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Compensatory Growth of Soybean after Shade during Vegetative Promotes Root Nodule Recovery

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ABSTRACT

Background: The recovery growth of soybean after environmental stress, especially shade stress, is essential for soybean to alleviate the yield loss caused by shade. Although the mechanism of recovery growth on leaves has been intensely researched, little is known about the mechanism of compensatory growth on soybean nodule formation.

Methods: This study aimed to investigate the effect of recovery growth after shade on soybean nodule formation and the distribution of carbohydrates in root nodules. Four shade treatments, including vegetative period shade (VS), reproductive period shade (RS), total growth period shade (TS) and no shade as control (CK), were applied on monoculture soybean by using a black mesh shading net with light transmittance of 50%.

Result: The aboveground dry weight, nodule number and weight and grains yield were lowest in TS and those values in VS were higher than in RS. Compared with RS, VS increased the nodule weight by 75.6% and the sucrose and starch content by 19.3% and 15.6%, respectively. We suggested that, compared with RS, VS improved the compensation growth of soybean and promoted the carbohydrate distribution into nodules, ultimately reducing the negative effect on soybean nodule formation and yield production.

Key words: Carbon metabolism, Nodule, Recovery growth, Shade, Soybean, Yield.

INTRODUCTION

Soybean [*Glycine max* (L.)] is a grain legume with considerable dietetic, industrial, medicinal and economic importance (Joshi *et al*., 2014). Shade stress negatively affected soybean growth and reduced soybean yield production (Ben Salah *et al*., 2009). Shade inhibits soybean yield by reducing the photosynthesis process to decrease the assimilate accumulation (Liu *et al*., 2020). Shade reduces the plant's leaf number, chlorophyll content and net photosynthesis rate, diminishing the crop's biomass (Baghdadi *et al*., 2016). Meanwhile, the decrease in biomass also represses the formation of soybean root nodules, which decline the soybean's symbiotic nitrogen fixation capacity (Chen *et al*., 2014).

The different shade periods showed various adverse effects on the plant's biomass accumulation and yield formation. And for a variety that showed late-maturing and a low ratio of the vegetative and reproductive periods, the shade during the vegetative periods had a slighter negative effect on soybean yield loss (Fan *et al*., 2018). Soybeans have evolved different strategies in response to competition for light: shade avoidance and shade tolerance (Raza *et al*., 2020). Besides focusing on the adaptability of crops aboveground to a shaded environment, the negative effect of shade on soybean nodule formation and nitrogen fixation capacity has been a concern by researchers in recent years. Previous researchers have proved that the optimal root nodule's function depends on the proper carbon metabolism since carbon metabolism provides an energy matrix and carbon skeleton for nitrogen assimilation (Chen *et al*., 2014). The distribution of carbohydrates in soybean roots regulated the formation of root and root nodules. The decline of soybean nodule number and weight was positively related to a photosynthate shortage under shade stress (Ben Salah *et al*., 2009).

After the shade stress has been removed in the vegetative shade period, recovery growth could compensate for the biomass and yield loss by increasing the formation of new leaves and the synthesis of chlorophyll, ultimately promoting the growth of plants (Luo *et al*., 2020; Wu *et al*., 2021). However, there is little known about the effect of recovery growth on the distribution of carbohydrates in root nodules and root nodule formation. Therefore, this study was conducted with a simulation experiment with a shade net to clarify the effects of shade in different growth stages on root nodule metabolism and yield formation of varying soybean varieties.

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MATERIALS AND METHODS

The experiment was conducted at Chong'zhou Modern Agricultural Research and Development Base (30°56′N, 103°64′E), Sichuan Province, China, in 2020. Shade tolerant soybean 'Nandou25' (*Glycine max* (L.)) was used in this experiment. This experiment was a completely randomized block design with three replicates with the following treatments: shade from 0 days after sowing (DAS) to 60 DAS (vegetative period shade, VS), shade from 60 DAS to 120 DAS (reproductive period shade, RS), shade from 0 DAS to 120 DAS (total growth period shade, TS) and no shade as a control treatment (CK). Shade treatment used a black mesh shading net with light transmittance of 50%. The light-transmitting radiation of the shade net was measured by an LI-COR line quantum sensor (LI-COR Inc., Lincoln, NE). The soybean was planted with 100 cm row spacing, 17 cm hole spacing and plant densities of 117,000 plants ha $^{-1}$. The plot size in each replication was 36 m^2 (six rows, each 6 m long).

