NITROGEN FIXATION IN HIGH YIELDING SOYBEAN *(Glycine Max., L. Merr)*

by

Fernando Salvagiotti

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Agronomy

Under the Supervision of Professor James E. Specht

Lincoln, Nebraska

June, 2008

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University of Nebraska, 2008

Adviser: James E. Specht

Under the hypothesis that grain yield is limited by nitrogen (N) supply in late reproductive stages when soybean is grown in high-yielding environments, a 2-year study was conducted in order to evaluate whether N fertilization increases grain yield and N uptake, and to what extent N_2 fixation is complemented when fertilizer N was applied both on surface as well as below the nodules. Four N fertilization treatments were established in the soybean main plots of each year's soybean/maize rotation in a long-term experiment. Treatments were: a non-fertilized plot (N1) and 180 kg N ha^{-1} applied as i) polymer-coated controlled release urea placed 20 cm below the surface before planting (N2, late N fertilization strategy); ii) ammonium nitrate on the surface before planting and at V6 (N3, early N fertilization strategy) and, iii) ammonium nitrate on the surface at R5 (N4, late N fertilization strategy). All treatments were superimposed on two contrasting fertilizer management histories for maize (M1=recommended nutrient management; M2=intensive nutrient management). Grain yield in N1 averaged 4850 kg ha^{-1}, and a 5% increase was observed in response to N fertilization. This response was accompanied by a 9% increase in N uptake. Biological N_2 fixation accounted for 50% of N uptake in N1, but only 32 and 38% in N3 and N4, respectively. All N fertilization strategies succeeded in providing the additional N needed to attain the apparent maximum grain yield, but the amount of fertilizer N needed to achieve grain yield response was larger with ammonium nitrate applied on the surface N. This was because a proportion of total N derived from surface applied ammonium nitrate depressed N_2 fixation. In contrast, deep placement of slow-release urea did not depress biological N_2 fixation, and thus, showed the advantage of providing the additional N needed to reach grain yield potential with minimal trade-off between fixed N and fertilizer N. The greater photosynthesis response to light observed in fertilized soybean suggests that N

fertilization in high-yielding soybean should simultaneously take into account management practices intended to maximize canopy light capture.

TO SILVIA, MILENA, AILEN AND MAURO FOR THEIR CONTINUOUS SUPPORT

IN OUR LIFE PROJECT

ACKNOWLEDGEMENT

I gratefully acknowledge Achim Dobermann who served as main advisor the first two years of the project and help me to devise the research project. Special thanks to James Specht who served as main advisor the last part of the PhD program and whose help in field activities was central for the present research. Sincere appreciation is directed to the rest of the supervisory committee, Daniel Walters, Kenneth Cassman, and Albert Weiss for their support, mentoring and their teachings about how to think science. My gratitude to Kathi Cockrill for her help in all administrative issues and to Jose Aponte, Darren Binder, Sarah Brownell, Jonathan Chaky, Sadal Hwang, Adam Liska, Mike Livingston, Paul Nabity, Piyapoprn Phansak, Kyla Ronhovde, Watcharin Soonswon, Greg Teichmeier, and Travis Wegner for their help in field activities. Special thanks to Arlene Adviento, whose help was central for developing the chemical determinations in xylem sap in the laboratory. The author also acknowledges Fulbright Foundation who granted the first two years of my studies, and to the Graduate Studies Department of University of Nebraska-Lincoln who granted the third year. I also thank the Department of Agronomy and Horticulture at the University of Nebraska-Lincoln for providing the funding for this study. The author is also very thankful to Tri Setiyono, with whom I enjoyed research discussions. The author is grateful of my friends in Lincoln, specially Octavio, Nayade and Patricio that make life in USA very comfortable. Obviously I also recognize the constant support from the family members in Argentina, and a special dedication to my dad where ever he is now.

