Phosphate fertilizers increase CO₂ assimilation and yield of soybean plants in a shaded environment

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Research Article

Keywords: Soybean, Shading, Phosphate fertilizer, Photosynthesis, Yield

Posted Date: November 24th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-112384/v1>

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Abstract

To study how phosphate fertilizer regulates the photosynthetic of summer soybeans in low light environment, this study used Qihuang 34 as the test variety, two light treatments[ambient sunlight (L1) during the whole growth period and 40% shade (L2) from 40 days after sowing to 24 days after flowering] with two phosphate fertilizer treatments including non-phosphate fertilizer application (P0), conventional phosphate fertilizer application (P1) in each light treatment was set up to measure the gas exchange, chlorophyll a fluorescence differences of photosynthetic performance as well as the yield and its components in Qihuang 34. The results showed that phosphorus significantly increased chlorophyll content, photosynthetic rate, and grain yield in shading treatment. The yield of soybean was significantly reduced after shading treatment, which was 29.9%, but compared with L2P0, L2P1 treatment yield increased by 36.7%, which was significantly higher than the 27.1% under L1 treatment. The photosynthetic rate under saturated light (Asat) under L2 treatment decreased by 31.5% and 15.6% in R1 and R3 periods, respectively, and average increased by 27.4% and 45% after phosphorus application under two light condition. Furthermore, photosynthetic measurements demonstrated a more significant increase in the maximum efficiency of CO₂ fixation, maximum rates of RUBP-carboxylation (V_{cmax}) in phosphorous treatment under shading conditions. Moreover, further analysis of OJIP curves revealed that phosphate fertilizer significantly improved the electron transfer and light energy absorption performance of the PSII reaction center in shading. The above results demonstrate that phosphate fertilizer contributes to light energy utilization efficiency, increase the low light utilization efficiency by improving PSII performance, promote RUBP regeneration, ensure the source of carboxylates substrates, and coordinate the balance of the photochemical reaction and the Calvin cycle, then alleviate the reduction of photosynthetic efficiency under shading, increase the rate of dry matter accumulation, and greatly increase yield.

1. Introduction

Soybean, as both a grain and oil crop, is one of the most important crops in the world, which native to China. Many factors are affecting the scale of soybean production, of which the low yield is one of the most prominent factors. Photosynthesis is the basis of crop growth and yield formation. As one of the most important environmental factors in agricultural production ^[1], light is not only the driving force of, but also affects the structure and function of photosynthetic mechanisms ^[2, 3]. In the past half-century, the decrease of sunshine hours and solar radiation of crops have shown a persistent decline trend, due to the increase in aerosol concentration caused by human activities and the associated light fog and low clouds, especially in the North China Plain (June-October), severely restricts soybean production ^[4].

Photosynthesis is a complex biochemical and biophysical process that comprises photosynthetic pigment synthesis, light energy electron transport, and the Calvin cycle ^[5, 6], these processes are all affected by the light intensity ^[7]. Leaf area, chlorophyll content, and antenna pigment-protein under weak light environment improve the light interception and the absorption efficiency ^[8, 9], but the decrease of

light conversion and electron transfer efficiency^[10, 11] as well as a significant decrease in expression level and activity of photosynthetic carbon immobilized enzyme^[12-14] resulting in the inevitable reduction of photosynthetic capacity under weak light. Increasing nutrients is one of the important ways for crops to improve the use of low light^[15]. Phosphorus has the effect of regulating the stability of thylakoid membranes and the activity of photosynthetic proteins penetrating inside and outside the membrane^[16, 17], thereby affecting photosynthetic electron transport, photosynthetic phosphorylation, Calvin cycle, assimilation synthesis, and transport efficiency^[18], and then regulate crop photosynthesis. Soybeans are sensitive to phosphorus and require a large amount of phosphorus. Reasonable application of phosphorus fertilizer is an important measure to regulate soybean growth and yield formation^[19].

