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EFFECTS OF NITROGEN FERTILIZER ON PHOTOSYNTHETIC AND ANTIOXIDATIVE TRAITS OF SUPER-HIGH-YIELD SOYBEAN

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Abstract

Soybean is an important cash crop in China which provides a key protein source for Chinese. In order to study the effect of N fertilizer application (0, 75, 150, 225 and 300 kg/ha) on photosynthetic rate, diurnal variation of photosynthetic rate, superoxide dismutase (SOD) activity, peroxidase (POD) activity, malondialdehyde (MDA) content and yield of three super-high-yield soybean genotypes and one common yield soybean variety a field experiment was carried out. The results showed that the suitable fertilizer treatment (75, 150, 225 kg/ha) could significantly increase super-high-yield soybean yield compared with common yield soybean. Super-high-yield soybean cultivars Zhonghuang 35 (2944.6 kg/ha), Shennong 12 (2914.9 kg/ha) and Liaodou 14 (2438.9 kg/ha) produced greater yield than that of common yield soybean Liaodou11 (1983.2 kg/ha). Photosynthesis of super-high-yield cultivar were significantly enhanced with nitrogen fertilizer level added except 300 kg/ha at the R5, R6 growth stages, which are the key periods for seed filling. Photosynthetic diurnal variation of soybean cultivars did not show “noontime snooze” under different fertilizer treatments at R6 stage, and the photosynthetic rate of super-high-yield cultivar is higher than that of common cultivar after 14 o'clock. Fertilizer treatment was also able to delay soybean leaf senescence effectively for super-high-yield at R7 growth stage by enhancing the SOD, POD activity, and lowering the MDA content of super-high-yield soybean.

Introduction

Soybean (*Glycine max* (L.) Merrill.) is an important crop in China, which provides most of protein meal and edible oil. In China, soybean yield increased from 7.5 million Mg in 1978 to 19.8 million Mg in 2015. However, the amount of produced soybean is not sufficient to meet the growing demand. In China, the largest production areas are in the northeast of China, Liaoning, Jilin and Heilongjiang province, where farmers seed soybean in spring and harvest in autumn. The three provinces accounts for 33 and 44% of country's soybean sown area and total yield, respectively (Liu *et al*. 2002).

In northeast China, the potential soybean yield has been estimated to be from 3500 kg/ha to 5000 kg/ha. For the purpose of achieving high yield potential, soybean must maintain high photosynthesis rate and accumulate large amounts of dry matters. There is generally a positive relationship between nitrogen exists in leaves and photosynthesis (Sinclair 2004). Therefore, the soybean should have sufficient amount of N in leaves to sustain efficiency photosynthetic apparatus for converting incoming radiation into new biomass and finally grain yield. Although soybean can fix nitrate in the soil, there are gaps between crop N demand and N supply by N_2 fixation in soil, which will decrease the photosynthetic capacity of leaves in canopy and thus limit soybean yield potential. Applying diammonium phosphate fertilizer has been proposed as a good way to increase available N in the soil (Liu *et al.* 2008).

In recent years, most Chinese breeders developed many super-high-yield soybean varieties. However, the fertilizer demand for the super-high-yield soybean compared to common yield soybean was poorly understood. The objective of this study was to investigate the effect of different

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amount of nitrate fertilizer on yield and the physiological aspects of growth of super-high-yield soybean and common soybean.

Materials and Methods

Three super-high-yield soybean cultivars Shennong12, Zhonghuang35, Liaodou14 and one common soybean cultivar Liaodou11 were tested in the experiments. The field experiment was conducted at Shenyang Agricultural University, Liaoning Province, China from May to September, 2011 and 2012.

The experimental design was a split block with three replicates, in which the main plot was the nitrogen fertilizer treatment with five levels (0, 75, 150, 225 and 300 kg/ha) and the subplot was the soybean cultivars (Shennong12, Liaodou 14, Zhonghuang 35 and Liaodou 11). The fertilizers used were diammonium phosphate. The whole dose of N, P, K was applied as basal. At the beginning of the experiment, the soil (pH 5.5) contained organic matter (18.9 g/kg), total nitrogen (2.1 g/kg), total phosphorus (0.087 g/kg), total potassium (22.2 g/kg) and available nitrogen (74.26 mg/kg), available phosphorus (1.71 mg/kg), available potassium (149.34 mg/kg). The experimental unit was 6 m × 3 m. The intra and inter row distance was 11 cm and 60 cm, respectively. The plant density was about 15 plants /m^2.

