

## Seed Yield and Biological Nitrogen Fixation for Historical Soybean Genotypes

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### Summary

Seed yield formation and biological nitrogen (N) fixation (BNF) were evaluated during the seed filling period (SFP) for historical soybean genotypes under contrasting N strategies. Overall, seed yield increased with the year of release, primarily associated with increments in the seed number component. The study showed that seed weight factor was maintained across decades regardless of the improvement in seed number. Nitrogen factor, evaluated as zero-N application via inorganic fertilizers versus high-N added, influenced seed yield via impacting seed weight factor. The latter plant trait improved with the high-N treatment, which was related to changes in the duration of the SFP rather than in the rate (seed biomass accumulation per day). The BNF parameter also reflected changes during the SFP related to the N treatment implemented, with high BNF (c.a. peak around 70-90%) under zero-N treatment, but still providing N via BNF at a lower rate (c.a. peak around 40-50%) for the high-N treatment. The latter demonstrated that the N fertilization reduced BNF by nearly 50% but did not completely inhibit this process. Thus, the zero-N plants counted on three sources of N to satisfy seed N demand: N-BNF, N-soil, and N-fertilizer. Lastly, the high-N treatment also positively impacted yields (+7 bu/a), which could potentially demonstrate a nitrogen limitation toward the end of the SFP for soybeans. Further testing will be performed during the next growing season to provide an improved yield and BNF characterization under different growing seasons (weather).

### Introduction

Seed yield in crops is defined by two main numerical components: seed number and weight. For many crops such as corn and sorghum, most of the variation in seed yield is explained through changes in the final seed number. However, variations in the final seed weight can be responsible for large changes in yield (Borrás et al., 2004; Sadras, 2007).

Changes in seed weight can be characterized by the amount of dry mass deposited per unit of time (rate) and the duration of this process from beginning of seed formation to physiological maturity, herein termed as seed filling period (SFP). In soybean (*Glycine max* L. Merrill), genetic variation has been reported for rate and duration of the SFP (Egli et al., 1987; Swank et al., 1987) but also largely influenced by the environmental factors such as water, heat, or nutrient stresses (Egli, 1997; Egli et al., 1978; Saini and Westgate, 1999). During the SFP, parallel to the seed changes, production translocation of assimilates and nutrients takes place from different plant organs to the seed in

order to provide sufficient supply for the seed storage components (i.e., starch, oil, and protein). Specifically for soybeans, an additional process occurs during the SFP (as a continuation of its onset during the early crop-growing season), the biological nitrogen (N) fixation (BNF), presenting the higher rates during this period and supplying a large quantity of the N available in seeds.

Hence, during the soybean SFP several processes are intertwined. Attaining a high-yield crop is not only related to the environmental conditions but also the plant physiological status during the SFP. The primary objective of this study was to investigate if N is limiting potential seed weight (via studying both rate and duration of SFP) and, in consequence, final seed yield, and to provide a better understanding of the role of BNF process during the SFP for soybean crop.

## Procedures

A field study was conducted at the Kansas River Valley research station (Rossville, KS) during the 2016 growing season. Experimental layout was a complete randomized block design with seven genotypes and two fertilizer N rates all replicated three times. For the genotype factor, 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) (Pioneer®). Application treatments for the fertilizer N factor, zero-N and high-N with 500 lb N/a applied in three timings (i.e., V1, R1, and R3 growth stages). The study was planted May 12 and plot size was 10-ft wide by 50-ft long. For all treatments, seeds were inoculated and plots were maintained weed- and pest-free during the growing season. In-season cumulative precipitation was 31 inches, with an average seasonal temperature of 73°F. Prior to planting, a soil test was conducted to characterize initial soil conditions; overall, the study presented 21 ppm of P (Mehlich), 153 ppm of potassium (K) at 6-inch soil depth, and a total N of 3 ppm at 24-inch soil depth.

