Historical Soybean Study: Grain Filling × Nitrogen Fixation

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Summary

Genetic gain is characterized by comparing the performance of genotypes from a different year of release. Historic studies are useful to understand changes in yield-related traits that also contribute to yield potential. This study aims to quantify yield improvement for soybean through a set of seven genotypes with different years of release, and their respective numerical components, with a focus on final seed weight generation under two different nitrogen (N) conditions. Changes in biological N fixation (BNF) were quantified during the seed-filling period (SFP). Non-linear models were fit to the data to characterize seed weight and BNF changes throughout the SFP. Genetic gain led to an overall yield increase of 0.49 bu/a/year mainly explained by increases in the seed number rather than seed weight. Nitrogen application increased yield equally across genotypes ($P < 0.01$), and final seed weight in all genotypes tested. Biological nitrogen fixation activity was reduced by 44% at the onset of the SFP, however, no N deficiencies were observed.

Introduction

Final seed yield is defined by the number of seeds and their individual weights. Historically, increases in yield have been explained by increases in the seed number for many crops, including maize (Duvick et al., 2004), wheat (Loss and Siddique, 1994), and soybean (de Felipe et al., 2016). However, less attention has been paid to responses under differential nitrogen (N) conditions when historical genotypes are compared. Seed production in soybean requires larger amounts of N compared to cereal crops because of its chemical composition, however, soybean response to N fertilization has not always increased seed yield (Mourtzinis et al., 2018). Moreover, N fertilization reduces the biological N fixation (BNF) activity by inhibiting the process. This study aimed to quantify yield improvement for soybean through a set of seven genotypes with different years of release and their respective numerical components, with a focus on final seed weight generation under two different nitrogen (N) conditions. We also quantified the effect of N fertilization on the BNF activity during the seed filling period.

Procedures

A field study was conducted at the Kansas River Valley research station (Rossville, KS) during the 2016 and 2017 growing season. Plots were arranged in complete randomized blocks with three replicates in a split-plot design with seven genotypes (subplots) and two fertilizer N rates (main plots). Seven soybean varieties with different years of release

were tested: P3981 (1980), 9391 (1987), 9392 (1991), 93B82 (1997), 93B67 (2001), 93M90 (2003), and P35T58R (2013).

An unfertilized condition without N applied was used as a control, and a high N condition was used with 600 lb/a equally split at planting, beginning of flowering (R1), and R3 growth stages. The high N condition provided a non-limiting N scenario, and induced variability in the BNF by partially inhibiting the process. Nitrogen treatments were side-dressed using liquid urea ammonium nitrate (UAN; N-P-K, 28-0-0), all applied via a hand-held backpack sprayer.

The study was planted on May 12 and May 18 for the 2016 and 2017 growing seasons, respectively. Both years were planted in corn-soybean rotations. The plot size was 10-ft wide × 50-ft long with rows spaced at 30 in. For all treatments, seeds were inoculated and plots were maintained weed- and pest-free during the growing season.

At harvest, the two center rows in each plot were harvested with a plot combine and 2.2 lb of seed sample was collected in each plot. Individual seed weight was measured from a 1000 seed subsample. Then, seed number was estimated from the seed weight and seed yield information. Seed yield is expressed as dry matter basis.

Seeds were sampled in all plots at R5 weekly until harvest maturity in order to characterize the seed-filling curve and estimate final seed weight. Samples were taken from nodes in the upper third of canopy height and nodes in the lower third to adjust for differences in seed development. After sampling, pods were immediately placed in plastic hermetic bags for transport to the lab. Seeds were excised from pods in a humid box to avoid water loss. Seed dry weight (mg/seed) was measured after drying the samples at 149°F until constant weight was reached. Maximum and minimum temperatures were used to calculate the thermal units as $TT = ((Max + Min)/2) - 8$, using 8°C as the base temperature.

Final seed weight was estimated adjusting a logistic growth curve nonlinear model:

$$SW(mg/seed) = Asym - Drop \times exp[-exp(lrc) \times TT^{pwr}] \qquad \text{Equation 1}$$

where the parameters describe the maximum weight (*Asym*), the difference between the minimum and maximum weights (*Drop*), the natural log of a rate constant (*lrc*), and the power to which thermal time (TT) during the SFP is raised (*pwr*).