Plants were sampled four times for leaf areas, MDA, SOD, POD and three times for main stem and branch pod dry weight. At V7 (7 nodes on the main stem beginning with the unifoliolate node), R1 (one flower at any node), R4 (pod 2 cm (3/4 inch) long at one of the four uppermost nodes with a completely unrolled leaf), R5 (beans beginning to develop (can be felt when the pod is squeezed) at one of the four uppermost nodes with a completely unrolled leaf), R6 (pod containing full size green beans at one of the four uppermost nodes with a completely unrolled leaf), R7 (pods yellowing, 50% of leaves yellow; physiological maturity) and R8 (95% of pods brown, harvest maturity) growth stages (Fehr WR *et al.* 1971), photosynthetic rate (between 9:00 am and 11:00 am) and daily changes of photosynthetic rate (8:00, 10:00, 12:00, 14:00, 16:00, 18:00) were measured using a portable photosynthesis system (Li-Cor Model 6400) of sunny day. The third leaf from the top of the plant was used, and three leaves per plot were sampled, and the leaves were also used for measuring SPAD value using a chlorophyll meter (Model Spad 502).

Fresh leaves (0.5 g) were homogenized using 5 ml of 50 mM cold phosphate buffer (pH 7.8) containing 5 mM EDTA, 2 mM AsA and 2% (w/v) polyvinylpyrrolidone in a chilled mortar and pestle. The homogenate was centrifuged at 10000 g for 30 min at 4ºC, and the supernatant was subsequently used in assays for superoxide dismutase (SOD) and peroxidase (POD) activities. superoxide dismutase (SOD), peroxidase (POD) activity and malondialdehyde (MDA) content was assayed.

When soybeans matured, the 3-long middle part of the inside three rows was harvested for yield. According to the yield and harvested area, the yield per kilogram (kg) for per hectare (ha) was known.

Statistical analysis was performed using SPSS software. Graphs were constructed using the means for each data points using Graph Pad Prism 6.

Results and Discussion

The results indicated that there was significant effect of N fertilizer on photosynthetic rate at all stages, and fertilizer × cultivar interaction. N fertilizer treatments resulted in significantly higher photosynthetic rate than no fertilizer treatment at six growth stages, and the highest photosynthetic rate reached its peak at the R5 stage (Fig.1). At V7, R1, R4 growth stages, the 150 kg/ha fertilizer treatment resulted in the maximum photosynthetic rate. Leaf photosynthetic rate of all soybean cultivars significantly enhanced with increasing N level from 0 to 225 kg/ha at R5 and R6 growth stages. At the R7 stage, the 225 kg/ha fertilizer treatment resulted in the maximum photosynthetic rate. Furthermore, Shennong12 gave the highest photosynthetic rate at V7 and R1 growth stage and Zhonghuang35 had the higher photosynthetic rate at R4 growth stage under the 150 kg/ha fertilizer treatment. At the R5 stage, Liaodou14 had the highest photosynthetic rate. At the R6 stage, Zhonghuang35 showed the highest photosynthetic rate. The common soybean variety Liaodou11 had the lower photosynthetic rate than other super-high-yield soybean varieties except at the V7 stage.

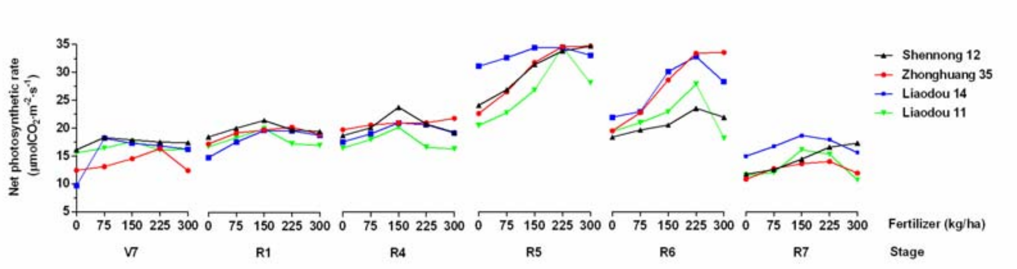

Fig. 1. Leaf photosynthetic rate of soybean cultivars under different nitrogen fertilizer treatments.