Previous reports mostly focused on light intensity or phosphorus as a single factor to increase photosynthetic capacity and physiological mechanisms in increased yield^[18-20]. According to field test results, there is a limited linear relationship between the amount of phosphorus application and photosynthetic rate. When the amount of phosphorus applied exceeds a certain ratio, the photosynthesis decreases rather than increases^[21], which shows that although the resource attributes of light and phosphorus are inconsistent, there is a certain complementary effect between them. There are few reports on the correlation between whether phosphorus applied or not and the relative inferiority efficiency of utilization of weak light in shading, the complementary mechanism still uncertain. The consequence of phosphate fertilizers compensate for the decrease in light intensity and an increase in photosynthesis needs to be further clarified. In this regard, we explored the complementary effects between phosphorus and light intensity under different light environments, as well as the connection and mechanism between phosphate fertilizer application and low light utilization efficiency, to innovate the agronomic production model for crops to efficiently use low light.

2. Materials And Methods

2.1 Field location

A field experiment was conducted at the Agronomy Station of Shandong Agricultural University in 2018, in Taian City, Shandong Province (117°09'E, 36°09'N). The region receives adequate light and has a suitable temperature, and rain and heat occur within the same season. The precipitation and atmospheric temperature dynamics of 2019 are shown in Figure 1. The major initial properties within the 0–20 cm soil depth were as follows: 12.53 g kg⁻¹ SOC (an Elementar Vario TOC), 1.15 g kg⁻¹ STN (the semi-micro Kjeldahl method), 11.27 mg kg⁻¹ Olsen P (sodium bicarbonate-molybdenum antimony anti-reagent colorimetric method), 80.9 mg kg⁻¹ exchangeable K (NH₄OAc extraction-flame photometer method) and a pH (calcium chloride solution extraction-potentiometric method) of 6.8.

2.2 Experimental design

Two treatments (L1: ambient sunlight; L2: 40% shade) were arranged in the field. Two light levels (L1: ambient sunlight; L2: 40% shade) and two phosphate levels (P1: 0 kg P₂O₅ ha⁻¹; P2:180 kg P₂O₅ ha⁻¹) were arranged in the field with a randomized block design. Shade treatment was applied at 40 days after planting and remained until 64 days after planting. Each plot had an area of 35 m² (5m×7m), and three replicates were included in the experiment. Qihuang 34 was selected as the plant material in this study with a planting density of 120,000 plant ha⁻¹, which was seeded on June 17, 2018, and harvested on October 15, 2018. N and K were applied with 300 kg ha⁻¹ urea (N content was 46.7%), 300 kg ha⁻¹ potassium sulfate (K₂O content was 50.0%), and calcium superphosphate, P₂O₅ content was 12%, is used as P fertilizer. P and K fertilizer were applied as a base fertilizer. Half of the N fertilizer at sowing and the other half was broadcast at the beginning pod stage. Maintain a water supply throughout the growth period. Disease, weeds, and pests were well controlled in each treatment.

2.3 Crop yield

Upon maturity, constantly collected 30 plants in each treatment with three replicates. The samples were air-dried and threshed, and then weighed to calculate the yield at 13% moisture content.

Soybean grain yield (kg ha⁻¹) = Harvest plant (plants ha⁻¹) × pods per plant×1000-grain weight (g 1000 grains⁻¹) /10⁶ × (1-sample moisture content%) / (1-13%)

2.4 Chlorophyll a and b measurement

Chlorophyll a and b were extracted by 80% acetone from the leaves of the soybean (fully expanded, exposed) at a similar position for each treatment group. The absorbance of the extract s was measured with a UV-2450 spectrophotometer (Shimadzu Suzhou instruments mfg. co., Ltd, China) at 663 nm (A663), 646 nm (A646), respectively. Chlorophyll a and b were calculated using equations established by Lichtenthaler and Wellburn [22]:

