (Figure 3B). It also suggested that sulfur-based fertilizers could promote the seed development of soybeans.

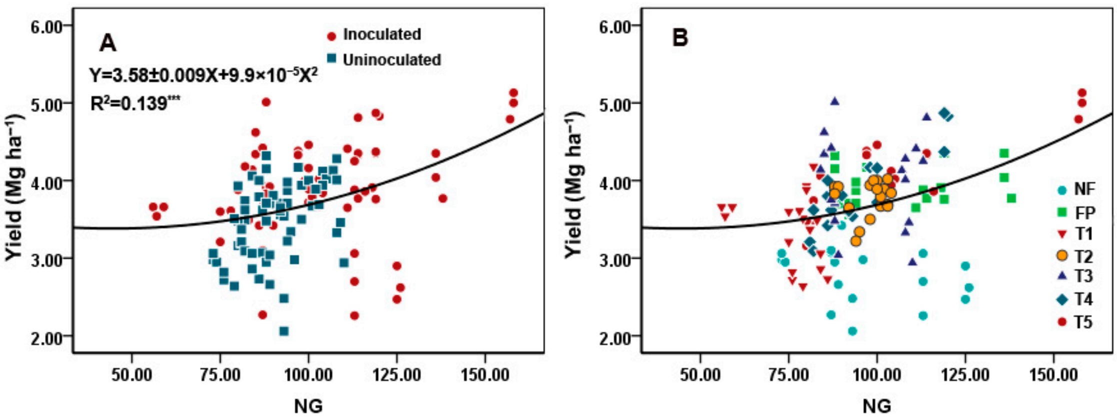

Figure 3. Relationship between yield and number of grains per plant (NG). Parameters of the fitted curve (solid line) and coefficient of determination (r^2) are also shown in (**A**). *** indicates $p < 0.001$. Soybean yield distribution between the rhizobia inoculation and no inoculation are shown (**A**) among the sulfur treatments are shown (**B**).

3.2. Effects of Sulfur Fertilizer on Soybean Properties

The results showed that the protein and oil content of soybeans varied significantly ($p < 0.05$) among fertilization treatments. On average, there was a higher oil but lower protein content in soybeans under treatment without fertilization than those with additional S application (Figure 4). T5 treatment had the highest protein content at 42.29% among all treatments (Figure 4A). A higher value of protein content was found in FP and T1, which increased protein content by 12% and 11% compared to the NP treatment, respectively. The oil content of soybean showed no significant difference among the treatments FP, T1, T2, T3, and T5, which had a lower value ranging from 18.37–18.75% (Figure 4B). Interestingly, the effect of rhizobia inoculation on protein and oil content showed a significant difference between treatments with and without fertilization, which also predicted the interaction between rhizobia and fertilizer application.

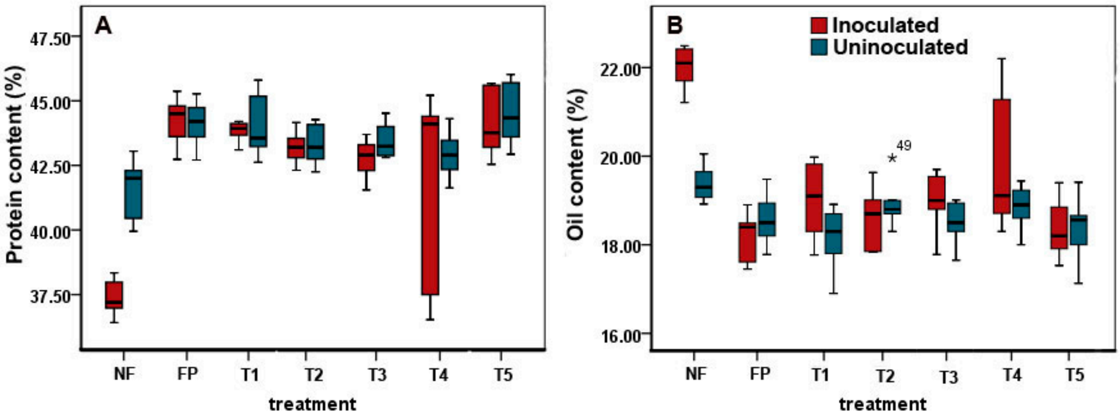

Figure 4. Quality traits of soybean under sulfur treatments supplemented with rhizobium inoculation and no inoculation. Protein content is shown (**A**) and oil content is shown (**B**).