In the present study, photosynthetic diurnal variation of soybean cultivars, Shennong12, Zhonghuang35, Liaodou14 and Liaodou11 did not show "noontime snooze" under different diammonium phosphate treatments at R6 growth stage (Fig. 2). Photosynthesis of the four cultivars was significantly increased with diammonium phosphate added, and the photosynthesis of super-high-yield cultivars was higher than that of common cultivar, Liaodou11, after 14 O'clock. The photosynthesis of super-high-yield variety Zhonghuang35 had the highest raised level with diammonium phosphate added, which was significantly higher than other super-high-yield cultivars after 14 O'clock.

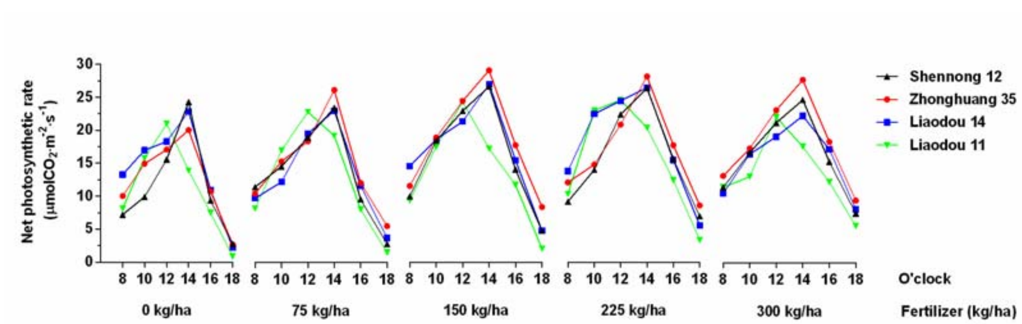

Fig. 2. Photosynthetic diurnal variation of soybean cultivars under different diammonium phosphate levels at the R6 growth stage.

The highest SPAD value occurred at R6 growth stage, which is a key duration for soybean seeds filling. The change of SPAD value was very complex in this study. The results showed that there was significant effect of N fertilizer and cultivar on SPAD value, and fertilizer × cultivar interaction at V7 and R5 growth stages (Fig. 3). Shennong12 and Zhonghuang35 had the highest SPAD value at those two growth stages. However, only varieties had significant effect on SPAD value at R1, R6, R7 growth stages, and the super-high-yield cultivars had higher SPAD value than common cultivar except Zhonghuang35, at R1 and R7 growth stage. At the R4 stage, both fertilizer treatments and cultivars had significant effect on SPAD value.

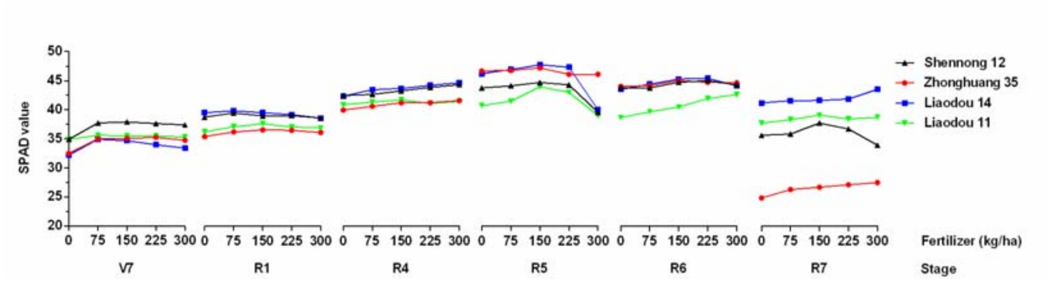

Fig. 3. Effect of nitrogen application on soybean leaf SPAD value at different growth stage.

It has been demonstrated that increasing fertilizer is a better way to enlarge soybean leaf area. In this study, there was significant effect of fertilizer on leaf areas at V7, R6 and R7 growth stages. The 225 kg/ha treatment and Zhonghuang35 gave the largest leaf area at V7 growth stage (Fig. 4). No significant interaction effects between fertilizer and cultivar was detected for leaf area at R1 growth stage. Significant fertilizer × cultivar interactions for leaf area at R6 and R7 growth stage were also observed. Furthermore, the 225 kg/ha treatment and Shennong12 had the biggest leaf area at R6 growth stage. At late seed filling stage, the 300 kg/ha treatment and Shennong12 had the largest leaf areas.

