

Effect of starter nitrogen fertilizer on photosynthetic characters of relay strip intercropping soybean under drought stress

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Abstract: A pot experiment was designed under drought stress condition to study the effect of different starter nitrogen fertilizer on photosynthetic characters in the leaves of relay strip intercropping soybean at branching stage. The Pn, Gs, Tr were measured, WUE was calculated. The results showed drought stress significantly decreased Pn, Gs and Tr. Mild drought stress reduced Ci, moderate drought stress and severe drought stress increased Ci. Under the same starter nitrogen fertilizer WUE was higher as the soil water content reduced. WUE of moderate drought stress was higher than that of mild drought stress, WUE of mild drought stress was higher than that of severe drought stress, and WUE of severe drought stress was higher than that of control. Appropriate starter nitrogen fertilizer was benefit to improve Pn, Gs and Tr, reduce Ci.

Introduction

Soybean is a species of legume widely grown for its edible bean which has numerous uses and is rather sensitive to water stress [1]. In order to increase land use efficiency which can produce a greater yield on a given piece of land by maximizing resources, wheat-corn/soybean relay strip intercropping system is popular in southwest China [2]. But because soybean under the system was affected by climate and hilly landforms, it always exposed to drought stress. In crop production, water is an important growth limiting factor and it is the most vital factor in physiological reaction at the same time [3]. Water stress could affect on plant's physiology, including growth [4], signaling pathways [5], gene expression [6] and leaf photosynthesis [7, 8]. Photosynthesis is an important determining factor of soybean yield [9]. Nitrogen for crop drought resistance has double role in water deficit [10]. Nitrogen is one of the major nutrients that are required for soybean growth and development. Starter nitrogen fertilizer benefited root activity, leaf photosynthesis, and consequently its yield [11]. Therefore, under drought stress condition, pot experiment was conducted to study the effects of nitrogen on Pn, Ci, Gs, Tr of relay intercropping soybean, and to provide an identification system for the study of soybean drought resistance, to provide theoretical basis and technical support in the application of nitrogen for soybeans.

Materials and Methods

Materials. Soybean cultivar Gongxuan No.1, a major component of southwestern soybean cultivars, was tested in the experiments.

Experimental Design. The experiment was conducted in a relay strip intercropping system at the farm of

Sichuan Agricultural University. Soybean seeds were sown in the pots. Only three plants were allowed to grow per pot. Urea was dissolved in water for base fertilizer, and the tray in the pelvic floor was used to avoid the leaching of nitrogen after the rain. Each treatment was conducted with three replicates, and each replicate had 6 pots. Four nitrogen treatments were used 0 g N pot⁻¹, 0.35 g N pot⁻¹, 0.70 g N pot⁻¹, 1.40 g N pot⁻¹, which were equivalent to 0 kgN·ha⁻¹, 45 kgN·ha⁻¹, 90 kgN·ha⁻¹, 180 kgN·ha⁻¹. Four drought stress treatments were imposed after the pots were moved in the shed, at the branching stage of soybean. 1/4 of the pots were kept continuously moist (WW, 75±2% of the field water capacity, short for FWC), and so did the light drought (LD, 60±2% of the field water capacity) and moderate drought (MD, 45±2% of the field water capacity) and severe drought (SD, 30±2% of the field water capacity). Soybean pots were placed under shade of maize to simulate light environment of the relay strip intercropping system of wheat-corn-soybean. Pots were moved into a shed with shading net at branching stage. The light under the net was 65% of environmental light. Net was moved, when maize in the field matured. The characters were determined 7 days after drought stress.

Photosynthetic parameters measurements. The net photosynthesis rate (Pn), stomatal conductance (Gs) and transpiration (Tr) were measured with a Portable Photosynthesis System (Model LI-6400, LI-COR Inc., Lincoln, NE). Water use efficiency (WUE) was calculated as Pn/Tr. The parameters were measured daily, after water stress was applied from 8:00 am to 12:00 am. Five plants were randomly chosen, and one of the most recently expanded leaves was selected from each plant four times. The photosynthetically active radiation (PAR), provided by an LED light source, was set to 1200 μmol m⁻² s⁻². The flow rate of air through the sample chamber was set at 500 μmol⁻¹ s⁻¹, and the leaf temperature was maintained at 25±0.8⁰C by thermoelectric coolers. The CO₂ concentration of the chamber was adjusted to 400 μl l⁻¹ with the system's CO₂ injector.

Statistic analyses. Results were analyzed by two-way analysis of variance (LSD) and means were compared by Duncan's multiple range tests at P<0.05. All data were organized in Excel (Microsoft) spread sheets and processed by the software Statistical Package for the Social Sciences (SPSS) version 11.5.

Results and Discussion

Pn. From Table 1, it was found that drought stress significantly decreased Pn, and Pn was lower as the degree of drought stress became severely. Under the same nitrogen treatment, Pn was the highest at control (75% of FWC), followed by mild stress (60% of FWC), and then moderate stress (45% of FWC), severe drought (30% of FWC). Under control (75% of FWC), appropriate N supply could increase Pn value, but differences of Pn among different N supply were not significant. Under 60%, 45% and 30% of FWC, appropriate N supply could significant increase Pn value. Under mild stress and moderate stress, Pn value was highest at 0.70 g N pot⁻¹, lowest at 0 g N pot⁻¹. Under severe stress Pn value was highest at 0.35 g N pot⁻¹, lowest at 1.40 g N pot⁻¹, which showed that when the soil water content was severely low, increase nitrogen application could not enhance Pn value, on the contrary Pn value decreased as the nitrogen application increased.