$$\text{chl a (mg mL}^{-1}\text{)} = 12.21A_{663} - 2.81A_{646}$$

$$\text{chl b (mg mL}^{-1}\text{)} = 20.13A_{646} - 5.03A_{663}$$

2.5 Gas exchange measurement

Gas exchange measurements were performed using a portable gas exchange system (CIRAS-3, PPSYSTEM Ltd, Ayrshire, UK). Measurements were made on the inverse fourth leaf of beginning flowering (R1), beginning pod (R3), and beginning seed (R5) plants. Responses of net CO₂ assimilation rate (A) to intercellular CO₂ concentration (C_i) were measured at a leaf temperature of 25°C, and a light intensity of 1400 μmol m⁻²s⁻¹. Plants were acclimated to these conditions until steady-state gas exchange was reached (20-30 minutes), and A/C_i curves measured with a sequence of reference CO₂ concentrations of 400, 350, 300, 250, 200, 150, 100, 75, 50, 400, 400, 400, 400, 500, 550, 600, 700, 800, 1000, 1200 and 1400 μmol mol⁻¹. The middle four measurements at 400 μmol mol⁻¹ were disregarded, as their purpose

was to allow the plant to re-acclimate to this condition after being exposed to low CO₂ concentrations. Data points were taken in sequential order with an equilibration time of 180-300 seconds at each CO₂ concentration. A_{sat} was measured on the inverse fourth leaf at R1, R3, R5, at a CO₂ concentration of 400 µmol mol⁻¹, a leaf temperature of 25°C, and a light intensity of 1400 µmol m⁻² s⁻¹.

2.6 Photosynthetic light response curves

A/Q response curves were performed using a portable gas exchange system (CIRAS-3, PP Systems USA). Measurements were made on the inverse fourth leaf of beginning flowering (R1), beginning pod (R3), and beginning seed (R5) plants. The response of the net CO₂ assimilation rate (A) to light intensity (Q) was measured at a leaf temperature of 25°C, the relative humidity of 50-60%, and a CO₂ concentration of 400 µmol mol⁻¹. Leaves were initially stabilized at saturating irradiance 2000 µmol m⁻² s⁻¹, after which A was measured at the following PPFD levels; 50, 100, 150, 200, 400, 800, 1200, 1600, 2000 µmol m⁻² s⁻¹. Measurements were recorded after A reached a new steady-state (1-2 min) before changing to the new light levels.

2.7 Chlorophyll fluorescence

Chlorophyll fluorescence measurements were performed using a Handy PEA fluorometer (Hansatech Instruments Ltd., King's Lynn, Norfolk, Great Britain). The leaves were dark-adapted using leaf clips for 20 min. The dark-adapted leaf samples of 4 mm diameter within each clip were illuminated with 660 nm light of 3500 µmol m⁻² s⁻¹ for 1 s. Descriptions and equations for calculating JIP-test parameters are explained in Table 1.

Table 1 Selected JIP-test parameters calculated based on fast fluorescence kinetics.

Fluorescence parameters	Description
F_o	Minimal recorded fluorescence intensity
F_m	Maximal recorded fluorescence intensity
$F_j = F_{2ms}$	Fluorescence at 2 ms
$F_k = F_{300\mu s}$	Fluorescence at 300µs
$F_v/F_m = (F_m - F_o)/F_m$	The maximum quantum yield of PSII primary photochemistry
$V_j = (F_{2ms} - F_o)/(F_m - F_o)$	Relative variable fluorescence at 2 ms
$W_k = (F_{300\mu s} - F_o)/(F_{2ms} - F_o)$	Relative variable fluorescence at 300 µs
$M_o = 4(F_{300\mu s} - F_o)/(F_m - F_o)$	The approximated initial slope of the fluorescence transient
$ABS/RC = M_o \cdot (1/V_j) \cdot (1/\phi_{Po})$	Absorption flux per RC
$\psi_o \equiv (1 - V_j)$	The probability that a trapped exciton moves an electron into the electron transport chain beyond Q _A
$\phi_{Eo} \equiv [1 - (F_o/F_m) \cdot \psi_o]$	Quantum yield of electron transport
$PI_{ABS} \equiv (RC/ABS) \cdot [\phi_{Po}/(1 - \phi_{Po})] \cdot [\psi_o/(1 - \psi_o)]$	Performance index on absorption basis

2.8 Statistical analysis

Excel 2016 was used to process the raw data. The ANOVAs (analysis of variance) of the data were analyzed via SPSS 16.0 Statistical Analysis Systems. Significant differences between treatments were assessed via Duncan's test at $P < 0.05$. The illustrations were prepared via Sigma-plot 10.0.