At 65, 79, 93 and 121 DAS, three soybean plants in each plot were sampled and cut at ground level. The soybean leaf area was measured as the scan method described by Luo *et al.* (2022). The aboveground parts of the soybean were oven-baked for drying at a constant temperature of 80°C for at least three days until a stable weight was reached and the biomass was weighed on an electronic balance. At 65 and 93 DAS, collect the root and nodules of soybeans in the soil block with length, width and depth of 68, 50 and 20 cm, respectively, containing four adjacent soybean plants. The root system and nodules extracted from the soil were washed in tap water, peeled off the nodules on the root, wiped the water on the nodules with absorbent paper, counted the nodule number and weighed the fresh nodule weight. After cleaning the soybean nodules, these nodules were frozen in liquid nitrogen and stored at -80°C for further enzyme activity analysis. The sucrose and starch content of nodules were measured as described by Liu *et al*. (2019). The activity of sugar metabolism-related enzymes using the assay kit provided by Solarbio (Solarbio, Beijing, China). The enzyme activity of sucrose synthase (SS), sucrose phosphate synthase (SPS), sucrose neutral invertase (NI) and sucrose acid invertase (AI) were measured according to the related assay kit instructions. When the soybean matured, investigate the numbers of plant grains, pods and hundred-grain weight in a 6 m^2 strip. Recorded the soybean grain yield when the grain moisture content reached about 13.5 per cent.

All data were presented as the mean of three replicates and were analyzed using the analysis of variance (ANOVA) followed by Fisher's significant difference test at $p<0.05$. The data analysis and figure drawing were conducted using Origin Pro 2022 (Learning version) (Origin Lab., Hampton, MA, United States).

RESULTS AND DISCUSSION

Soybean leaf area, aboveground dry weight and grain yield

This experiment showed that shade negatively affects soybean leaf area, aboveground dry weight and grain yield. These results were consistent with the previous results that shading reduced soybean biomass accumulation and yield formation (Zhang *et al*., 2011). In this experiment, the total leaf area in VS was significantly lower than under CK and RS at 65 DAS, but the total leaf area in VS was not different from CK and RS after 79 DAS (Fig 1). The recovery growth after the restoration of the canopy light environment under

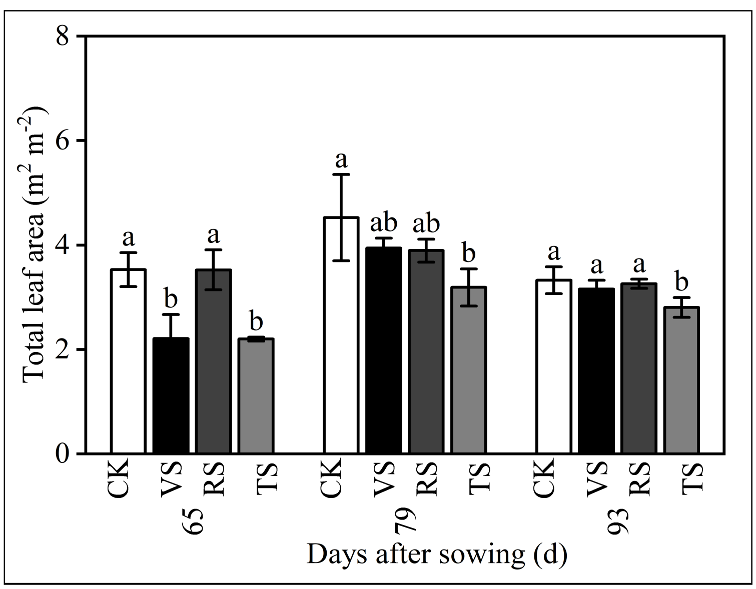

Fig 1: The effect of shade treatment on soybean leaf area. Shade treatment: CK, No shade control; VS, shade soybeans from 0 to 60 days after sowing; RS, shade soybeans from 60 days to 120 days after sowing; TS, shaded soybeans from 0 to 120 days after sowing. Different lowercase letters indicated a significant difference between treatments at 0.05 ($p<0.05$) probability levels.

