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Effect of starter nitrogen fertilizer and drought stress on fluorescence parameters of relay strip intercropping soybean

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Abstract: A pot experiment was designed under drought stress condition to study the effect of different starter nitrogen fertilizer on fluorescence parameters in the leaves of relay strip intercropping soybean at branching stage. The Fo, Fv/Fm, ΦPSII, qP, NPQ and ETR were measured or calculated. The results showed drought stress significantly increased Fo and NPQ, reduced Fv/Fm, ΦPSII, qP and ETR. Nitrogen application soybean plants had higher qP, ΦPSII, NPQ and ETR, as compared to the zero nitrogen application soybean plants when under drought stress. This could be because drought did not have a serious effect on the nitrogen application soybean plants compared to zero nitrogen application soybean plants.

Introduction

Soybean is one of the major and widespread crops in the world and is rather sensitive to water stress [1]. In southwest China, wheat-corn/soybean relay strip intercropping system is popular, due to the high land use efficiency which can produce a greater yield on a given piece of land by maximizing resources [2]. But soybean under the system always exposed to drought stress as affected by climate and hilly landforms. Water is another most important growth limiting factors in crop production and at the same time it is the most vital factors in physiological reactions [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 the actual photochemical efficiency (ΦPS II) and photochemical quenching (qP) maximum photochemical efficiency (Fv/Fm), lower non-photochemical quenching (qN) 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. 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. Only three plants were allowed to grow per pot. 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}(N_1),45 kgN·ha^{-1}(N_2),90 kgN·ha^{-1}(N_3),180 kgN·ha^{-1}(N_4). 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.

Chlorophyll fluorescence measurements. Chlorophyll fluorescence was measured by a fluorescence monitoring system (MINI-PAM, WALZ Co., Germany) on randomly selected leaves (third fully expanded leaf) of plants at 0:00-4:00 am. Following 30 min of dark adaptation, the minimum chlorophyll fluorescence (Fo) was determined using a measuring beam of 0.2 μmol m^{-2} s^{-1} light intensity. A saturating pulse (1s white light with a PPFD of 7500 μmol m^{-2} s^{-1}) was used to obtain the maximum fluorescence (Fm) in the dark-adapted state. Maximum quantum yield of PSII (Fv/Fm) was calculated as (Fm−Fo)/Fm [12]. Following on from this, an actinic light (PPFD of 300 μmol m^{-2} s^{-1}) was applied, subsequently, further saturating flashes were applied at appropriate intervals to measure the Fm' (maximum fluorescence in a light saturated stage). Ft is the steady-state fluorescence in the light-adapted state. 3 seconds after removal of actinic light, Fo' (minimum chlorophyll fluorescence in a light-adapted state) was measured using a far-red light of 5 W m^{-2}. Quantum yield of PSII (ΦPSII) is calculated as: ΦPSII = (Fm’−Ft) / Fm’ [13]. Photochemical quenching (qP) was calculated as: qP =(Fm’–Ft)/(Fm’–Fo’). Non-photochemical quenching (NPQ) was calculated as: NPQ= (Fm−Fm’)/Fm’ according to Maxwell and Johnson [12]. Apparent photosynthetic electron transport rate was calculated as: ETR=ΦPSII×PAR×0.5×0.84 (PAR=350 μmol m^{-2} s^{-1}) [14]. Transport of one electron requires absorption of two quanta, as two photosystems are involved (factor 0.5). It is assumed that 84 % of the incident quanta are absorbed by the leaf (factor 0.84).

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

Fo. From Table 1, it was found that drought stress significantly increased Fo, and Fo became higher as the degree of drought stress became severely. Under the same nitrogen treatment, Fo was the lowest 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 Fo value, but differences of Fo among different N supply were not significant. Under

60%, 45% and 30% of FWC, appropriate N supply could significant increase Fo value. Under mild stress and moderate stress, Fo value was highest at 0.70 g N pot^{-1}, lowest at 0 g N pot^{-1}. Under severe stress Fo value was highest at 0.35 g N pot^{-1}, lowest at 0 g N pot^{-1}.Which showed that when the soil water content became lower, appropriately increase nitrogen application could enhance Fo value.