In each sampling time, plants were removed to use the stem fraction to measure ureides and nitrates concentration using a hot water extraction method, (Hungria and Araujo, 1994). Both concentrations were used to calculate the relative abundance of ureides (%RAU) as a parameter to estimate BNF throughout the SFP. The percentage of BNF was quantified using established calibrations from Unkovich et al. (2008). A quadratic function was fitted to characterize the dynamics during the SFP:

$$BNF(\%) = a + b \times TT + c \times TT^2 \qquad \text{Equation 2}$$

where the parameter represents the BNF percentage in the beginning of the SFP and b and c are parameters of the function.

The effect of genotype, N condition, and their interaction on the seed yield and seed number was tested with a mixed model. Genotype and N condition were considered fixed effects, while blocks and years were considered random. Nitrogen condition factor was nested in blocks, and blocks were nested in the year factor. To test if there was a genetic gain over seed yield and seed number, release year was used as a quantitative variable in the model.

A non-linear mixed model was adjusted to fit the data for seed weight and BNF. Different models were compared including different fixed factors and their interactions. Thus, data were fitted first to a full model including all fixed effects and their interactions, a model for main effects, a model considering only the variety effect, and a model with the N condition effect. The best model was selected using the Akaike information criterion (AIC). All statistical analyses were performed using R software (R Core Team, 2017). Nonlinear mixed models were fitted using the "nlme" function from the nlme package, while linear mixed models were fitted using the "lme" function from the lme4 package.

Results
Genetic Gain in Seed Yield and Seed Number Across Different Nitrogen Conditions
The analysis for seed yield showed no significant ($P > 0.05$) interaction of the N conditions with the varieties tested in this experiment (Table 1). However, the effect of N application showed a positive increase in seed yield in all genotypes showing constant difference ($P < 0.01$) among genotypes with a different year of release (Figure 1A). Genetic gain in seed yield showed an improvement of 0.49 bu/a/year of seed yield increase for both N conditions.

For the seed number trait, the interaction factor was not significant, nor was the N condition (Table 1). However, seed number increased across years of release and can potentially explain yield increases as depicted in Figure 1B. Moreover, when the overall data set for seed yield and seed number is regressed (Figure 1C) a significant relationship can be observed. However, graphical distribution of the data depicts, in many cases, a similar number of seeds for different treatments that reach different final seed yield. This dispersion in the data can be partially explained by changes in the other numerical component of the final seed yield, such as the seed weight.

Final Seed Weight Responses to Nitrogen Conditions
Grain filling dynamics were found significantly different between genotypes and N conditions. The best model to report the accumulation of biomass in the developing seeds was the one including only the main effects (i.e. variety and treatment). Thus, the interaction was not significant, meaning that changes in seed weight were constant among varieties. For all genotypes in this study, the high N condition resulted in a higher seed weight compared to the control condition with large genotypic variability for this trait (Table 2).

Even though large variability was observed for the dynamics in the SFP (Figure 1), the final seed weight did not show any relationship with the year of release ($P > 0.05$). Hence, for the set of genotypes used in this study, yield increases can be fully attributed to increases in seed set per area. While seed weight showed differences among genotypes and treatment, its contribution to the overall yield was lower compared with the seed number.

Genetic Variability and Nitrogen Responses to BNF Dynamics during the SFP

Comparisons between models for BNF dynamics during the SFP showed the best fit for the model included only the N condition factor. There were no interactions or differences between genotypes for BNF dynamics.

Percentage of BNF at the beginning of the SFP was significantly higher in the control compared with the high N condition (Figure 3). The magnitude of this response can be attributed to the effect of the nitrates in the soil that originated from fertilizer applications, which inhibited the activity in the nodules.

Given the parameters from Equation 2, the magnitude of the reduction was significant ($P < 0.05$) and represented a 44% reduction in the percentage of BNF at the beginning of the SFP from the control condition (Table 1). Even though BNF activity is the main source of N during the SFP, the amount of the nutrient provided by the fertilizer application was enough to maintain photosynthesis levels to still supply photoassimilates to the seeds and increase their respective seed weights.

