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Cover Page Footnote

Thanks to the Kansas State University CROPS Crop Production Team; the National Agricultural Technology Institute (INTA), Argentina; Pioneer; and the Fluid Fertilizer Foundation.

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Soybean: Genetic Gain × Fertilizer Nitrogen Interaction

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Summary

The United States (US) and Argentina (ARG) account for more than 50% of the global soybean production. Soybean yields are determined by the genotype, environment, and management practices ($G \times E \times M$) interaction. Overall, 50-60% of soybean nitrogen (N) demand is usually met by the biological nitrogen fixation (BNF) process. An unanswered scientific question concerns the ability of BNF process to satisfy soybean N demand at varying yield levels. The overall objective of this project was to study the contribution of N via utilization of different N strategies, evaluating soybean genotypes released in different eras. Four field experiments were conducted during the 2016 season: Ottawa (east central Kansas, US), Ashland Bottoms (central Kansas, US), Rossville (central Kansas, US), and Oliveros (Santa Fe province, Argentina). A wide variety of historical and modern soybean genotypes were used (from the 1980s, 1990s, 2000s and 2010s release decades) in the US and ARG, all tested under three N management strategies (S1: non-N applied but inoculated, S2: all N provided by fertilizer, and S3: late-N applied) and all seeds inoculated. At Ottawa, the study was planted in an area without previous soybean history with yields ranging from 21 to 30 bu/a. Modern genotype (2010) increased yields by 15% relative to the other varieties. As related to the N management approach, higher yields occurred when the N nutrition was based on S2 (overall 10% increase). At Ashland Bottoms, yields ranged from 47 to 65 bu/a, and the 1990s variety out-yielded the rest of the varieties by 13%. There was not statistical significance for N management at this location. At Rossville, yields ranged from 37 to 85 bu/a, with higher yields observed for the modern genotype (released after 2010). Regarding N strategies, S2 increased yields by 18% compared to S1. At ARG, yield ranged from 40 to 74 bu/a, with modern soybean varieties (released after 2010) yielding 34% greater than the rest of the varieties. Nitrogen application S2 increased yields by 5% when compared to the S1 strategy. Relative to yield potential, yield levels in Argentina were similar to those in central Kansas (Ashland Bottoms and Rossville).

Introduction

The United States (US) and Argentina (ARG) account for more than 50% of the global soybean production (USDA, 2016). In the US, more than 85% of the soybean land area is located in the Corn Belt region, where two-year corn-soybean rotation (>60%) is the main system. In Argentina, soybeans are planted in the Pampas and Chaco regions, under rainfed conditions, as monoculture or in rotations with corn and wheat.

Soybean yield potential is genetically determined. Yield potential (Y_p) can be attained under “ideal” conditions (genotype (G) $\times$ environment (E) $\times$ management practices (M)), assuming no limitations of water and nutrient supply and absence of biotic and abiotic yield-limiting factors (e.g., insects, diseases, etc.). Yield gaps between Y_p and actual on-farm yield (Y_A) are primarily defined by crop management practices (e.g., row spacing, planting date, fungicide and nutrient application, among others) and the interactions of those with the E (weather factor). Maximum soybean yields are dependent on a balanced nutrition, with N nutrition as the main nutrient limiting soybean yields and seed quality (Ciampitti et al., 2016).

Interaction between soybean genotypes and fertilizer N response is not well understood. Rowntree et al. (2013) documented an annual genetic US soybean yield gain of approximately 0.37 bu/a for maturity group (MG) III released from 1920s to 2000s when planted around May. Yield gain for high yielding soybean was achieved in detriment to the protein concentration (Rowntree et al., 2013). Thus, it is valid to hypothesize that high-yielding soybean will have higher nutrient demand to sustain protein levels, which represents the bio fortification issue.

Soybean plants have the capacity to fix nitrogen (N) from the atmosphere through the symbiosis process of the plant with the bacteria *Bradyrhizobium* that needs to be present in the soil or added as inoculant. Nitrogen fixation is the result of the conversion of atmospheric N_2 into ammonia (NH_3), and later on into N-containing organic components that become available to the plant (Wright & Lenssen, 2013). However, it had been documented that the biological nitrogen fixation (BNF) process is not able to supply the total requirement of the plant. Overall, only 50-60% of soybean nitrogen (N) demand is usually met by the BNF process (Salvagiotti et al., 2008). An unanswered scientific question is, “How well does the BNF process satisfy soybean N demand at varying yield levels?”