shade stress increased the soybean leaf number, total leaf area and soybean biomass(Wu *et al*., 2021). Thus, in this experiment, the aboveground dry weight was lowest in TS and VS increased the aboveground dry weight by 14.1% compared to RS at 93 DAS (Fig 2). The increase in soybean biomass under VS contributed to the yield formation of soybeans. VS increased the pod number, grains number and grain yield by 10.1%, 52.8% and 36.1% compared to RS, respectively (Table 1). And the decrease in soybean pod number and grains number was consistent with the previous results that the reproductive shade inhibited the carbohydrate supply during the flower and pod formation process to decrease soybean pods and grains number (Baghdadi *et al*., 2016). And the pods and grains number and grain yield in VS was higher than in RS, mostly related to the recovery growth of soybeans in VS. Previous studies proved that the biomass loss caused by the shading stress during the vegetative stage could be alleviated during the recovery growth period, which increased the production of carbohydrates and energy to supply the growth and yield formation (Holubek *et al*., 2020; Li *et al*., 2001). Comprehensive the results in soybean leaf area, biomass and grain yield, we suggested that, compared with RS, VS showed a higher grain yield due to its promoted increase in leaf area and aboveground dry weight during the later growth period.

Soybean nodule phenotype and the sucrose and starch metabolism

As a legume crop, soybean could convert atmospheric nitrogen into biological ammonium with the help of symbiotic bacteria in root nodules by biological nitrogen fixation (Zhou *et al*., 2019). And the soybean nodule number and nodule weight were important phenotype indicators that reflect the ability of root nodules on biological nitrogen fixation. This experiment showed that shade treatments decreased the soybean nodule number and nodule weight at the 93 DAS and those values were lowest in TS (Fig 3). At the 93 DAS, the nodule number between VS and RS was not different, but the soybean nodule weight in VS was significantly higher than in RS. Compared with RS, VS increased the soybean root nodule number, nodule weight and average nodule weight by 13.8%, 75.6% and 54.4%, respectively. And the result that the nodule number and weight in VS were higher than in RS might be related to the change in soybean aboveground dry weight. Since the increase in soybean aboveground biomass might supply more carbohydrates to support the formation of root nodules growth and

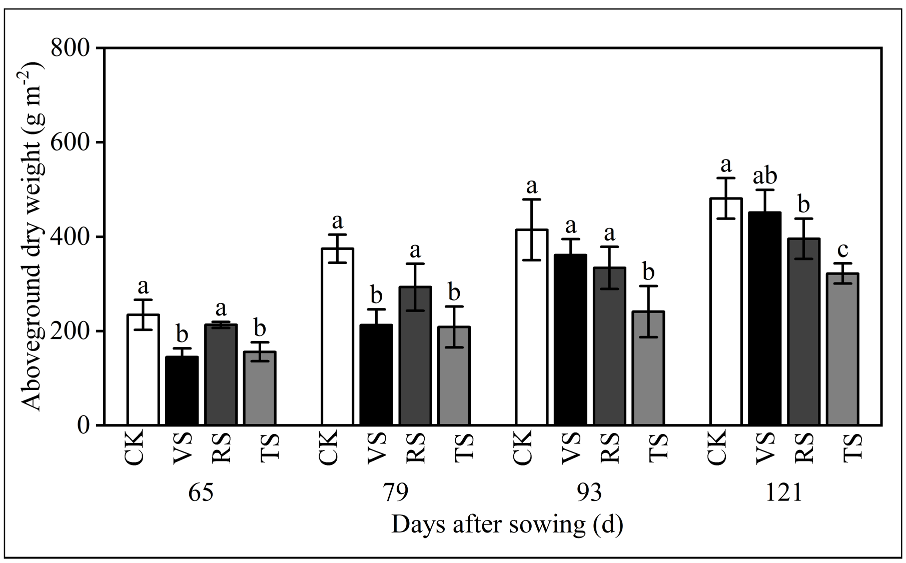

Fig 2: The effect of shade treatment on soybean aboveground dry weight. CK, No shade control; VS, shade soybeans from 0 to 60 days after sowing; RS, shade soybeans from 60 days to 120 days after sowing; TS, shaded soybeans from 0 to 120 days after sowing. Different lowercase letters indicated a significant difference between treatments at 0.05 ($p < 0.05$) probability levels.

Table 1: Effect of shade treatment on soybean yield and yield components.

Treatment	Pod number (No.10^4 ha^{-1})	Grain number (No.10^4 ha^{-1})	Grains per pod (grains pod^{-1})	Yield (kg ha^{-1})
CK	22.13±0.26a	624±133.1a	1.42±0.09b	2283±350a
VS	21.19±0.66ab	493±64.4ab	1.6±0.07ab	1939±162a
RS	19.25±0.17b	324±35.7b	1.7±0.1 a	1439±145b
TS	20.45±0.4b	316±11.7b	1.54±0.07b	1166±71b

Note: CK: No shade control; VS: Vegetative period shade; RS: Reproductive period shade; TS: Total growth period shade. Different lowercase letters indicated a significant difference between each treatment at 0.05 probability levels.