3.3. Effect of Sulfur Treatments on Photosynthesis Characteristics

To verify the variations of photosynthesis characteristics in response to different fertilizer treatments, the change curves of photosynthesis parameters measured under different fertilizer treatments during the V5–R6 stages of soybean growth were plotted (Figure 5). Compared with NF treatment, the chlorophyll index value of leaves under all treatments showed a similar tendency to significantly decrease, with a higher value in the V5 to R2 stages followed by a slight increase from the R2 to R6 stages (Figure 5A). It was reasonable to postulate that stronger photosynthetics could support the development of plants during the late reproductive stages of the soybean. In general, stomatal conductance (gs) followed different temporal patterns for the different reproductive stages of the soybean compared with chlorophyll index value (Figure 5B). The stomatal conductance of most treatments reaches its maximum value in R2 and then decreases gradually in the following stages, while the highest means of stomatal conductance were found in the R4 stage under T2 treatment. Among them, T5 treatment had the highest gs mean at 0.49 mmol H_2O m^{-2} s^{-1} in the R2 stage. The fertilizer treatments showed a similar trend relating to photosynthetic rate (Pn), with a higher value at the R4 stage and a lower value at later stages. The maximum Pn value of treatment T4, which appeared at the R4 stage, was 22.5 (mmol m^{-2} s^{-1}), which was more than that at the R6 stage, 12.9 (mmol m^{-2} s^{-1}). However, the changes in the photosynthetic rate of FP and T1 over the four developmental stages were relatively smooth. The Pn of soybean plants were decreased by 27% and 36%, under FP and T1 treatment, respectively, compared to the corresponding values at the R4 stage. The intercellular CO_2 concentration (Ci) and transpiration rate (E) of soybean plants under six treatments at the four development stages were further evaluated. Among these treatments, T3, T4, and T5 were found to exhibit a peak value of E and Ci at the R2 stage (Figure 5D,F). It was noted that the transpiration rate of T2 and the intercellular CO_2 concentration of FP showed different tendencies over the four development stages of the soybean plant. These results showed that the changes in E and Ci values for T5 were stable and could be the main contributing factors to crop yield formation. The leaf area index (LAI) of the plants under most treatments showed a trend of continuous increase during the V5–R6 stages of soybean growth (Figure 5E). The maximum LAI mean values of 4.9 and 4.8 were recorded at the R6 stage under T1 and T2 treatment, respectively. At the same time, the lowest average LAI value was found in the FP treatment at R4 or R6 when compared with other treatments.