In summary, for the genotype $\times$ N interaction, the main question is, “Do high yielding soybeans need to be fertilized with nitrogen?” The understanding of genetic gain $\times$ N in conditions for expressing high yield potential is a critical factor for advancing soybean yield improvement. For instance, is genetic improvement (genetic gain) accompanied by changes in N uptake (and partition) in soybean?

The objectives of this study were to 1) study the contribution of N in soybean under different N nutrition scenarios: i) soybean planted under normal production conditions, ii) all N requirement met by N fertilizer, and iii) under normal production conditions + late additional N on soybean yields and plant N content; and to 2) Evaluate the yield performance of historical and modern soybean genotypes released from the 1980s to the 2010s.

Procedures

Four locations were evaluated, three of them located in Kansas, US (Ottawa, Ashland Bottoms, and Rossville) and one in Santa Fe, Argentina (Oliveros) (Figure 1).

Experimental Design

The study was conducted in experimental plots that measured 10-ft wide by 50-ft long at Ottawa and Ashland Bottoms (US); and 10-ft wide by 30-ft long at Rossville (US). Target seeding rate was 140,000 seeds per acre at Ottawa, 180,000 seeds per acre at Ashland Bottoms, and 103,000 seeds per acre at Rossville. Row spacing of 30 inches was used at the three US locations. At Ottawa and Ashland Bottoms each treatment was replicated 6 times in a split-plot layout with a complete block arrangement (soybean variety as the main plot). Nine treatment combinations were evaluated for the genotype by N approach interaction at Ottawa and Ashland Bottoms (Table 1). Nitrogen fertilizer application (expressed in lb N/a) per treatment is also presented in Table 1. Rossville experiment had 3 replications. At this location, the experiment was structured for 13 genotypes and 3 N strategies for a total of 39 treatment combinations.

At Argentina, experimental plots were 8.5-ft wide by 23-ft long. Each treatment was replicated 4 times. Eight varieties and 3 N strategies were evaluated for a total of 24 treatment combinations.

Herbicides and hand weeding were implemented to minimize weed interference for the entire growing season, and soil nutrient concentrations (other than N) were maintained above the recommended critical levels (through inorganic P/K applications).

Fertilizer Applications

The fertilizer N applications were performed using liquid urea-ammonium-nitrate (UAN at 32-0-0) as needed per each treatment combination. The three N strategies were the same at all 4 locations. Strategy 1 (S1) was a control with no N applied but seeds were inoculated. Strategy 2 (S2) was all N provided by fertilizer at a rate of 600 lb/a which was split in 3 timings: planting, R1, and R3. Strategy 3 (S3) has a late season application (at R3) of 50 lb N/a. Nitrogen applications at planting and R1 were performed using an all-terrain vehicle (ATV) equipped with spraying technology. The last N applications (at R3) were performed using a CO₂ backpack sprayer with drop tubes attached to the spraying boom in order to place the liquid fertilizer directly into the soil.

Site Characteristics

Soil samples were taken before planting at 6 and 24 inches depth for US locations (Ottawa, Ashland Bottoms, and Rossville). Parameters analyzed from these samples were pH; Mehlich P; cation exchange capacity (CEC); organic matter (OM); Ca, Mg, and K availability; and nitrate concentration (N-NO₃) (Table 2).

At the Argentina site, soil samples were taken at 8 inches depth. Parameters analyzed were pH; Bray 1 P; OM; and N-NO₃ (Table 2).

In-Season Measurements

A variety of in-season measurements were performed at the US locations. Main in-season activities are listed below:

- Stand counts (early in the season).
- Plant height (ground to the last developed leaf): At V4, R2, and R5 stages.
- Light bar interception (above and below canopy): At V4, R2, and R5 stages.

- Leaf area index (above and below canopy): At V4, R2, and R5 stages.
- Biomass sampling at V4, R2, R5, and R8 stages.