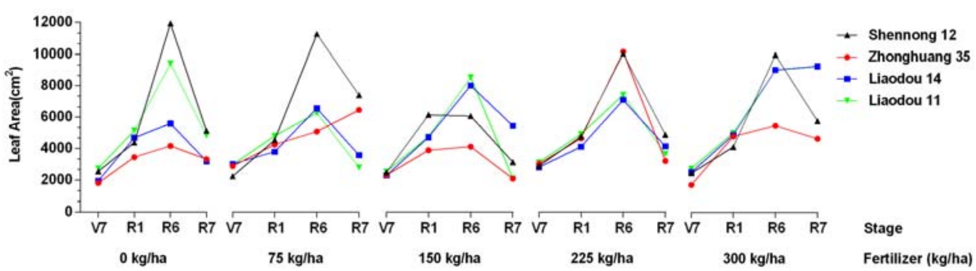

Fig. 4. Effect of nitrogen application on soybean leaf areas at the four growth stages.

Significant effect on main stem branch number of the soybean cultivars were observed at all growth stages. The 75 kg/ha treatment and Shennong12 had the biggest main stem branch number at R7 growth stage. The 225 kg/ha treatment and Shennong12 gave the maximum main stem branch number at the V7 growth stage (Fig. 5). At the R1 stage, 225 kg/ha treatment and Liaodou14 and Shennong12 had the biggest main stem branch number. The 300 kg/ha treatment and Shennong12 gave the biggest main stem branch number at R6 growth stage. All the super-high-yield soybean varieties had more main stem branch number than common variety except Zhonghuang35 at every growth stage, which was correlated to characteristic of the variety.

Significant effect of fertilizer and cultivars on MDA content was at V7, R1, R6 and R7 growth stages (Fig. 6). Super-high-yield soybean cultivars showed much lower MDA content than that of common soybean variety, Liaodou11, at R7 growth stage. Super-high-yield soybean varieties had a gradual decrease in MDA content under all fertilizer treatments at all growth stages.

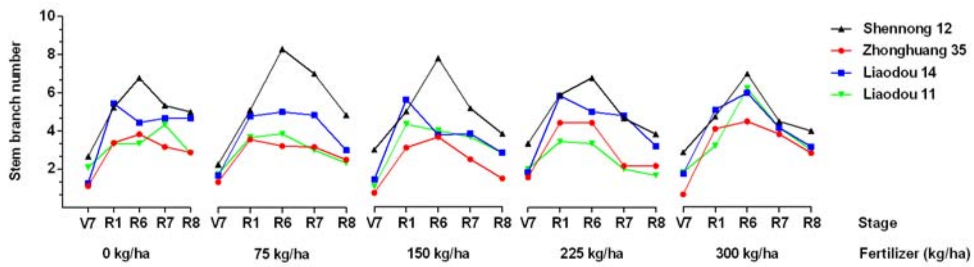

Fig. 5. Effect of nitrogen application on main stem branch numbers at different growth stages.

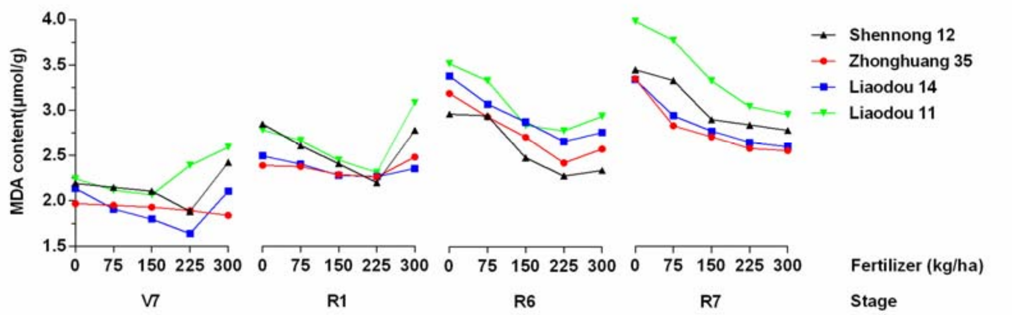

Fig. 6. Response of MDA content of different soybean cultivars to nitrogen application levels at different growth stages.

Results showed that fertilizer treatments significantly increase the POD activity at all the four growth stages. The 225 kg/ha treatment and super-high-yield Zhonghuang35 had the highest POD activity at the V7 and R7 stages, while 300 kg/ha treatment and Shennong12 had the biggest POD activity at R1 growth stage (Fig. 7). The 225 kg/ha treatment and Shennong12 had the higher POD activity than that of other fertilizer treatments and cultivars at R6 growth stage.