3. Results

3.1 Phosphorus significantly improves dry matter accumulation and yield of soybean plants in shading

Table 1 shows the yield and formation factors of each treatment. The yield of soybean increased significantly after phosphorus application under the same light conditions. Soybean yield significantly reduced under shading conditions, showed a trend of $L1P1 > L1P0 > L2P1 > L2P0$.

The effects of phosphate fertilizer on soybean yield were different under ambient light (L1) and shade treatment (L2). Two-way ANOVA revealed a significant interaction effect between light intensity and phosphorus concentration on effective pods per plant, number of seeds per plant, and yield of soybean. Effective pods per plant and the number of seeds per plant were closely related to irradiance ($P < 0.01$), phosphate fertilizer ($P < 0.01$), and the interaction between the two ($P < 0.01$), but the effect of 1000-grain weight is not affected by phosphate fertilizer. Compared to P0, the yield of P1 treatment is 27.1% and 36.7% increased under L1 and L2 treatment. It can be seen that under low light conditions, soybean yield increased significantly with the application of phosphate fertilizer, and its increase was significantly higher than that under full light conditions. Phosphate fertilizer can promote the flowering and pod formation of soybean, which increases soybean yield via promoting the number of effective pods per plant and the number of seeds per plant.

3.2 Phosphorus fertilizer increases chlorophyll content of plant leaves under low light

As shown in Figure 2, the content of chlorophyll a and b at each growth stage after anthesis increased significantly with the decrease of light intensity and the application of phosphate fertilizer. The shading is the principal reason for the significant increase in soybean chlorophyll content. Phosphorus fertilizers have different effects on the improvement of chlorophyll content under different light environments, compared to L1P0 treatment, the chlorophyll content of L1P1 treatment increased by 9.6% and 16.1%, respectively, which was significantly less than 13.8% and 21.8% under low light environment; however, chlorophyll b increased by 16.8%, 29.3% and 23.2%, 24.9% under L1 and L2 after application of phosphate fertilizer, during R3 and R5 growth stage, respectively. It can be seen that the application of phosphate fertilizer has a significant effect on the chlorophyll content under shading, especially the content of chlorophyll b was more notable.

3.3 Phosphate fertilizer improves the plant's light energy utilization capacity in shading

To explore the changes of photosynthetic rate under shading condition, light-saturated rate of photosynthesis (A_{sat}) were determined. As shown in figure 3, the A_{sat} in the R1 period after shading was significantly lower than that in L1 treatment. The A_{sat} of L2P0 decreased by 38.3% and L2P1 decreased by 26.3% under the same phosphate fertilizer treatment. The CO_2 assimilation rate of P1 treatment increased significantly in shading compared with P0 treatment as the processing time increases. After relighting in the R5 period, L1P1 was 4.6% higher than L2P1, the difference was not significant ($P>0.05$), indicating that phosphorus application under shading condition is beneficial to maintain a high rate of CO_2 assimilation after re-lighting in the later stages of plant growth.

To further explore the reasons for the significant increase in the A_{sat} of the L2P1 treatment during the R3 period, CO_2 assimilation rates were determined as a function of light intensity. From these light response curves the CO_2 assimilation rates were shown to be significantly lower in L2 treatment when compared to L1 treatment, showing a trend of $\text{L1P1} > \text{L1P0} > \text{L2P1} > \text{L2P0}$, but as the shading treatment time increased, the CO_2 assimilation rate of the L2P1 treatment gradually increased, and showed to be $\text{L1P1} > \text{L2P1} > \text{L1P0} > \text{L2P0}$ (Figure 4), and the CO_2 assimilation rate of L2P1 remains at a high level after re-lighting.

We focused on the response of the CO_2 assimilation rate to low light intensity ($\leq 200 \text{ mmol m}^{-2} \text{ s}^{-1}$) to make a thorough probe of change in apparent quantum yield (PQY) during low light. The initial slope of the A / Q curve reflects the plant's weak light utilize the ability. The PQY of plants under weak light shows a significant upward trend, especially with L2P1 treatment. Figure 5 compares the photosynthetic capacity unit light intensity, the weak light ($100 \text{ mmol m}^{-2} \text{ s}^{-1}$) utilization capacity of plants in a shading environment was significantly higher than that in the full light environment. As the adaptability to weak light improves, its high light intensity ($800 \text{ mmol m}^{-2} \text{ s}^{-1}$) utilization capacity also increased synchronously. The low light utilization capacity of L2P1 plants after relighting maintaining high, indicating that phosphorus application under shading makes the plants quickly adapt to low light environments while ensuring the restoration of photosynthetic capacity of plants after re-lighting, ensure the accumulation of photosynthetic products, and significantly increase yield.