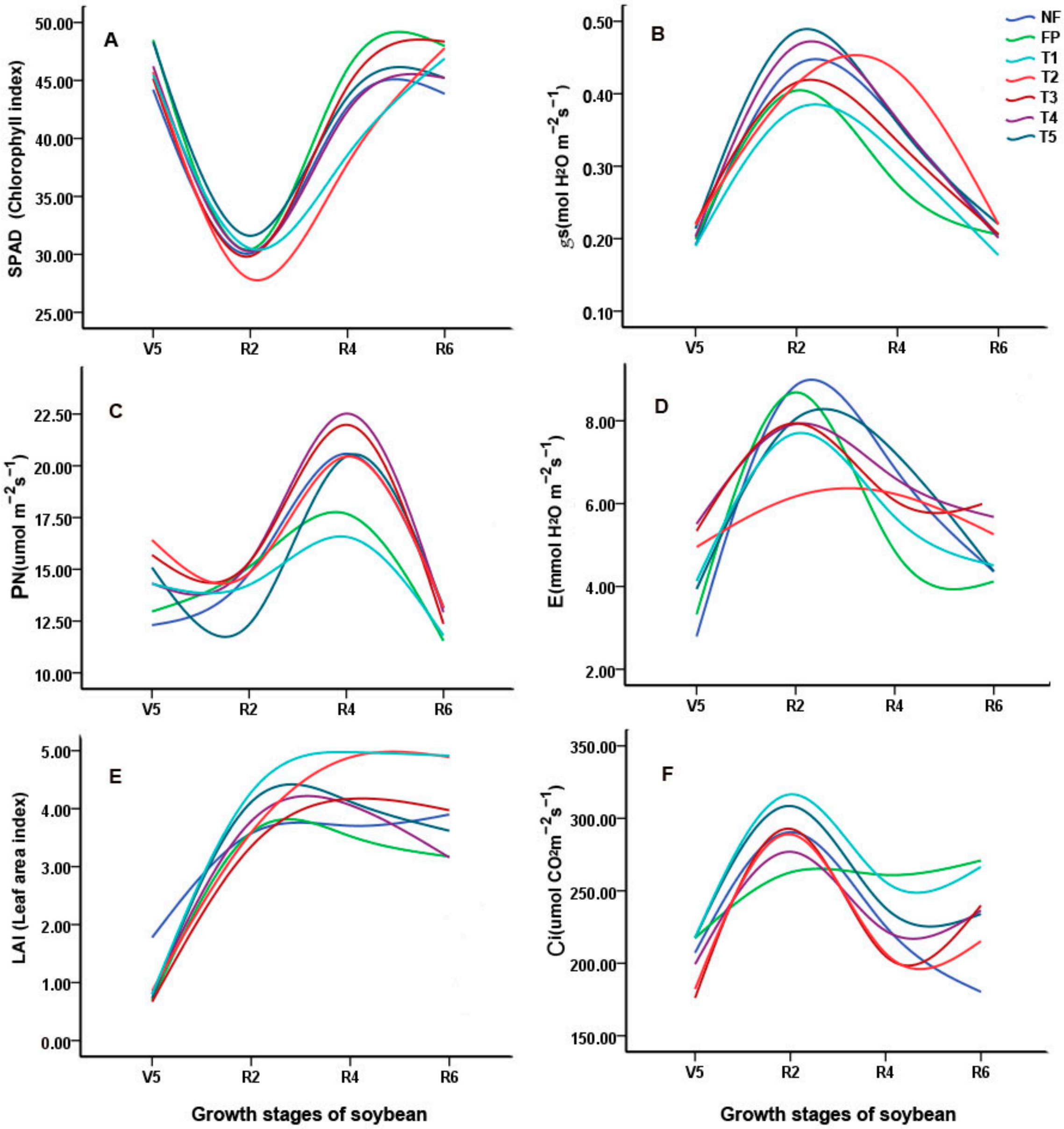

Figure 5. Variations of photosynthesis characteristics as affected by fertilizer treatments during the V5–R6 stages of soybean growth. (**A**) the chlorophyll index (SPAD) of different treatments during the V5–R6 stages; (**B**) the stomatal conductance (Gs) of different treatments during the V5–R6 stages; (**C**) the net photosynthesis rate (Pn) of different treatments during the V5–R6 stages; (**D**) the transpiration rate (**E**) of different treatments during the V5–R6 stages. (**E**) the leaf area index (LAI) of treatments during the V5–R6 stages. (**F**) the intercellular CO_2 concentration (Ci) of different treatments during the V5–R6 stages.

3.4. Correlation of Grain Yield and Photosynthetic Traits

In our study, the yield of soybean illustrated strong correlations with photosynthetic traits at the V5 and R6 stages. For V5, grain yield showed a positive significant relationship with the SPAD, Pn, Gs, and E, in which r^2 was 0.27, 0.35, 0.39, and 0.42, respectively. For R6, the grain yield of soybean presented a positive significant relationship with the SPAD, E, and Ci, in which r^2 was 0.24, 0.28, and 0.44, respectively (Figure 6). Interestingly, the grain yield of soybeans exhibited no significant correlation with any photosynthetic trait during the R2–R4 periods. At the same time, LAI demonstrated a highly significant but negative correlation with yield at the V5 and R6 stages.