Conclusions

In this study, genetic gain represented an overall yield increase of 0.49 bu/a/year and it was mainly explained by increases in the seed number rather than seed weight. The effect of N application did not show a differential response for the release year, moreover, N application increased yield equally across genotypes.

Seed weight was the main yield component affected by N treatments. Dynamics in seed biomass accumulation were different across genotypes and treatments. However, increases in seed weight remained constant given the lack of significant interaction effect.

Biological nitrogen fixation activity was affected by the fertilizer N application, showing an overall reduction close to 44% at the onset of the SFP. Despite the significant inhibition of the process, N sourcing from the fertilizer application was still enough to increase seed yield and seed weight.

References

de Felipe, M., Gerde, J.A., Rotundo, J.L., 2016. Soybean genetic gain in maturity groups III to V in Argentina from 1980 to 2015. Crop Sci. 56, 3066–3077. https://doi.org/10.2135/cropsci2016.04.0214

Duvick, D.N., Smith, J.S.C., Cooper, M., 2004. Long-term selection in a commercial hybrid maize breeding program. Plant Breed. Rev. 24, 109–152.

Hungria, M., Araujo, R.S., 1994. Manual de métodos empregados em estudos de micro-biologia agrícola. Embrapa-Serviço de Produção e Informação, Brasilia, Brazil.

Loss, S.P., Siddique, K.H.M., 1994. Morphological and Physiological Traits Associated with Wheat Yield Increases in Mediterranean Environments, in: Advances in Agronomy. pp. 229–276. https://doi.org/10.1016/S0065-2113(08)60625-2

Mourtzinis, S., Kaur, G., Orlowski, J.M., Shapiro, C.A., Lee, C.D., Wortmann, C., Holshouser, D., Nafziger, E.D., Kandel, H., Niekamp, J., Ross, W.J., Lofton, J., Vonk, J., Roozeboom, K.L., Thelen, K.D., Lindsey, L.E., Staton, M., Naeve, S.L., Casteel, S.N., Wiebold, W.J., Conley, S.P., 2018. Soybean response to nitrogen application across the United States: A synthesis-analysis. F. Crop. Res. 215, 74–82. https://doi.org/10.1016/j.fcr.2017.09.035

R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Unkovich, M., Herridge, D.F., Peoples, M., Cadisch, G., Boddey, B., Giller, K., Alves, B., Chalk, P., 2008. Measuring plant-associated nitrogen fixation in agricultural systems. ACIAR Monograph No. 136.

Table 1. Analysis of variance and mean values of seed yield and seed number for each genotype and nitrogen (N) condition

	Seed yield		Seed number	
	bu/a		seed/ft^2	
9391	42.7	bc	187	bc
9392	41.0	bc	180	bc
93B67	42.6	bc	182	bc
93B82	46.6	b	202	ab
93M90	45.8	b	197	b
P35T58R	55.8	a	241	a
P3981	36.3	c	151	c
Control	40.9	B	179	
High N	47.8	A	202	
Variety	***		***	
Treatment	**		ns	
Variety × Treatment	ns		ns	

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively.
NS = not significant.
Means followed by the same letter are not significantly different based on Tukey's HSD test ($P < 0.05$).

Table 2. Final seed weight and their standard error for each combination of variety and nitrogen (N) condition

Treatment	Variety	Seed weight
		mg/seed
Control	9391	126 ± 3.26 *hi*
	9392	122 ± 3.15 *i*
	93B67	135 ± 3.52 *fgh*
	93B82	143 ± 3.22 *cdef*
	93M90	139 ± 3.38 *defg*
	P35T58R	129 ± 3.48 *ghi*
	P3981	132 ± 3.36 *gh*
High N	9391	146 ± 3.35 *cdef*
	9392	143 ± 3.21 *efg*
	93B67	155 ± 3.64 *abc*
	93B82	164 ± 3.28 *a*
	93M90	160 ± 3.47 *ab*
	P35T58R	150 ± 3.60 *bcde*
	P3981	152 ± 3.46 *bcd*

Means followed by the same letter are not significantly different based on Tukey's HSD test ($P < 0.05$).