3.4 Phosphate fertilizer increases the rate of CO_2 assimilation in shading

To explore the changes in the carboxylation system under different treatments, we also determined A as a function of internal CO_2 concentration (C_i) in the same plants. The CO_2 assimilation rate of L2P0 and L2P1 decreased significantly after shade treatment. As the plant's adaptability increased, the CO_2 assimilation rate of L2P1 increased significantly during the R3 period, which maintaining a high level compared to L2P0 treatment when re-lighting at R5 (Figure 6).

Further analysis of the A/ C_i curves using the equations published by von Caemmerer and Farquhar (1981) [23] illustrated that the light environment significantly affects the maximum rate of carboxylation by Rubisco (V_{cmax}) and maximum electron transport rates (J_{max}). As shown in figure 7, V_{cmax} and J_{max} in the natural light environment were significantly higher than in the shading environment, showing to be

L1P1> L1P0> L2P1> L2P0, except for the R5 period. V_{cmax} and J_{max} respond differently to phosphate fertilizer under two light conditions. The use of phosphate fertilizer has no significant effect on V_{cmax} in ambient light environment, L2P1 and L2P0 show significant difference only in the R3 period in shading. After applying phosphate fertilizer under different light environments, J_{max} changed significantly. Compared with P0 treatment, J_{max} increased by 8.1% and 16.9% in P1 treatment during R3 and R5 periods under L1 treatment, and 38.8% and 32.3% in L2 treatment, respectively. It can be seen that the application of phosphate fertilizer significantly improves the regeneration rate of RUBP in shading.

3.5 Chlorophyll fluorescence analysis reveals increased photosynthetic efficiency in plants in shading

Variable fluorescence curves and $D V_t$ [For ΔV_t analysis ($\Delta V_t = D(F_t - F_o) / (F_m - F_o)$) fluorescence in L1P0 treatment on each day of the experiment (was a used as a reference and) equaled 0.] were constructed to compare differences between treatments on each measuring day during the experiment (Figure 8). Compared with plants under ambient light, where the K and J-bands showed significantly higher values for both shading treatments, indicating that the PSII donor side and acceptor side were reduced to different degrees in shading and it has alleviated during the R3 period. J-bands were relatively low with phosphorus treatment, especially in the shade environment, which means phosphate fertilizer can alleviate the effect of low light on the performance of the leaf receptor.

Figure 9 shows the change of the relative variable fluorescence intensity at points K and J of chlorophyll a fluorescence transient (OJIP) curves. After shade treatment, W_k and V_j both increased, especially V_j , indicating that the performance of the donor side and the receptor side was reduced to varying degrees, and the receptor side was significantly affected by the low light. W_k and V_j of phosphorus-applied plants showed a downward trend in two light treatments. The effect of phosphate fertilizer on W_k was not significant, but V_j was significantly reduced. After re-lighting, the V_j of L2P1 was significantly lower than that of L2P0 treatment, but not significantly different from that of L1P1 treatment. The above results indicate that phosphorus application improves the electron transport performance on the receptor side in shading stress, and is beneficial to the retention of the receptor side performance after the plant was relighted.

The analysis of the relative variable fluorescence at points K and J of the OJIP curve found that both light and phosphate fertilizer had a significant effect on V_j . The change in the relative fluorescence at point J is closely related to the electron transport performance of the PSII acceptor side, so we studied the maximum quantum yield of PSII primary photochemistry (F_v/F_m), the probability that a trapped exciton moves an electron into the electron transport chain beyond QA^- (y_o), the quantum yield of electron transport (j_{E0}) and performance index on absorption basis (PI_{ABS}).