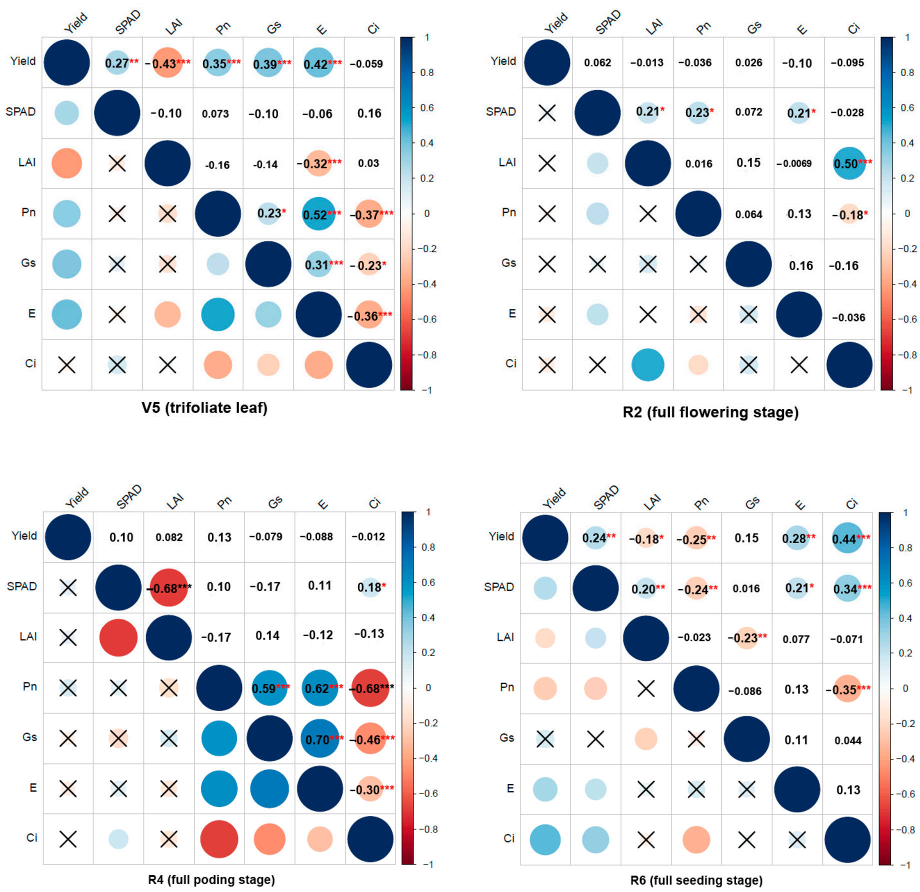

Figure 6. Correlation coefficients of leaf photosynthetic traits during the V5–R6 stages and grain yield. Yield (Mg ha^{-1}), the chlorophyll index (SPAD), the net photosynthesis rate (Pn), the stomatal conductance (Gs), the transpiration rate (E), the leaf area index (LAI), and the intercellular CO_2 concentration (Ci). A correlation matrix with numeric values is displayed as a graphical representation. Blue bubbles represent a positive relationship, orange bubbles represent a negative relationship, and black-colored values are not significant ($p > 0.05$ level). * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p < 0.001$.

4. Discussion

4.1. The Influence of Sulfur and Rhizobium Inoculation on Soybean Yield

Adequate fertilization has been essential in achieving optimum grain yield for crops. Several mineral fertilizers have been evaluated for their effect on the the growth and grain yield of soybeans [24–26]. Previous reports have highlighted the impact of S fertilization on plant growth and crop yield [27]. Keren Brooks et al. conducted experiments to test the effects of S and N on soybean yield in a diverse range of environments. However, the results suggest that S did not affect grain yield in most environments [28]. The objectives of this study were to determine the effects of different S treatments on seed yield and its impact on photosynthesis dynamics at different growth stages. The highest soybean yield was obtained in treatment T5. Higher NP and NG values were also found in the treatments T4 and T5, which helped in better understanding that it is beneficial for plant growth and yield formation to gradually increase S supply in treatments. As emphasized by Alamgir

Kabir, the application of sulfur fertilization has the potential to increase biological yield by enhancing crop growth and improving yield attributions [29–31]. In this study, the largest yield of soybeans was recorded under T5 treatment with a dose of 19.6 kg S ha^{-1}, but it was only higher than T3 treatment with a dose of 9.8 kg S ha^{-1} by 2.5% (by 0.1 Mg ha^{-1}) and higher than T1 treatment in the absence of fertilization with S by 22.1% (by 0.74 Mg ha^{-1}). Additionally, the lowest yield of soybeans was observed under NF treatment in the absence of fertilizer. Likewise, it has also been inferred that Starter-N application led to improved early plant growth and an increased soybean yield, as we observed that greater LAI due to S application indicated that the plants obtained more leaf expansion due to intercepting more incident light earlier. The improvement of pod number per plant, grain number per plant, and 100-grain weight may be the result of improved plant growth and physio–biochemical characteristics due to the S supplied. In our studies, the maximum 100-seed weight under conditions of fertilization with S supply at a dose of 19.6 kg S ha^{-1} resulted in an increasing yield of soybean. Higher NP and NG were obtained under treatment with fertilizers supplied with S. These yield-related parameters might be attributed to seed yield per plant so the yield value of S–supplied plants is higher. Prior studies have indicated that the combination of fertilization and the application of a suitable rhizobium strain may represent a more effective agronomic strategy for achieving high yields in soybean cultivation [32,33]. In this study, a phenomenon was confirmed. When inoculated with rhizobium, plants of higher NP and NG exhibited a higher yield compared to those without inoculation. Furthermore, there was an increasing trend of yield, accompanied by rhizobium inoculation, as sulfur concentrations rose.