Seeds were sampled in all plots at R5 weekly in order to characterize the seed filling curve and estimate rate, duration, and seed weight. In each sampling time, plants were removed to use the stem fraction to measure ureides and nitrates concentration using the hot water extraction method, following Hungria and Araujo (1994). Both concentrations were used to calculate the relative abundance of ureides (%RAU) as a parameter to characterize BNF throughout the SFP.

An analysis of variance was performed to test the effect of genotype, N level, and their interaction in all traits measured. Seed growth rate and duration were determined for each combination of genotype × replication by fitting a bi-linear model (Equations [1] and [2]) as in (Gambín and Borrás, 2011) together with knowledge on heritability estimates and possible trade-off relations among traits. Sixty-five sorghum inbred lines were

evaluated for grain filling and other agronomic traits during 2008 and 29 re-evaluated in 2009. Time to anthesis, final grain weight (GW):

$$\text{Seed weight (mg seed}^{-1}\text{)} = a + b * d \text{ for } d < c \text{ linear function} \quad [1]$$

$$\text{Seed weight (mg seed}^{-1}\text{)} = a + b * c \text{ for } d > c \text{ plateau function} \quad [2]$$

where  $d$  are the days after R5,  $a$  is the y-intercept (mg seed<sup>-1</sup>),  $b$  is the linear rate of dry mass accumulation (mg seed<sup>-1</sup> d<sup>-1</sup>), and  $c$  is the duration of the SFP (days).

## Results

### *Seed Yield and Numerical Components*

Differences for seed yield were significant between genotypes and N levels ( $P < 0.01$  and  $P < 0.05$ , respectively; Table 1). There was a positive trend between year of release and seed yield, with higher yield for modern genotypes (i.e., 64.5 bu/a for 2013). This trend was also observed for the seed number component, but presented only significant differences for the genotype effect ( $P < 0.001$ ), with greater seed number for modern soybean varieties. Furthermore, the treatment depending solely on the fertilizer N source produced an increase in overall yields relative to only the inoculated scenario ( $P < 0.01$ ).

Differences between genotypes and N levels were highly significant for the final seed weight ( $P < 0.001$ ; Table 1). However, for this plant trait, a different trend was documented as related to the release year of the genotypes (e.g., 1997 > 2003 > 1980 > 2013) when compared with yield and seed number factors. Nitrogen application increased seed weight as a result of an increase in the duration of the SFP, but without altering the seed growth rate (Figure 1A). Changes in seed filling duration were also previously documented in the scientific literature, and were also in agreement that the seed filling rate was less sensitive to environmental changes.

### *Biological Nitrogen Fixation During Seed Filling Period*

Regardless of the genotype evaluated, superior %RAU was observed for the zero-N treatment and with low %RAU values for the high-N scenario (Figure 1B). As expected, application of exogenous N during the growing season partially inhibited the BNF process, depicting a fairly constant value of %RAU during the seed filling period (ca. 40-50%; Figure 1B). Meanwhile, the %RAU evolution during the SFP for the zero-N application depicts superior BNF values for the first 20 days (ca. 70-90%) and then consequently dropping until a final N fixation value was attained 50 days after the onset of the SFP (ca. 50%; Figure 1B).

Even though the BNF process was partially inhibited or reduced, the high-N treatment supplied sufficient N in order to achieve larger seed weight and higher seed yield.

Key conclusions from this study are:

- Even though the response was relatively low (7 bu/a) for the environment tested, there was a positive and significant response in seed yield to N applications in soybean. This study does not warrant application of N to soybeans, but demonstrates that the crop can be limited for this nutrient at the end of the growing season.
- Seed weight was the main yield component affected by N treatments. Larger seed weight was primarily explained by changes in duration, rather than on the rate of the SFP.
- Biological nitrogen fixation activity was affected by the fertilizer N application, showing an overall reduction close to 50% at the onset of the SFP when zero-N was compared to the high-N treatment.