Table 3. Values for parameters (Equation 2) and their standard errors from the curves in Figure 3

Parameter	Treatment	Value
a	High nitrogen	52.2 ± 5.75*a*
	Control	92.6 ± 5.74*b*
b	High nitrogen	0.03986 ± 0.0157*a*
	Control	-0.00696 ± 0.0156*b*
c	High nitrogen	-0.0000267 ± 0.000011
	Control	-0.0000423 ± 0.000011

Means followed by the same letter are not significantly different based on Tukey's HSD test ($P < 0.05$).
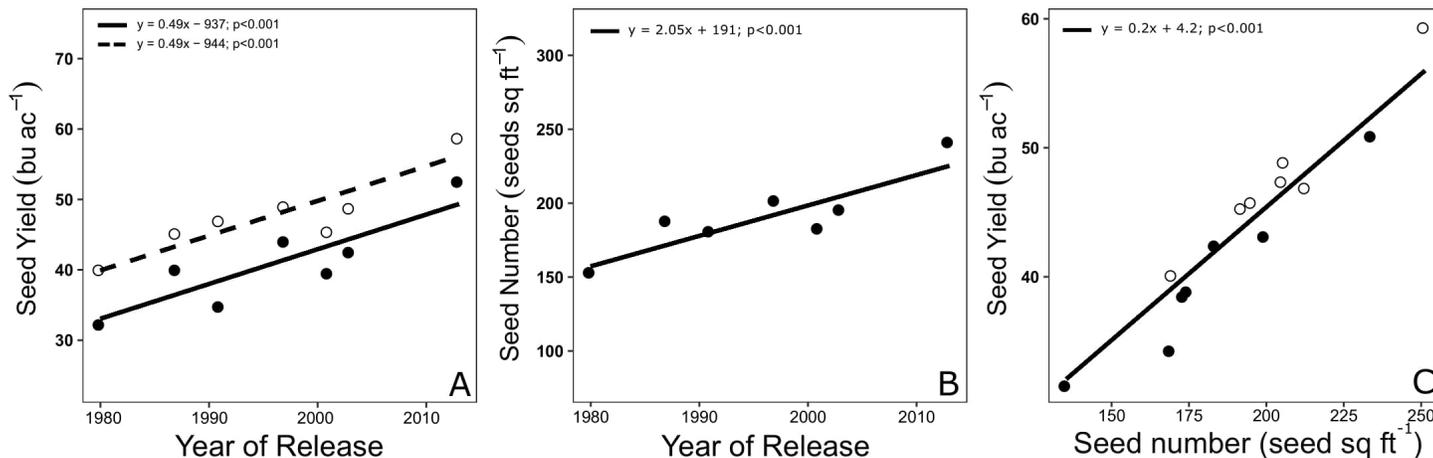
Figure 1. Relationship of the year of release with seed yield (A) and seed number (B) and the relationship between seed number and seed yield (C). Dashed curve and open circles represent the high nitrogen condition and full curves and circles represent the control condition. Each data point is the average of two years of experiment.
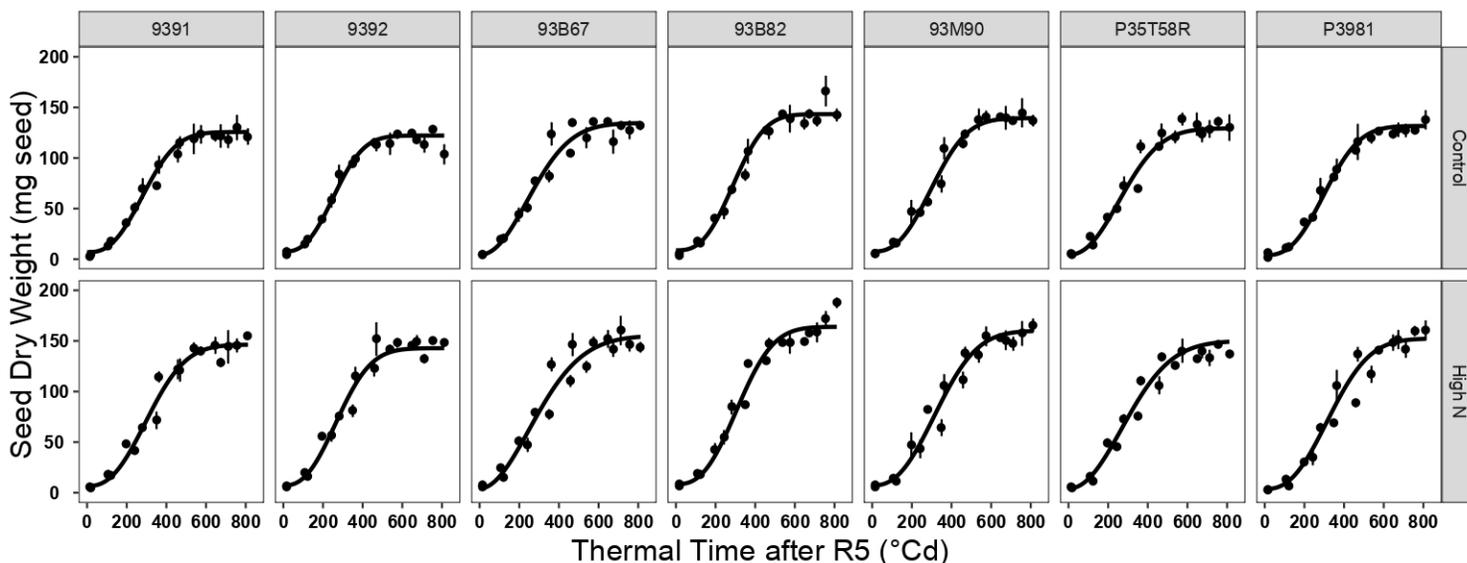