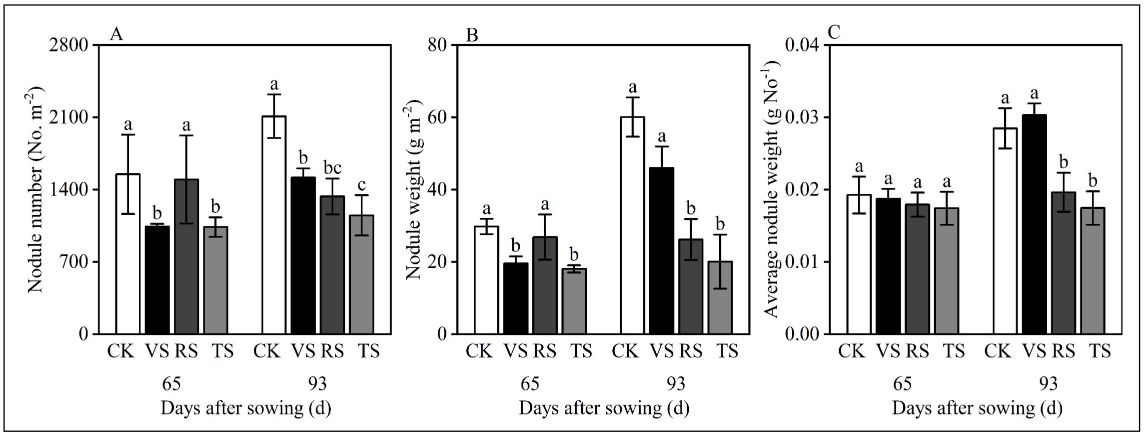

Fig 3: The effect of shade treatment on soybean nodule number and nodule weight. A and B represent the soybean nodule number and nodule weight, respectively. CK, No shade control; VS, shade soybeans from 0 to 60 days after sowing; RS, shade soybeans from 60 days to 120 days after sowing; TS, shaded soybeans from 0 to 120 days after sowing. Different lowercase letters indicated a significant difference between treatments at 0.05 ($p<0.05$) probability levels.

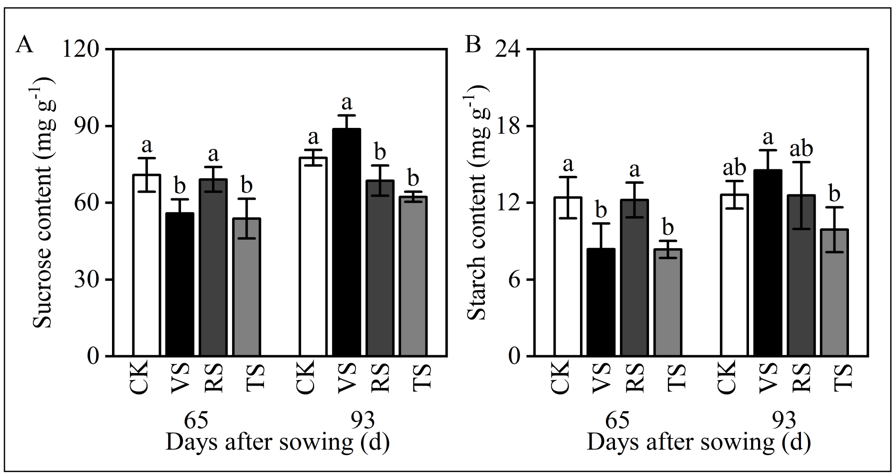

Fig 4: The effect of shade treatment on sucrose and starch content of soybean nodules. A and B represent the sucrose content and starch content, respectively. CK, No shade control; VS, shade soybeans from 0 to 60 days after sowing; RS, shade soybeans from 60 days to 120 days after sowing; TS, shaded soybeans from 0 to 120 days after sowing. Different lowercase letters indicated a significant difference between treatments at 0.05 ($p<0.05$) probability levels.

development. In this experiment, the decrease in soybean nodule number and weight was consistent with previous studies that the shade inhibited soybean nodule formation by reducing the carbohydrate distribution in roots (Ben Salah *et al*., 2009).