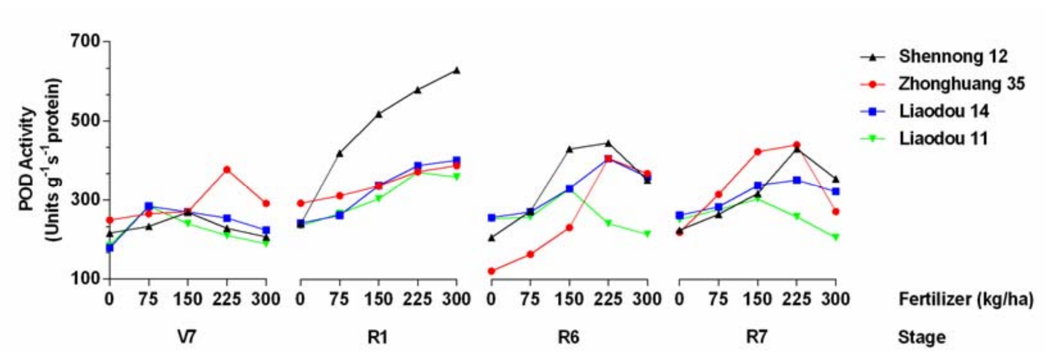

Fig. 7. Response of POD activity of different soybean cultivars to nitrogen application levels at different growth stages.

The results indicated that the SOD activity of leaves were less in cultivars at late developing stages than that of cultivars at early development stage. There was significant effect of fertilizer and cultivars on SOD activity, and fertilizer × cultivar interaction at all four stages (Fig. 8). The fertilizer could enhance the SOD activity compared with control at every growth stage. The 150 kg/ha treatment and Liaodou14 had the highest SOD activity at V7 stage, and 225 kg/ha treatment and Liaodou14 had the biggest SOD activity at R1 stage. At the R6 and R7 growth stage, 300 kg/ha treatment and Zhonghuang35 had the highest SOD activity. All the super-high-yield cultivars had the higher SOD activity than that of common soybean variety Liaodou11 at all the four growth stages.

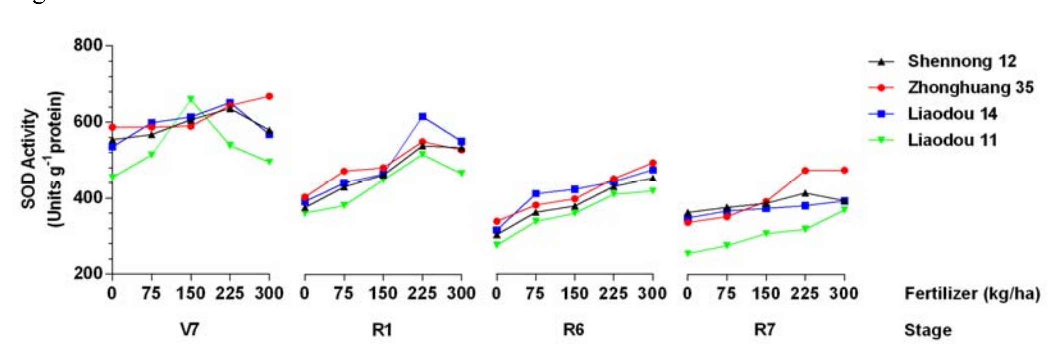

Fig. 8. Response of SOD activity of different soybean cultivars to nitrogen application levels at different growth stages.

The results showed that there was significant effect of fertilizer on soybean yield, and fertilizer × cultivar. Super-high-yield soybean varieties had higher yield than that of common soybean Liaodou11. With 150 kg/ha treatment both Shennong12 and Zhonghuang35 gave the highest yield (Fig. 9). The change of yield presented as a single peak curve, every cultivar had its appropriate fertilizer amount.

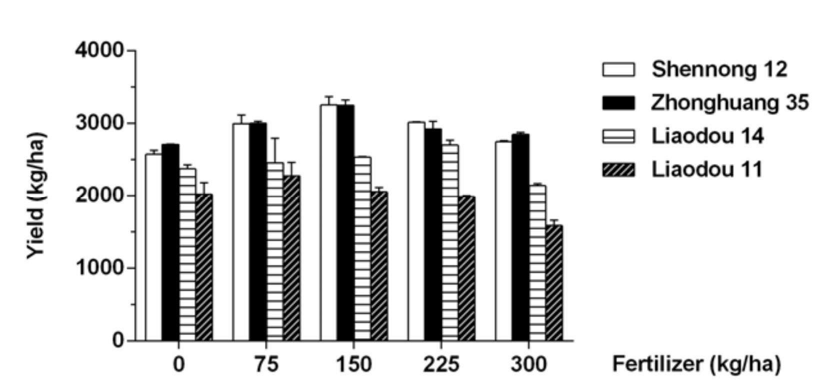

Fig. 9. Effect of nitrogen application on yield of cultivars.