At the Argentina location, the same measurements were collected at the R2, R5, and R7 stages. Biomass determination was performed from a sample of five linear feet per plot. Each individual plant was cut at the stem base out in the field. Total fresh weight of the sample was taken and then it was sub-sampled to ten plants per plot. These 10-plant sub-samples were separated into different fractions: 1) leaves and stem (vegetative phase); and 2) pods, grain, leaves, and stems (reproductive phase). Each independent fraction was separately chopped and dried to constant weight at 140°F. When samples were dry, they were ground to fine particles that later were sent to a laboratory for analysis of nutrient concentrations.

At Ottawa and Ashland Bottoms, 2016, root samples were collected at the V4 stage. Ten roots per plot were sampled for root scanning and nodules count at three repetitions for each treatment. In addition, five ground pictures per plot were taken with a professional camera for future software analysis of canopy cover. As a complement, at Ashland Bottoms and Rossville (US) imagery analysis was performed, collecting information from different parameters using drones. The main focus was canopy cover and normalized difference vegetation index (NDVI) at different growth stages during the season.

Results

Weather Information

Seasonal precipitation, maximum (max) and minimum (min) temperatures, and solar radiation values were documented throughout the entire 2016 growing season at all sites. In the US, similar mean temperatures were observed with max of 91, 87, and 89°F and min of 46, 47, and 44°F for Ashland, Ottawa, and Rossville, respectively. Cumulative precipitation was higher in the high yielding environments (Ashland and Rossville) with 28- and 32-inch relative to the low yielding environment (Ottawa) with 21-inches (Figure 2). In Oliveros, max temperatures were close to those in the US with 90°F, although min temperatures were higher with 63°F. Cumulative precipitation was similar to the US high yield environments, totaling 27 inches. Solar radiation indexes were similar across locations with 82, 76, and 83 × 1000 cumulative Langleys (Ly), except at Ottawa, with 58,000 Ly.

Stand Counts

Early-season stand counts were collected in two 5-ft sections per plot at the V4 stage (Table 3). Stand count efficiency, when compared to seeding rate at Ottawa, ranged from 39% to 89% with an average of 64%. At Ashland Bottoms, stand count efficiency ranged from 30 to 86%, and its average was 60%. Average stand count efficiency at Rossville was 66%. Efficiency in stand count at all US locations was between 60 and 66% overall. In Oliveros, stand count efficiency averaged 84%.

Nodules Information

Nodules information was compiled for Ottawa and Ashland Bottoms (US) during the 2016 growing season, and expressed in nodules per plant at the V4 stage. Nitrogen strat-

egies showed statistical effects ($P < 0.05$) while genotypes did not present differences in final nodules number. Overall, Ashland Bottoms presented a higher number of nodules per plant (Figure 3) than Ottawa (no soybean history for the last 20 years). As expected, S2 resulted in the lowest number of nodules per plant at both locations (5 nodules per plant at Ottawa and 6 nodules per plant at Ashland Bottoms) when compared to S1 and S3 treatments.

Genetic Gain

Twenty-one soybean genotypes from different releases were used in this experiment. At Rossville, 13 genotypes released in the decades of the 1980s, 1990s, 2000s, and 2010s were tested. At Oliveros, 8 genotypes (two from each of the previously listed decades) were used. At both locations, maximum yield was recorded for the modern variety (2010s), with relative yields improving with the year of release of the commercial material (Figure 4).

Yields

Yield information, expressed in bu/a, was adjusted to 13.5% of moisture content. Yields were recorded with a plot combine and from the two central rows in all plots.

Soybean Genotypes by Nitrogen Fertilization Strategies

Yields for 13 genotypes are presented for Rossville (US) and 8 genotypes for Oliveros (ARG), all considering the three N strategies. Yields were similar for both locations, ranging between 37 and 87 bu/a. Nitrogen strategy and genotypes presented statistical significance ($P < 0.05$), but there was not interaction. Greater yields, 18% increase at Rossville and 21% increase at Oliveros, were obtained with modern soybean genotypes (release year > 2000s). On the N applications, S2 (600 lb N/a) increased 18% yields at Rossville and 5% at Oliveros compared to S1 (non-N applied but inoculated) (Figure 5).