Table 1 Effect of drought stress and nitrogen levels on Fo

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	341.67b	397.33a	391.33a	388.67a
45% of FWC	287.00d	303.67cd	326.22bc	315.67bcd
60% of FWC	281.67d	284.67d	311.00bcd	288.33cd
75% of FWC	278.67d	280.00d	289.33cd	283.67d

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

Fv/Fm. The results showed that Fv/Fm reduced significantly when drought happened, and the Fv/Fm value was lower as the degree of drought stress became severely. Under the same nitrogen supply, the Fv/Fm value was highest at 75% of FWC, followed by mild stress, and then moderate stress, severe stress. Under 75% and 60% of FWC, Fv/Fm was highest at the treatment of 1.4 g N pot^{-1}, but the differences were not significant. Under 45% of FWC Fv/Fm was highest at the treatment of 0.35 g N pot^{-1}, and also the differences were not significant. Under 30% of FWC Fv/Fm was significantly lower at the treatment of 0.35 g N pot^{-1} than the other nitrogen treatments.

Table 2 Effect of drought stress and nitrogen levels on Fv/Fm

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	0.7147c	0.7167c	0.6963c	0.7613b
45% of FWC	0.791a	0.7947a	0.7822ab	0.7910a
60% of FWC	0.798a	0.7980a	0.7953a	0.7990a
75% of FWC	0.8013a	0.8077a	0.8027a	0.8093a

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

Table 3 Effect of drought stress and nitrogen levels on ΦPSII

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	0.2516ef	0.1731f	0.1919f	0.2589def
45% of FWC	0.2802def	0.2676def	0.2917def	0.2733def
60% of FWC	0.3952bcd	0.3364cde	0.4374bc	0.4262bc
75% of FWC	0.5003ab	0.5751a	0.6100a	0.5761a

qP. The results showed that qP reduced significantly when drought happened, and the qP value was lower as the degree of drought stress became severely. Under the same nitrogen supply, the qP value was highest at 75% of FWC, followed by mild stress, and then moderate stress, severe stress. Under control, mild drought stress, moderate drought stress and severe drought stress, qP was highest at the treatment of 0.7 g N pot^{-1}, lowest 0 g N pot^{-1}.

Table 4 Effect of drought stress and nitrogen levels on qP

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	0.5534d	0.6631c	0.8440b	0.7357bc
45% of FWC	0.6300c	0.7468bc	0.9714a	0.7873bc
60% of FWC	0.6296c	0.7791bc	0.9986a	0.8771ab
75% of FWC	0.8296b	0.9095ab	1.0375a	1.0088a

NPQ. The results showed that NPQ increased significantly when drought happened, and the NPQ value was higher as the degree of drought stress became severely. Under the same nitrogen supply, the NPQ value was lowest at 75% of FWC, followed by mild stress, and then moderate stress, severe stress. Under control, mild drought stress, moderate drought stress and severe drought stress, NPQ was highest at the treatment of 1.4 g N pot^{-1}, lowest 0 g N pot^{-1}.

Table 5 Effect of drought stress and nitrogen levels on NPQ

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	3.3249abc	3.4383abc	3.9867ab	4.3132a
45% of FWC	2.004cdef	2.3902bcde	2.9905abcd	3.0067abcd
60% of FWC	1.3008def	1.8571cdef	1.8809cdef	2.1879bcdef
75% of FWC	0.8671ef	0.5048f	0.5891ef	0.8418ef

ETR. The results showed that ETR reduced significantly when drought happened, and the ETR value was lower as the degree of drought stress became severely. Under the same nitrogen supply, the ETR value was highest at 75% of FWC, followed by mild stress, and then moderate stress, severe stress. Under control, mild drought stress, moderate drought stress and severe drought stress, ETR was highest at the treatment of 1.4 g N pot^{-1}, lowest 0 g N pot^{-1}.

Table 6 Effect of drought stress and nitrogen levels on ETR

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	11.48e	11.72e	12.83e	16.29de
45% of FWC	13.87de	22.39cde	28.59bcde	30.69bcd
60% of FWC	31.03bcd	34.97bc	37.27abc	39.28abc
75% of FWC	36.90abc	38.98abc	42.29ab	53.83a

Conclusion

Drought stress significantly increased Fo and NPQ, reduced Fv/Fm, ΦPSII, qP and ETR. Nitrogen application soybean plants had higher qP, ΦPSII, NPQ and ETR, as compared to the zero nitrogen application soybean plants when under drought stress. This could be because drought did not have a serious effect on the nitrogen application soybean plants compared to zero nitrogen application soybean plants.

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