Effect of nitrogen and drought stress on membrane lipid peroxidation and protective enzyme activities 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 nitrogen levels on lipid peroxidation and protective enzyme activities in the leaves of relay strip intercropping soybean. The activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and the content of malondialdehyde (MDA) were measured. Results showed that under the drought stress condition, activities of SOD, POD, CAT, content of MDA were significantly increased when drought happened, and the value increased as the degree of drought stress became severely. Nitrogen application could reduce MDA content, increase SOD, POD, CAT activities, and then the membrane lipid peroxidation was reduced. The effects of different nitrogen application levels are different. Appropriate nitrogen fertilizer can increase the activity of enzyme and reduce the content of MDA, but much too high nitrogen treatment will be contrary.

Introduction

The demand for food is also constantly increasing, as a result of population increases [1, 2]. Multiple cropping systems using intercropping can produce a greater yield on a given piece of land by maximizing resources [3]. In China half of the total grain yield is produced by multiple cropping [4]. The wheat-corn/soybean relay strip intercropping system is popular in Southern China. Soybeans are typically exposed to summer drought in southwest China as affected by climate and hilly landforms. The research results showed that the activity of protective enzymes SOD, POD, CAT, photosynthesis rate and biomass could be increased by suitable nitrogen level under water deficit [5]. But the activity of SOD rises to CK. Nitrogen for crop drought resistance has double role in water deficit [5]. Therefore, under drought stress condition, pot experiment was conducted to study the effects of nitrogen on membrane lipid peroxidation and protective enzyme activities 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.

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

SOD. From Table 1, it was found that drought stress significantly increased the activity of SOD, and the activity of SOD was higher as the degree of drought stress became severely. Under the same nitrogen treatment, the activity of SOD was the highest at 30% of FWC, followed by moderate stress (45% of FWC), and then mild stress (60% of FWC), control (75% of FWC). The activity of SOD was the lowest at 71.81 μ•g^{-1}FW at the treatment of 1.4 g N pot^{-1} under 75% of FWC, and the activity of SOD in the severe stress was the highest at 320.48 μ•g^{-1}FW under treatment of 0.70 g N pot^{-1}. Under 75% of FWC SOD activity was highest at the treatment of 0.35 g N pot^{-1}. Under 60% of FWC SOD activity was highest at the treatment of 0.7 g N pot^{-1}. Under 45% of FWC SOD activity was highest at the treatment of 0.35 g N pot^{-1}. Under 30% of FWC SOD activity was highest at the treatment of 0.7 g N pot^{-1}. Suitable N application increased SOD activity when drought happened.

Table 1 Effect of drought stress and nitrogen application level on the SOD activity of soybean leaves(μ•g^{-1}FW)

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	254.73def	308.59ab	320.48a	300.34ab
45% of FWC	229.02fg	286.75bc	270.99cd	209.61g
60% of FWC	166.42h	231.44efg	257.88de	160.71h
75% of FWC	124.70i	231.93efg	217.61g	71.81j

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

POD. From Table 2, it was found that drought stress significantly increased the activity of POD. Under 75% of FWC POD activity was highest at the treatment of 1.4 g N pot^{-1}. Under 60%, 45%

and 30% of FWC POD, activity was highest at the treatment of 0.35 g N pot^{-1}. The activity of POD was the lowest at 4477.78 μ(0.01A_{470}•min^{-1}) at the treatment of 0 g N pot^{-1} under 75% of FWC, and the activity of POD in the severe stress was the highest at 6838.89 μ(0.01A_{470}•min^{-1}) under treatment of 0.35 g N pot^{-1} .

Table 2 Effect of drought stress and nitrogen application level on the POD activity of soybean leaves μ(0.01A_{470}•min^{-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	6419.44abc	6838.89a	6686.11ab	6263.89bcd
45% of FWC	6041.67cd	6763.89ab	6472.22abc	6291.67abcd
60% of FWC	5333.33f	6211.11bcd	5394.44ef	5844.44de
75% of FWC	4477.78g	4761.11g	4894.44fg	4988.89fg

CAT. From Table 3, it was found that drought stress significantly increased the activity of CAT, but the differences were not significant. Under 75% of FWC CAT activity was highest at the treatment of 0.35 g N pot^{-1}. Under 60% of FWC CAT activity was highest at the treatment of 0.7 g N pot^{-1}. Under 45% of FWC CAT activity was highest at the treatment of 0.35 g N pot^{-1}. Under 30% of FWC CAT activity was highest at the treatment of 0.7 g N pot^{-1}. The activity of CAT was the lowest at 1.8511 mg H_2O_2/g•min at the treatment of 1.4 g N pot^{-1} under 75% of FWC, and the activity of CAT in the severe stress was the highest at 34.7829 mg H_2O_2/g•min under treatment of 0.7 g N pot^{-1} . Suitable N application increased CAT activity when drought happened.

Table 3 Effect of drought stress and nitrogen application level on the CAT activity of soybean leaves (mg H_2O_2/g•min)

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	8.4575bc	16.1394b	34.7829a	8.6497bc
45% of FWC	6.0831bc	12.0592bc	11.7259bc	7.0586bc
60% of FWC	3.4441bc	7.5548bc	9.1909bc	6.2685bc
75% of FWC	2.8328bc	5.8783bc	4.7521bc	1.8511c

MDA. From Table 4, it was found that drought stress significantly increased the content of MDA, and the MDA content was higher as the degree of drought stress became severely. Under the same nitrogen treatment, the content of MDA was the highest at 30% of FWC, followed by moderate stress (45% of FWC), and then mild stress (60% of FWC), control (75% of FWC). Under the same water supply, MDA content was lower as the N application increased. The content of MDA was the lowest at 25.85 nmol g^{-1}FW at the treatment of 0.35 g N pot^{-1} under 75% of FWC, and the content of MDA in the severe stress was the highest at 60.41 nmol g^{-1}FW under treatment of 0 g N pot^{-1} under 30% of FWC**.** Suitable N application decreased MDA content when drought happened.

Table 4 Effect of drought stress and nitrogen application level on the MDA content of soybean leaves (nmolg^{-1}FW)

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	60.41a	59.07a	46.87b	44.78bc
45% of FWC	41.54bcd	31.00de	32.39cde	34.04cde
60% of FWC	38.16bcde	27.57e	30.45de	28.04e
75% of FWC	32.75cde	25.85e	29.34de	27.47e

Conclusion

Drought is a major abiotic factor that limits agricultural crop production. To improve agricultural productivity within limited land and water resources, it is imperative to ensure high crop yields against unfavorable environmental stresses. Nitrogen for crop drought resistance has double role in water deficit. In this study, appropriate nitrogen fertilizer (not too much) can increase the activity of enzyme and reduce the content of MDA, but high nitrogen treatment will be contrary.

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