The accumulation of sucrose and starch in soybean nodules could reflect the plant's ability to provide sufficient carbon sources and energy for the growth of underground roots (Bruening and Egli, 2000). The SS and SPS enzymes are essential for normal nodule development and function (Seger *et al*., 2008). Results in this experiment showed that, at 93 DAS, the sucrose and starch content were highest in VS treatment (Fig 4). Compared with RS, VS increased the sucrose and starch content by 29.3% and 15.6%, respectively. And the increase in sucrose and starch content might suggest that VS promote more carbohydrate allocated in nodules to encourage the formation. And the change in sucrose and starch-related enzymes also proved our suggestion, where VS increased the SPS and AI enzyme activity by 48.7% and 58.3%, compared with RS (Fig 5). Either SS or AI might hydrolyze this sucrose and follow the glycolytic pathway to provide energy and carbon skeletons for bacteroid respiration and ammonia assimilation (Du *et al*., 2020). The increase in AI enzyme activity provides a carbon source for the rapid growth of tissue (Burger and Schaffer, 2007). Thus, the increase in SS, SPS and AI enzymes in VS might indicate that VS enhanced the ability of nodules to synthesize sucrose. Comprehensive the above results, we suggested that VS increased the activity of SPS and AI and allocated more carbohydrates in soybean

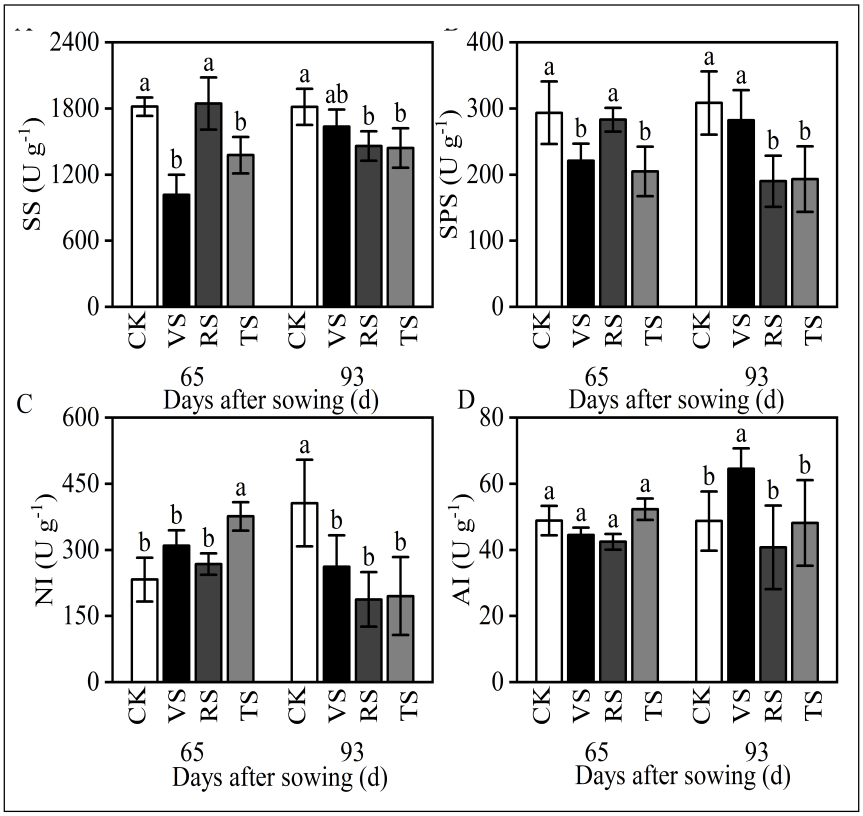

Fig 5: The effect of shade treatment on carbon metabolism of soybean nodules. A, B, C, and D represent SS,SPS, NI, and AI enzyme activities, respectively. CK, No shade control; VS, shade soybeans from 0 to 60 days after sowing; RS, shade soybeans from 60 days to 120 days after sowing; TS, shaded soybeans from 0 to 120 days after sowing. Different lowercase letters indicated a significant difference between treatments at 0.05 ($p < 0.05$) probability levels.

nodules to improve the formation of root nodules during the recovery growth.

CONCLUSION

Shade decreased soybean nodule number and weight, leaf area and grain yield, but those values in VS were higher than in RS and TS treatment. Compared with RS, VS increases enzyme activity of SS, SPS and AI to improve the sucrose and starch allocated in root nodules to increase the soybean nodule number and nodule weight, ultimately reducing the negative effect on soybean nodule formation and yield production caused by shade.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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