Figure 10 shows that F_v/F_m , y_o , j_{E0} , and PI_{ABS} all decreased significantly after shading. With the improvement of the plant's low light adaptability, the photosynthetic mechanism changed adaptively. During the R3 period, P1 F_v/F_m , y_o , j_{E0} and PI_{ABS} increased by 0.9%–3.4%–9.9%, and 20.5% in L1 treatment

compared to P0, and which were 1.5%, 6.4%, 48.5%, and 69.7% under L2, respectively. Phosphate fertilizer can strengthen PSII performance, especially under low light. Compared with other treatments, L2P1 has the least decreased in the R5 period after re-lighting, which was not significantly different from the L1P1 treatment, indicating that phosphorus application benefits maintain the performance of the photosynthetic system after relighting, ensuring that the absorbed light energy is fully used to promote electron transfer, effectively improve the soybean photosynthetic capacity in the later period.

Table 2. Effects of different treatments on yield and its components of summer soybean

Treatment		1000-seeds weight	Effective pods per	Seeds per	Yield (kg ha ⁻¹)
Light (L)	Phosphorus (P)	(g)	plant	plant	
L1	P0	276.2 b	66.9 b	117.2 b	3883.8 b
	P1	272.6 b	77.7 a	150.9 a	4936.7 a
L2	P0	298.1 a	39.2 d	73.0 d	2611.4 c
	P1	301.2 a	45.9 c	98.8 c	3570.4 b
Significance					
L		**	**	**	**
P		ns	**	**	**
L×P		**	**	**	*

L1: ambient sunlight; L2: 40% shade; P0: phosphate fertilizer application of 0 kg P₂O₅ ha⁻¹; P1: phosphate fertilizer application of 180 kg P₂O₅ ha⁻¹. L: light factors, P: phosphate fertilizer factors, L×P: interaction of light and phosphate fertilizer. Different lowercase letters in the same column indicate significant differences (P<0.05). *P<0.05, ** P<0.01, ns: not significant.

4. Discussion

Photosynthesis is the basis of crop growth and yield formation, which is susceptible to light intensity [25] and decreases significantly under low light [3]. By increasing the chlorophyll content, the plants can improve the light absorption ability of chloroplasts [26], make full use of weak light, and adapt to it. In this experiment, the chlorophyll a (chl a) and chlorophyll b (chl b) in the leaves increased significantly after shading (Figure 2), but the A_{sat} decreased significantly, indicating that the increase in chlorophyll was insufficient to compensate for the photosynthetic rate caused by the decrease in light intensity. However, the leaves chl a, chl b, and A_{sat} all increased significantly in P1 treatment, especially in the shading environment (L2), indicating that the plants increased the photosynthetic rate of the leaves by increasing the light energy capture capacity (Figure 3). After a long period of shading treatment, the plant has an adaptive response. The initial slope of the A/Q curve (PQY) is considered to be a symbol of the plant's low light usability. Under shading treatment, the PQY of the plant was significantly higher than that of the ambient light environment, and the phosphorus application was significant to improve its weak light utilization ability, the PQY of L2P1 treatment was significantly higher than that of other treatments in the R3 period (Figure 4), and the plant's light energy utilization efficiency (P_n/PAR) increased synchronously (including PAR=100 mmol m⁻² s⁻¹ and PAR=800 mmol m⁻² s⁻¹) (Figure 5), which shows that the

reasonable application of phosphorus in the shading environment can slow down the reduction of the CO_2 assimilation rate, and quickly improve the plant's adaptability to shading environment simultaneously and make full use of light energy to produce photosynthetic products, thereby significantly increasing production. Compared with P0, the output of P1 treatment under shading increased by 36.7% (Table 2), but due to the restricted of light, the phosphate fertilizer can only partially alleviate but not make up for the decrease of CO_2 assimilation rate and product caused by insufficient light energy.