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CHAPTER 1

General Introduction

Nitrogen (N) is essential in crop production because it is directly involved in the photosynthesis process. In soybean (*Glycine max*. [L] Merr), high yields are associated with greater N remobilization from vegetative tissues to the seed. Then, this N mobilization will diminish leaf N content and thus, it will reduce leaf photosynthesis rate. Therefore, in a high-yielding environment, grain yield potential may be restricted by N supply. Soil fertilizer may complement the N requirements of high-yielding soybean, but soil nitrate supply causes an inverse down-regulation of biological N_2 fixation which may limit the use of fertilizer N. This dissertation is concerned with the role of biological N_2 fixation and N fertilization in high-yielding soybean, the likely ways in which N fertilizer may be efficiently used in complementing soybean N requirements and, the likely mechanisms by which this additional N may improve grain yield.

The first chapter of this dissertation shows a quantitative meta-analysis of published reports in the scientific literature, exploring relationships among soybean seed yield, N uptake, biological N_2 fixation, and the yield response to N fertilization. The extent to which available information explains the role of biological N_2 fixation and fertilizer N in high-yielding soybean is discussed.

The successive chapters summarizes the results of a 2-year study on soybean within an intensive maize-soybean system experiment aimed at understanding the yield potential of maize and soybean. The second chapter shows that biological N_2 fixation was not sufficient to meet the N demand required to reach the apparent maximum grain yield. Also, this chapter shows that different N fertilization strategies supplied this additional N, but with a different efficiency. Physiological mechanisms associated with the response, the contribution of biological N_2 fixation and the offset with N fertilizer in the different strategies are also discussed.

In the third chapter, the increase in carbon assimilation observed in response to N fertilization at the whole plant level is discussed in terms of photosynthesis response to light at the leaf level. Changes in the light response curve associated with N fertilization were scaled up to the canopy level to determine the significance of these modifications. Finally, the last chapter provides a general summary of the main results of these experiments and future needed research.

CHAPTER 2

Nitrogen uptake, fixation and response to fertilizer N in soybeans: a review

Abstract

Although relationships among soybean (*Glycine max*. [L] Merr) seed yield, nitrogen (N) uptake, biological N_2 fixation (BNF), and response to N fertilization have received considerable coverage in the scientific literature, a comprehensive summary and interpretation of these interactions with specific emphasis on high yield environments is lacking. Six hundred and thirty seven data sets (site-year-treatment combinations) were analyzed from field studies that had examined these variables and had been published in refereed journals from 1966 to 2006. A mean linear increase of 0.013 Mg soybean seed yield per kg increase in N accumulation in aboveground biomass was evident in these data. The lower (maximum N accumulation) and upper (maximum N dilution) boundaries for this relationship had slopes of 0.0064 and 0.0188 Mg grain kg^{-1} N, respectively. On average, 50 to 60% of soybean N demand was met by biological N_2 fixation. In most situations the amount of N fixed was not sufficient to replace N export from the field in harvested seed. The partial N balance (fixed N in aboveground biomass – N in seeds) was negative in 80% of all data sets, with a mean net soil N mining of -40 kg N ha^{-1}. However, when an average estimated belowground N contribution of 24% of total plant N was included, the average N balance was close to neutral (-4 kg N ha^{-1}). The gap between crop N uptake and N supplied by BNF tended to increase at higher seed yields

for which the associated crop N demand is higher. Soybean yield was more likely to respond to N fertilization in high-yield (>4.5 Mg ha^{-1}) environments. A negative exponential relationship was observed between N fertilizer rate and N_2 fixation when N was applied on the surface or incorporated in the topmost soil layers. Deep placement of slow-release fertilizer below the nodulation zone, or late N applications during reproductive stages, may be promising alternatives for achieving a yield response to N fertilization in high-yielding environments. The results from many N fertilization studies are often confounded by insufficiently optimized BNF or other management factors that may have precluded achieving BNF-mediated yields near the yield potential ceiling. More studies will be needed to fully understand the extent to which the N requirements of soybean grown at potential yields levels can be met by optimizing BNF alone as opposed to supplementing BNF with applied N. Such optimization will require evaluating new inoculant technologies, greater temporal precision in crop and soil management, and most importantly, detailed measurements of the contributions of soil N, BNF, and the efficiency of fertilizer N uptake throughout the crop cycle. Such information is required to develop more reliable guidelines for managing both BNF and fertilizer N in high-yielding environments, and to also improve soybean simulation models.