The results presented in this study include soil-environment conditions experienced in one growing season. Further studies are needed to explore a wider range of environmental conditions in order to provide a large dataset of the overall effect of N in limiting maximum seed weight, and thus, potentially impacting seed yields.

## References

- Borrás, L., Slafer, G.A., Otegui, M.E., 2004. Seed dry weight response to source–sink manipulations in wheat, maize and soybean: a quantitative reappraisal. *F. Crop. Res.* 86, 131–146.
- Egli, D.B., 1997. Cultivar maturity and response of soybean to shade stress during seed filling. *F. Crop. Res.* 52, 1–8. doi:10.1016/S0378-4290(97)00005-1
- Egli, D.B., Leggett, J.E., Duncan, W.G., 1978. Influence of stress on leaf senescence and N redistribution in soybeans. *Agron J* 70, 43–47. doi:10.2134/agronj1978.00021962007000010011x
- Egli, D.B., Swank, J.C., Pfeiffer, T.W., 1987. Mobilization of leaf N in soybean genotypes with varying durations of seedfill. *F. Crop. Res.* 15, 251–258. doi:10.1016/0378-4290(87)90014-1
- Gambín, B.L., Borrás, L., 2011. Genotypic diversity in sorghum inbred lines for grain-filling patterns and other related agronomic traits. *Crop Pasture Sci.* 62, 1026–1036. doi:10.1071/CP11051
- Hungria, M., Araujo, R.S., 1994. Manual de métodos empregados em estudos de microbiologia agrícola. Embrapa-Serviço de Produção e Informação.
- Sadras, V.O., 2007. Evolutionary aspects of the trade-off between seed size and number in crops. *F. Crop. Res.* 100, 125–138.
- Saini, H.S., Westgate, M.E., 1999. Reproductive Development in Grain Crops during Drought. *Adv. Agron.* 68, 59–96. doi:10.1016/S0065-2113(08)60843-3
- Swank, J.C., Egli, D.B., Pfeiffer, T.W., 1987. Seed growth characteristics of soybean genotypes differing in duration of seed fill. *Crop Sci.* 27, 85–89.

**Table 1. Analysis of variance and means for seed yield (13.5% moisture), seed number, final seed weight, seed filling rate, and duration for all genotypes and nitrogen (N) levels**

Genotype	Release year	N level	Seed yield		Seed number		Seed weight		SFP rate		SFP duration
			bu/a		seed m <sup>-2</sup>		mg seed <sup>-1</sup>		mg d <sup>-1</sup>		days
P3981	1980		42.7	d	2080	c	148	b	3.81	b	41
9391	1987		51.2	bcd	2636	b	134	c	4.08	ab	35
9392	1991		44.6	cd	2214	bc	133	c	4.34	a	32
93B82	1997		56.2	ab	2583	bc	166	a	4.31	a	40
93B67	2001		44.2	cd	2054	bc	135	c	3.86	b	36
93M90	2003		53.4	bc	2453	bc	151	ab	4.08	ab	39
P35T58R	2013		64.5	a	2664	a	137	c	4.01	b	36
		Zero-N	47.5	b	2270		133	b	4.06		34
		High-N	54.5	a	2469		154	a	4.08		40
Genotype			**		***		***		*		***
N Level			*		ns		***		ns		***
Genotype × N level			ns		ns		ns		ns		*

Different letters indicate significant differences at  $P \leq 0.05$

\* Significant at  $P \leq 0.05$ ; \*\* significant at  $P \leq 0.01$ ; \*\*\* significant at  $P \leq 0.001$ .

Ns: non-significant.

SFP = seed filling period.

