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## Towards improved N<sub>2</sub> fixation and yield in soybean

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## Summary

Soybean has developed into one of the important food and cash crops in China. It plays a key role in many cropping systems, and can be planted as a vegetable to supplement the limited vegetable supply in early summer in Central China. To maximize soybean yield, farmers in China apply high rates of nitrogen (N) fertilizer as a single dressing before flowering, which seriously suppresses symbiotic N<sub>2</sub> fixation. This thesis deals with questions related to improve N<sub>2</sub> fixation in soybean cultivation. The objective of the study is to contribute to the design of a fertilizer management system that results in: (i) maximum contribution of symbiotic N<sub>2</sub> fixation to total crop nitrogen supply, (ii) minimum losses of nitrogen to the environment, and (iii) maximum economic return to the farmer. Field, greenhouse and climate room studies were conducted to generate basic data on crop response to nutrient supply.

The effect of N fertilizer application at different phenological stages (V2 [early vegetative], V4, R1, R3, R5 [late reproductive]) on growth, biomass production, N<sub>2</sub> fixation and yield of three soybean varieties was studied (Chapters 2 and 4). Starter N, at 25 kg/ha, was neither sufficient for maximum soybean yield nor for maximum N<sub>2</sub> fixation, which was applied before sowing. The timing of N fertilizer application played a decisive role in the response of soybean yield and N<sub>2</sub> fixation: in the vegetative growth phase the V2 (nodule formation and development) stage appeared optimum, in the reproductive phase the R1 (flowering) stage. Experiments in the greenhouse, with plants in nutrient solution, confirmed these results and indicated that increased leaf area development was the most important process in the response to mineral N supply (Chapter 7).

Phosphorus plays a key role in biological nitrogen fixation (BNF) and in soybean, P-deficiency results in restricted nodule formation, impaired nodule growth and reduced nitrogenase activity as a consequence of reduced N<sub>2</sub> fixation, and consequently in lower seed yield. The effect of basal P fertilizer application (0, 50, 100 kg P<sub>2</sub>O<sub>5</sub>/ha) on growth, biomass production, N<sub>2</sub> fixation and yield of soybean was studied (Chapter 4). P fertilization significantly increased nodulation, N<sub>2</sub> fixation and seed yield. Under the conditions of the field experiment, the optimum dose was 50 kg P<sub>2</sub>O<sub>5</sub>/ha and in the greenhouse the optimum concentration in the nutrient solution was 0.5 mM PO<sub>4</sub><sup>3-</sup> (Chapter 5). Growth parameters, that contributed to the increased biomass under mineral N and P supply were, in decreasing order of importance: (i) total leaf area, (ii) individual leaf area, (iii) shoot/root ratio, (iv) leaf area ratio and (v) specific leaf area. The higher yield under external mineral N and P supply was mainly associated with a larger number of pods and seeds per plant.

Plant density is a major factor in growth, biomass production, seed yield and N<sub>2</sub> fixation in soybean, through its effect on leaf area dynamics and light interception, and hence on

canopy photosynthesis. In a field experiment with three soybean varieties, the effect of density on growth,  $N_2$  fixation and yield was studied (Chapter 3I). Total biomass, total  $N_2$  fixed and grain yield of all three varieties responded positively to higher density. Density effects on individual plant characteristics were variable: double density planting had no significant effect on individual plant biomass and seed weight per plant in the early maturing variety Wuyin9, with a determinate growth pattern, but higher density had significantly negative effects in the later maturing varieties, Jufeng with an indeterminate growth pattern and You91-19 with a semi-determinate growth pattern. Experiments in a growth chamber with plants in nutrient solution and in the greenhouse with potted plants, confirmed these results (Chapter 3II). Yield reduction of individual plants at higher density was mainly associated with lower numbers of pods and seeds per plant, while seed number per pod and seed weight were not affected.

Nitrate is a major inhibitor of symbiotic  $N_2$  fixation in legumes, but no consensus exists on the mechanism(s) involved. In a climate room with plants in nutrient solution, the effect of nitrate supply (3.75 mM) on plant biomass, nodulation and  $N_2$  fixation and pod yield of two nodulated soybean varieties was investigated (Chapter 6I). Plant biomass,  $N_2$  fixation and pod yield were highest with free access to nitrate, and additional  $N_2$  fixation did not result in a further increase in biomass. Plants with free access to nitrate produced more roots, a higher total nodule number and higher nodule dry weight, than 'control' plants, relying for nitrogen supply only on  $N_2$  fixation. In addition, mineral N supply increased total  $N_2$  fixed. Both, nodule number and nodule dry weight per unit plant dry weight were lower under nitrate supply. Nodule number and/or nodule weight, per unit plant dry weight are useful parameters for a quantitative study on the effect of N fertilization on  $N_2$  fixation in soybean.

On fertile soils, nitrate is generally abundantly available as N source to crop plants. In acid soils and/or infertile clay soils, nitrification is restricted, and ammonia may be more abundant than nitrate. Differences in response of soybean to fertilizer N might, at least partly, be the result of differences in soil pH, the relative abundance of nitrate and ammonium in the soil, and/or the relative efficiency with which the two forms of N are taken up and assimilated by the plant. To increase insight in the relative importance of these factors, a greenhouse experiment was conducted to study the effect of different N sources ( $NO_3^-$ ,  $NH_4^+$  and  $NH_4NO_3$ ) and concentrations (2 and 20 mM), daily supplied with nutrient solution, on growth, nodulation, N compounds (ureides) in the xylem sap, and  $N_2$  fixation of soybean (Chapter 6II). The high N concentration of all three sources resulted in higher biomass production and total N accumulation than the low concentration. However, nodule number, nodule dry weight and total  $N_2$  fixed were lower. Under the condition with high  $K^+$  supply,  $NH_4^+$  was a more efficient N source than nitrate, as expressed by the higher biomass, nodule dry weight, total N accumulation, and total  $N_2$  fixed.

The thesis concludes that in terms of nutrient use efficiency, the harvest, which is the result of the nodulation, should be made a priority to induce them to leghaemoglobin nodulation, that density it may even stimulate

Our results have shown that in climate rooms or greenhouses, suggesting that similar means to predict soybean yield, both in practice in Hubei, are highly variable, but that is a basal application of topdressing of 50 kg

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The thesis concludes that soybean may not always be beneficial for the subsequent crop in terms of nutrient availability, particularly if the aboveground residues are also removed at harvest, which is the normal practice for soybean production in Central China. The farmers should be made aware of the potential benefits of these residues in terms of N supply, to induce them to leave the residues in the field. Although N fertilizer application suppresses nodulation, that does not necessarily lead to a decrease in total N<sub>2</sub> fixation, on the contrary, it may even stimulate it, if N fertilizer is applied at a suitable time.

Our results have shown that the results of experiments under controlled conditions, either in climate rooms or in the greenhouse, are consistent with those of field experiments, suggesting that small-scale experiments under controlled conditions, may be an economic means to predict crop performance under field conditions (Chapter 8). For farmers' practice in Hubei, Central China, the results of our investigations suggest that to optimize soybean yield, both N- and P-fertilizers are necessary. Though crop response to fertilizers is highly variable, both in time and in space, the best fertilizer management regime suggested, is a basal application of 25 kg N/ha combined with 50 kg P<sub>2</sub>O<sub>5</sub>/ha, followed by a topdressing of 50 kg N/ha at the early vegetative or early flowering stage.