The workers reported that producing the same biomass in soybean needs twice the amount of nitrogen compared with wheat and oat (Fei 1960). This is due to protein concentration of soybean with approximately 40% more than other crops, which needs more fertilizer. Soybean nutrition is associated with photosynthesis and yield. Although soybean can provide 50 - 60% N for itself by biological N_2 fixation, that is not sufficient enough to total demand. Therefore, increasing fertilizer is an essential measure for enhancing soybean yield. It is also found that super-high-yield soybean varieties need large amounts of N to support both above ground biomass and high protein seed (Liu *et al.* 2008).

Photosynthetic rate is positively associated with yield for similar high yield cultivars (Zhang *et al.* 1986). At the seed filling stage, a significant relationship (r = 0.92) was found. The greater photosynthetic rate can produce more biomass, which is the basis for high yield. In the present study, super-high-yield soybean cultivars had the greater photosynthetic rate than that of common soybean cultivar Liaodou11 at the R5, R6 stage under all fertilizer treatments. This is consistent with previous study (Jin *et al*. 2004b, 2005). Ma *et al.* (2014) showed that the diurnal variation in photosynthesis of super-high-yield variety did not appear "noontime snooze" at seed filling stage. In this study, the super-high-yield cultivars had greater photosynthesis after 14 o'clock under all fertilizer treatments, and the fertilizer treatment prolonged the photosynthetic time. These indicated that super-high-yield cultivars had greater photosynthetic assimilation capacity than that of common cultivar under the fertilizer treatment at R6 growth stage, which was one of the important physiological reasons for super-high-yield cultivars' greater yield. The previous study showed that higher chlorophyll content could increase the light absorption of the crop, which was significantly related to photosynthetic rate (Li *et al.* 1987). In this experiment, the super-high-yield soybean had the greater SPAD value at the R5, R6 growth stage, which are the key stages of seedling filling. This was the basis for high photosynthetic rate of super-high-yield soybean cultivars. Zhonghuang35 is an early soybean variety compared with other soybean cultivars. Because of it's short growth period, the leaves of Zhonghuang35 senesce earlier than that of other soybean cultivars. At the same time, Zhonghuang35 has a stronger taproot compared with other varieties, which provide a greater absorbing nutrition ability and stronger lodging-resistance capability (data not shown).

Higher SOD activity in soybean cultivars indicated higher superoxide anion scavenging activity during growth period. In this study, SOD activity of four soybean cultivars declined with the developmental process. That may be related to physiological status of soybean cultivars. In the early growth and development, the soybean cultivar has a high rate of metabolism sustaining faster vegetative growth compared with late stages of development. And SOD activity of the super-high-yield soybean varieties were higher than that of common soybean Liaodou11 at R1, R6, R7 growth stage under all fertilizer treatments, which indicated that super-high-yield maintained a good growing status with less ROS. All the fertilizer treatments could enhance the SOD activity, which was effectively resistant to senescence. This was an important reason for fertilizer treatment sustaining better physiological status. This was consistent with previous study (Zhang *et al*. 2009).

A higher POD activity is beneficial for plants adjusting to varieties of stresses, which was confirmed by other study (Hediye *et al*. 2007). In our study, SOD activity enhanced with increasing amount of nitrogen in a certain range. At the same time, the content of MDA in soybean leaves declined, which indicating fertilizer treatment could be resistant to senescence in some extent. The results also showed that POD activity of the super-high-yield soybeans are higher than that of common soybean at R6, R7 stage under 225 kg/ha, 300 kg/ha, indicating that appropriate fertilizer amount could delay senescence effectively for super-high-yield.

The previous study showed that the nitrogen fertilizer could efficiently increase the crop yield. However, the efficiency of increasing yield became smaller with the increasing fertilizer (Sun *et al.* 2013, Xu *et al*. 2006, Ji *et al*. 2013). In this study, the nitrogen fertilizer significantly enhanced the soybean yield of both super-high-yield or common yield soybean cultivars.

Acknowledgments

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