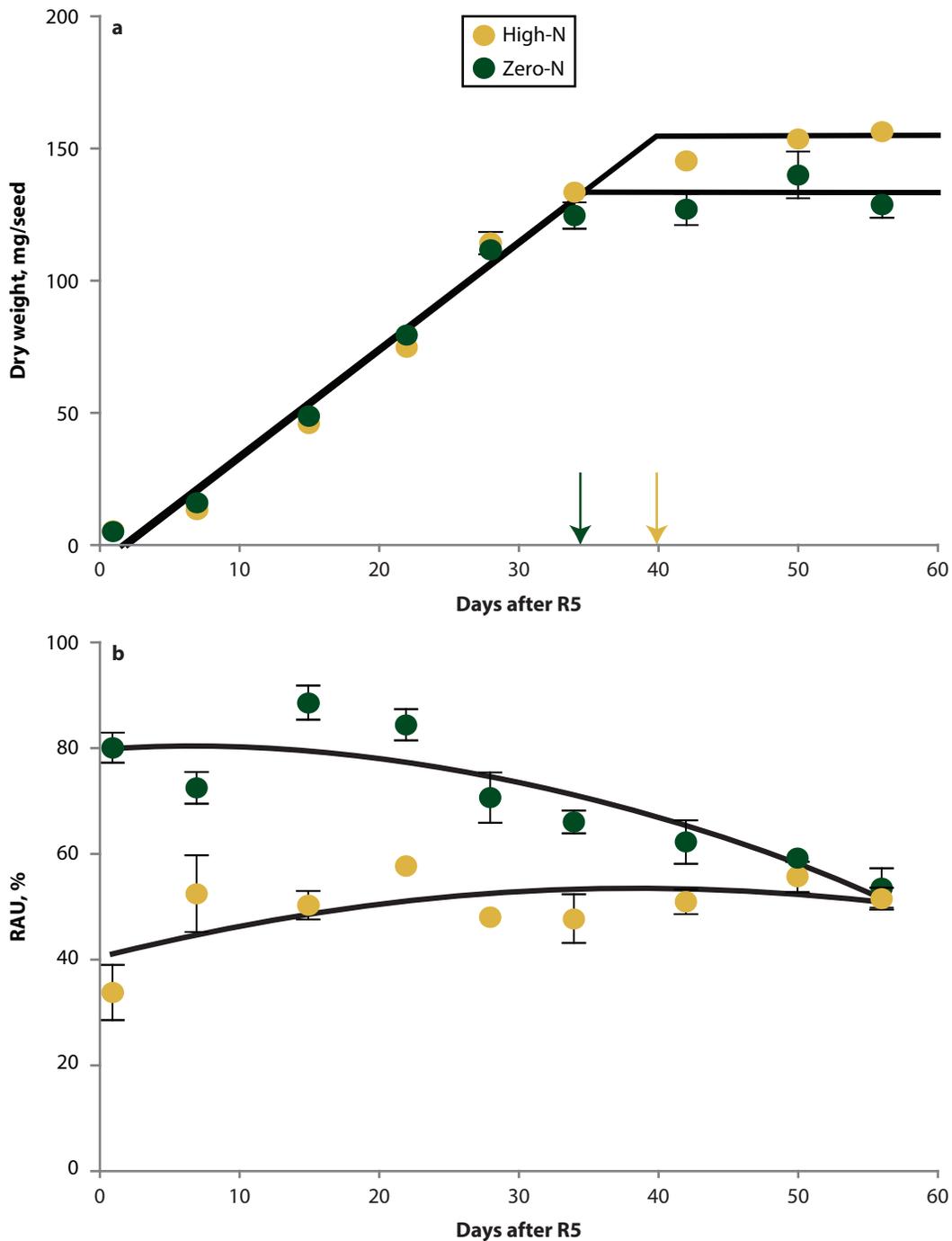


Figure 1. Evolution of the seed dry weight, a, and percentage of relative abundance of ureides (%RAU; b) after R5 growth stage (onset of seed filling) for two different N levels. The arrows in Figure 1a portray the duration of the seed filling period for zero- (green symbols (dark)) and high-nitrogen (N) (yellow symbols (light)) treatments, respectively.