4.2. The Quality Traits Response to Sulfur Treatments and Rhizobia

Research has indicated that N and S fertilization in different stages of crop growth could increase protein concentrations [34]. Our study revealed that soybeans demonstrated a higher protein content in response to S fertilizer treatments, ranging from 42.44% to 44.29%, compared to S-free soybean plants under similar conditions. Consequently, oil content decreased in response to S fertilizer treatments, ranging from 18.37% to 19.25%. Daniel E. Kaiser et al. found that N increased early plant weight, while S increased concentration, uptake, and removal of S in plants and the protein concentration in seeds, but decreased seed oil concentration [35]. Another related study showed that it is necessary to boost the yield and storage of proteins in soybean grains using an appropriate dose of S [22]. The maximum protein content was obtained at 42.29% with S supply at a dose of 19.6 kg S ha^{-1} in our study. However, Zerihun Getachew et al. revealed that the protein content of soybean was elevated to 44% with sulfur fertilization at 40 kg S ha^{-1}, along with Rhizobium inoculation [36]. S fertilization appeared to improve the process of oil composition and biosynthesis of acetyl CoA, which is involved in oil biosynthesis. Moreover, it is possible that the formation of certain saturated fatty acids or oil storage organs was promoted due to S supply [37,38]. However, our study did not observe a significant increase in oil content with the application of S. However, the results indicated that the simultaneous application of S and rhizobium could impact the protein quality and oil composition of grains.

4.3. Effects of Photosynthesis on Soybean Yield

In general, sulfur fertilizer improved PN, gs, and E under the T3, T4, and T5 treatments during the V5–R6 stages (Figure 5B–D). The improvements of PN, gs, and E might be due to the increased activity of photosynthetic enzymes and the biosynthesis of photosynthetic pigments [39,40]. The increase in metabolites and enzyme activity could improve photosynthetic parameters and, hence, their values. Our results are in accordance with previous studies that revealed that the application of S increased plant photosynthetic rates in *Brassica juncea* [41]. Furthermore, gs and E increased significantly under most fertilizer treatments at full bloom (R2) compared with other investigated growth stages, indicating that the photosynthetic capacities of plants at the R2 stage are more important for grain yield. The seed yield increase might be due to the role of S in improving growth traits, increasing chlorophyll content synthesis, and leading to higher total photosynthetic productivity, cell division, and more photosynthetic product transport to seeds [42]. Our study revealed that there was a highly significant correlation between grain yield and photosynthetic traits such as SPAD, Pn, and E at the V5 and R6 stages. This indicates that the improvements in chlorophyll content and photosynthetic rate when S is supplied might increase yield characteristics. However, we did not detect a significant correlation between grain yield and photosynthetic traits at the R2 and R4 stages, which might extend the duration of green leaves and cause little variation in photosynthetic traits.

5. Conclusions

Sulfur facilitates growth, photosynthesis rate, enzymatic activities, and development and influences the grain yield and seed quality of oilseed crops. Our findings revealed that the soybean yield was significantly affected by the combination of sulfur and rhizobia inoculation application. Sulfur application, especially at rates of 19.6 kg S ha^{-1}, led to significant enhancements in the number of grains per plant (NG), 100-seed weight, and maximum seed yield compared to the unfertilized control (NF). In addition, the enhancement of soybean growth and yield through inoculation with rhizobia alone was not found to be statistically significant in the absence of sulfur supplementation. It may be concluded that sulfur supply results in improved yield components; there was a slight upward trend in soybean production capacity. It highlights the importance of balanced sulfur-nitrogen nutrition for soybean growth and yield performance.

More studies are required to investigate the physiological and molecular mechanisms underlying the sulfur–inoculant interactions. Moreover, we should conduct more research on the long-term effects of S application and rhizobia inoculation on soil health and sustainability.

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