One question we aimed to address was whether the high CO_2 assimilation rates observed in soybean under shading condition is a consequence of the significant improvement of the photosynthetic system or the activity of the related reaction process of the Calvin-Benson cycle. Related studies have shown that C3 cycle-related enzymes are notably affected by light intensity ^[12, 14]. As the most abundant enzyme in plants, the proportion of activated Rubisco in C3 plants is about 25% ^[27, 28], the content may be excessive, so the reduction of Rubisco carboxylation rate can be compensated by rapid activation. FBPA and SBPase are the key enzymes in the C3 cycle for RUBP regeneration, small changes in its content will have a significant impact on CO_2 assimilation, and the activity is affected by the light intensity ^[29-30]. The activity of FBPA and SBPase significantly decreased in the low light, affecting RUBP regeneration, reducing the source of the Rubisco carboxylation substrate ^[31]. It can be seen that the carboxylation efficiency was reduced after shade treatment from the A/C_i curve, and it is effectively relieved in phosphorus application treatment (Figure 6). Further analysis showed that Rubisco's maximum carboxylation efficiency (V_{cmax}) and RUBP maximum regeneration rate (J_{max}) decreased significantly with the decrease of light. The V_{cmax} difference between P0 and P1 treatment was not significant under the same light environment, while J_{max} increased significantly and the increase in shading environment is significantly higher than that in the ambient light environment (Figure 7), indicating that the application of phosphate fertilizer in shading mainly increases CO_2 assimilation by increasing the regeneration rate of RUBP rather than the efficiency of Rubisco carboxylation, which also shows that phosphorus application in shading the increase of CO_2 assimilation rate may be closely related to the improvement of the performance of the photosystem.

Based on the above results, we have analyzed PSII. As the primary site of photosynthesis, photosystem II has the function of absorbing and converting light energy ^[32-33], and its performance is significantly affected by the light intensity ^[34]. The chlorophyll a fluorescence transient (OJIP) curves can reflect the process and state of the original photochemical reaction of PSII. The maximum photochemical efficiency (F_v/F_m) is an important parameter to measure the activity of PSII under various environmental stresses ^[35]. This experiment found that the changes of $F_v/F_m < 5\%$ before and after phosphorus application in L1 and L2 treatment (Figure 10A). Therefore, the effect of applying phosphate fertilizer on the photosynthetic rate of soybean under shading conditions seems to be very small according to the maximum photochemical efficiency of photosystem II. However, after weak light treatment, compared with P1 treatment, the relatively variable fluorescence at point J (2 ms) in the OJIP curve treated with P0

was significantly increased (Figure 8-9), indicating that the electron transfer from Q_A to Q_B is obstructed, that is, the PSII receptor Side electron transfer is blocked. Besides, we also observed that the relative fluorescence yield of P0 treatment at point K also showed a significant increasing trend, but not as obvious as point J, which proved that the application of phosphate fertilizer can improve the activity of the oxygen-evolving complex, but the influence on the smoothness of electron transfer is more significant.

To further explore the effect of phosphate fertilizer on photosystem activity, we compared j_{E0} , y_o , and PI_{ABS} . Studies on the PSII reaction center showed that electron transfer efficiency and light energy absorption performance were significantly reduced under shading (Figure 10 B-D). The performance of the receptor side of the plant, electron transfer efficiency, and light energy conversion efficiency of the PSII reaction center were all significantly improved after applying phosphorus fertilizer in shading, which is consistent with the changing trend of A_{sat} . Indicating that the increase in the rate of CO_2 assimilation after the application of phosphate fertilizer in shading is closely related to the improvement of PSII electron transport performance. The higher performance of the photosystem provides more assimilation force (NADPH, ATP) for RUBP regeneration, use the available photochemical energy to provide Rubisco with a sufficient source of the substrate.

After resuming light in the R5 period, the V_{cmax} and J_{max} of the L2P1 treatment showed an upward trend, and the performance of the PSII reaction center has a minimum reduction. There was no significant difference in A_{sat} compare to L1P1, and the light energy utilization efficiency was significantly higher than other treatments. Such results indicate that after restoring the light, L2P1 treatment can use more absorbed light energy for photochemistry due to restored balance between the light-dependent and light-independent reactions, maintain the electron transfer efficiency of the photosystem, and improve the regeneration rate of RUBP to provide sufficient substrate for Rubisco and ensures that soybeans can make full use of light energy after re-lighting, and increase the rate of photosynthesis products accumulation to increase yield.