At Ottawa, yields were lower (ranging from 21 to 30 bu/a) when compared to Ashland Bottoms (47 to 65 bu/a) (Figure 6). At Ottawa and Ashland Bottoms, genotypes had statistical effect ($P < 0.05$) on soybean yields, and N application was also significant but just for Ottawa. At Ottawa, higher yields were observed for modern soybean genotypes (2010s decade) and for the S2 and S3 N-management approaches relative to past varieties and the S1 treatment.

Historical Genotypes by Nitrogen Strategies Interaction

At Oliveros, genotype by N strategy presented a significant ($P < 0.05$) interaction (Figure 7). Highest yield (74 bu/a) was observed with the modern soybean genotype (2010s release decade) and the S2 N-management approach. On the other hand, lower yields were documented for the 1990s variety regardless of the N-management approach.

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Table 1. Treatment description for Ottawa and Ashland sites (US), 2016 growing season

Treatment	Release decades	Varieties	Nitrogen (N) application
1	1990s	non-RR	Non-N applied
2			All N provided by fertilizer (600 lb/a)
3			Late-season N (50 lb/a)
4	2000s	RR-1	Non-N applied
5			All N provided by fertilizer (600 lb/a)
6			Late-season N (50 lb/a)
7	2010s	RR-2	Non-N applied
8			All N provided by fertilizer (600 lb/a)
9			Late-season N (50 lb/a)

Table 2. Pre-plant soil characterization at 6- and 24-inch depth for Ottawa, Ashland Bottoms, and Rossville, US sites; and 8-inch depth for Oliveros, ARG

Soil parameters	Location and soil depth			
	Ashland Bottoms 6 in.	Ottawa 6 in.	Rossville 6 in.	Argentina 8 in.
pH	6.7	5.7	6.9	5.55
Mehlich P (ppm)	22	14	21	12
CEC (meq/100g)	9	18.5	11	-
Organic matter (%)	1.5	4.3	2.17	2.14
Potassium (ppm)	181	80	153	-
Calcium (ppm)	1599	2665	2074	-
Magnesium (ppm)	179	393	202	-
N-NO ₃ (ppm)*	2.5	5	3	6.3

* Nitrate concentration (N-NO₃ ppm): all 3 US locations samples were taken at 24-inch depth.

Table 3. Final stand counts per repetition block and seeding rate for Ottawa, Ashland Bottoms, and Rossville, US sites, and Oliveros, ARG, during the 2016 growing season

Field sites	Repetitions (× 1,000 plants/a)						Seeding rate (× 1,000 plants/a)
	1	2	3	4	5	6	
Ottawa	94	94	93	97	82	81	140
Ashland Bottoms	104	112	97	111	88	119	180
Rossville	67		72		68		103
Oliveros	120		126		120		146

Figure 1. Map of the state of Kansas and Argentina identifying the four studies conducted during the 2016 season: Ottawa, Ashland, Rossville (US), and Oliveros (ARG).

Figure 2. Daily precipitation, from January to December, (left panels) and seasonal minimum and maximum temperatures (right panels) for the 2016 growing season at Ottawa, Ashland Bottoms, and Rossville (US locations).

Figure 3. Per-plant nodule number affected by soybean genotype and nitrogen interaction at Ottawa (top) and Ashland Bottoms (bottom) sites, US, at V4 stage during the 2016 growing season.

Figure 4. Genetic improvement for soybean genotypes presented in relative yields (%) for four release decades at Rossville, US, and Oliveros, ARG, during the 2016 growing season.

Figure 5. Seed yield for soybean genotypes with different nitrogen fertilization strategies at Rossville, US (top), and Oliveros, ARG (bottom), during the 2016 growing season.

Figure 6. Seed yield for soybean genotypes with different nitrogen fertilization strategies at Ottawa (top) and Ashland Bottoms (bottom), US, during the 2016 growing season.

Figure 7. Seed yield for soybean genotypes with different nitrogen fertilization strategies at Oliveros, ARG, during the 2016 growing season.