Figure 2. Changes in seed weight after R5 stage for all genotypes in the control condition and the high nitrogen condition. Vertical bars represent standard error for each data point.
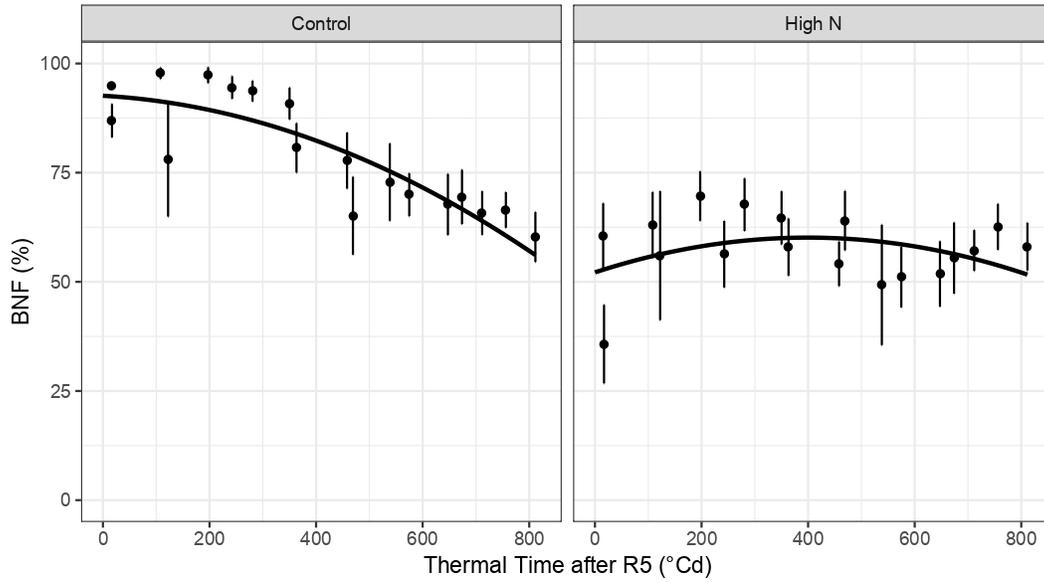
Figure 3. Changes in biological N fixation (BNF) percentage during the seed-filling period (SFP) for both nitrogen (N) conditions. Each data point is the average for each year and the vertical lines represent their standard errors.

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