Declarations

Acknowledgments

This work was financially supported by National Key Research and Development Program of China(2016YFD0300205). National Natural Science Foundation of China (31401339). Shandong Agricultural Science and Technology Fund (2019YQ014). Funds of Shandong “Double Tops” Program.

Authors' contribution

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

Precipitation and mean temperature of the month in cropping seasons of 2018. The bars indicate the precipitation and the lines indicate the mean temperature of the month.

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Figure 2

Chlorophyll a, b content of different treatments. L1: ambient sunlight; L2: 40% shade; P0: phosphate fertilizer application of 0 kg P₂O₅ ha⁻¹; P1: phosphate fertilizer application of 180 kg P₂O₅ ha⁻¹. The data is mean of 5 replications. Lowercase letters above bars indicate significant difference between the treatments at $P < 0.05$.

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Figure 3

The photosynthetic rate at a light intensity of 1400 mol m⁻² s⁻¹ of different treatments. L1: ambient sunlight; L2: 40% shade; P0: phosphate fertilizer application of 0 kg P₂O₅ ha⁻¹; P1: phosphate fertilizer application of 180 kg P₂O₅ ha⁻¹. The data is mean of 5 replications. Lowercase letters above bars indicate significant difference between the treatments at P < 0.05.

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Figure 4

Response of assimilation rate to the light intensity of different treatments. L1: ambient sunlight; L2: 40% shade; P0: phosphate fertilizer application of 0 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$; P1: phosphate fertilizer application of 180 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$. The data is mean of 5 replications. Lowercase letters above bars indicate significant difference between the treatments at $P < 0.05$.

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Photosynthetic capacity unit light intensity of different treatments. L1: ambient sunlight; L2: 40% shade; P0: phosphate fertilizer application of 0 kg P₂O₅ ha⁻¹; P1: phosphate fertilizer application of 180 kg P₂O₅ ha⁻¹. The data is mean of 5 replications. Lowercase letters above bars indicate significant difference between the treatments at $P < 0.05$.

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Response of assimilation rate to intercellular CO₂. L1: ambient sunlight; L2: 40% shade; P0: phosphate fertilizer application of 0 kg P₂O₅ ha⁻¹; P1: phosphate fertilizer application of 180 kg P₂O₅ ha⁻¹. The data is mean of 5 replications. Lowercase letters above bars indicate significant difference between the treatments at P < 0.05.

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Figure 7

Maximum rates of RUBP-carboxylation (V_{max}) and maximum rates of RUBP-regeneration (J_{max}) of different treatments. L1: ambient sunlight; L2: 40% shade; P0: phosphate fertilizer application of 0 kg P2O5 ha⁻¹; P1: phosphate fertilizer application of 180 kg P2O5 ha⁻¹. The data is mean of 5 replications. Lowercase letters above bars indicate significant difference between the treatments at $P < 0.05$.

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Figure 8

Chlorophyll fluorescence induction kinetics of different treatments. The upper curves represent the relative variable fluorescence rise normalized between F_o and F_m and plotted as V_t changes on a logarithmic scale [$V_t = (F_t - F_o) / (F_m - F_o)$]. From these OJIP curves[24] ΔV_t values were derived (lower curves). L1: ambient sunlight; L2: 40% shade; P0: phosphate fertilizer application of 0 kg P2O5 ha⁻¹; P1: phosphate fertilizer application of 180 kg P2O5 ha⁻¹. The data is mean of 5 replications. Lowercase letters above bars indicate significant difference between the treatments at $P < 0.05$.

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Figure 9

The donor and acceptor side performance of different treatments. L1: ambient sunlight; L2: 40% shade; P0: phosphate fertilizer application of 0 kg P2O5 ha⁻¹; P1: phosphate fertilizer application of 180 kg P2O5 ha⁻¹. The data is mean of 5 replications. Lowercase letters above bars indicate significant difference between the treatments at P < 0.05.

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Figure 10

PSII reactive center performance of different treatments. L1: ambient sunlight; L2: 40% shade; P0: phosphate fertilizer application of 0 kg P₂O₅ ha⁻¹; P1: phosphate fertilizer application of 180 kg P₂O₅ ha⁻¹. The data is mean of 5 replications. Lowercase letters above bars indicate significant difference between the treatments at $P < 0.05$.

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