Table 1 Effect of drought stress and nitrogen levels on Pn ($\mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$)

Treatment	0 g N pot ⁻¹	0.35 g N pot ⁻¹	0.70 g N pot ⁻¹	1.40 g N pot ⁻¹
30% of FWC	0.9187g	1.3553g	0.599h	0.5107h
45% of FWC	3.5683f	4.423e	5.4501e	3.7044f
60% of FWC	7.2227cd	9.3608c	11.2961b	8.7658c
75% of FWC	14.0188a	14.7867a	15.9408a	14.6445a

Note: The same small letters indicate the significant differences at $P < 0.05$, the same below.

Gs. The results showed that Gs reduced significantly when drought happened, and the Gs value was lower as the degree of drought stress became severely. Under the same nitrogen supply, the Gs value was highest at 75% of FWC, followed by mild stress, and then moderate stress, severe stress. Under 75% of FWC, Gs was highest at the treatment of 0.35 g N pot⁻¹. Under 60% of FWC Gs was highest at the treatment of 0.7 g N pot⁻¹. Under 45% of FWC Gs was highest at the treatment of 0.7 g N pot⁻¹. Under 30% of FWC Gs was highest at the treatment of 0.35 g N pot⁻¹.

Table 2 Effect of drought stress and nitrogen levels on Gs ($\text{mmol m}^{-2} \text{ s}^{-1}$)

Treatment	0 g N pot ⁻¹	0.35 g N pot ⁻¹	0.70 g N pot ⁻¹	1.40 g N pot ⁻¹
30% of FWC	0.0037e	0.0060d	0.0037e	0.0036e
45% of FWC	0.0065d	0.0114cd	0.0117cd	0.0124cd
60% of FWC	0.0160cd	0.0265c	0.0367b	0.0217c
75% of FWC	0.0462b	0.0966a	0.0900a	0.0702ab

Ci. We observed a reduction and then an increase in Ci under the same nitrogen supply when soil water content reduced. Under the same nitrogen supply, the Ci value was lowest at mild stress, followed by control, and then moderate stress, severe stress. Under 75%, 60%, 45% and 30% of FWC, Ci was lowest at the treatment of 0.7 g N pot⁻¹, and was highest at the treatment of 0 g N pot⁻¹.

Table 3 Effect of drought stress and nitrogen levels on Ci (mg kg^{-1})

Treatment	0 g N pot ⁻¹	0.35 g N pot ⁻¹	0.70 g N pot ⁻¹	1.40 g N pot ⁻¹
30% of FWC	578.03a	458.67b	448.09b	537.90ab
45% of FWC	449.71b	338.99c	241.72cde	325.36cd
60% of FWC	235.18de	230.66de	186.60e	210.99e
75% of FWC	251.48cde	241.89cde	238.41cde	247.49cde

Tr. The results showed that Tr reduced as the soil water content decreased. Under the same nitrogen supply, the Tr value was highest at 75% of FWC, followed by mild stress, and then moderate stress, severe stress. Under 75% , 60% and 45% of FWC, Tr was lowest at the treatment of 0 g N pot⁻¹, and was highest at the treatment of 0.7 g N pot⁻¹. Under 30% of FWC, Tr was lowest at the treatment of 0 g N pot⁻¹, and was highest at the treatment of 0.35 g N pot⁻¹.

Table 4 Effect of drought stress and nitrogen levels on Tr ($\text{mmol m}^{-2} \text{ s}^{-1}$)

Treatment	0 g N pot ⁻¹	0.35 g N pot ⁻¹	0.70 g N pot ⁻¹	1.40 g N pot ⁻¹
30% of FWC	0.15d	0.23d	0.15d	0.16d
45% of FWC	0.39d	0.51d	0.60d	0.48d
60% of FWC	0.80cd	1.21cd	1.68c	1.03cd
75% of FWC	2.96b	3.95a	3.97a	3.81ab

WUE. The results showed that mild drought and moderate drought can increase WUE under four nitrogen treatments. Under 0 g N pot⁻¹ and 0.35 g N pot⁻¹ treatments severe drought could also enhance WUE. WUE of severe drought reduced under 0.7 g N pot⁻¹ and 1.4 g N pot⁻¹ treatments. Under 0 g N pot⁻¹ and 0.35 g N pot⁻¹, WUE was highest at moderate stress, followed by mild stress, and then severe stress, control. Under 0.7 g N pot⁻¹, WUE was highest at moderate stress, followed by mild stress, and then control, severe stress. Under 1.4 g N pot⁻¹, WUE was highest at mild stress, followed by moderate stress, and then control, severe stress.

Table 5 Effect of drought stress and nitrogen levels on WUE

Treatment	0 g N pot ⁻¹	0.35 g N pot ⁻¹	0.70 g N pot ⁻¹	1.40 g N pot ⁻¹
30% of FWC	6.12c	5.89cd	3.99de	3.19e
45% of FWC	9.15a	8.67ab	9.08a	7.72b
60% of FWC	9.03a	7.74b	6.72c	8.51ab
75% of FWC	4.74d	3.74de	4.02de	3.84de

Conclusion

Stomatal closure primarily causes a decline in the photosynthesis rate [12]. The variation in C_i can be used as a standard to estimate the reasons for decreased P_n , and whether decreases in G_s or reductions of mesophyll can result in changes in the cell's photosynthetic capacity. In this study, we observed a decrease in G_s and increase in C_i in the moderate drought and severe drought stressed plants. These results suggest that the strong decrease in P_n in mild drought-stressed plants may be caused by the closure of stomata. Reduction of moderate and severe drought-stressed plants in the photosynthetic capacity of mesophyll cells, which in turn results in increased C_i . Appropriate starter nitrogen fertilizer was benefit to improve P_n , G_s and Tr , reduce C_i .

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