2018

Effects of Nitrogen in Soybean Seed Quality Definition During Seed-Filling Period

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Recommended Citation
Tamagno, S.; Adee, E. A.; and Ciampitti, I. A. (2018) "Effects of Nitrogen in Soybean Seed Quality Definition During Seed-Filling Period," Kansas Agricultural Experiment Station Research Reports: Vol. 4: Iss. 7.
https://doi.org/10.4148/2378-5977.7609

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Abstract
During the seed filling period (SFP), parallel to the seed changes, 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) that ultimately will determine the seed quality. There are two processes that define the final seed weight in any crop: 1) the amount of dry mass deposited per unit of time (rate) and 2) the duration of this process from beginning of seed formation to physiological maturity. As seed number is defined, any source limitation during the SFP can affect the final weight and quality of the seeds. This study aims to investigate if nitrogen (N) is limiting potential seed weight and, in consequence, final seed yield as well as the characterization of the deposition of seed components (i.e., oil and protein) that define soybean seed quality among different N conditions and genotypic background.

Keywords
soybean, seed quality, seed filling, late-season N application

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Effects of Nitrogen in Soybean Seed Quality Definition During Seed-Filling Period

S. Tamagno, E.A. Adee, and I.A. Ciampitti

Introduction
During the seed filling period (SFP), parallel to the seed changes, 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) that ultimately will determine the seed quality. There are two processes that define the final seed weight in any crop: 1) the amount of dry mass deposited per unit of time (rate) and 2) the duration of this process from beginning of seed formation to physiological maturity. As seed number is defined, any source limitation during the SFP can affect the final weight and quality of the seeds. This study aims to investigate if nitrogen (N) is limiting potential seed weight and, in consequence, final seed yield as well as the characterization of the deposition of seed components (i.e., oil and protein) that define soybean seed quality among different N conditions and genotypic background.

Procedures
A field study was conducted at the Kansas River Valley research station (Rossville, KS) during the 2016 growing season (Table 1). Experimental layout was a complete randomized block in a split-plot design with seven genotypes (subplots) and two fertilizer N rates (main plots) all replicated three times. For the genotype factor, seven soybean varieties with different years of release were tested (Pioneer). Fertilizer was applied in three timings (i.e., V1, R1, and R3 growth stages). Plot size was 10-ft wide × 50-ft long. For all treatments, seeds were inoculated and plots were maintained weed- and pest-free during the growing season.

Seeds were sampled in all plots at the onset of R5 growth stage (beginning of seed filling) weekly in order to estimate seed filling rate and duration, seed weight and chemical composition (protein and oil content). Protein and oil content (mg/seed) for each seed sample were estimated as the product between individual seed dry weight and component concentration. Protein concentration (%) was estimated as N concentration multiplied by 6.25 using the Kjeldahl method. Oil concentration (%) was determined gravimetrically after extraction with hexane in another 0.5-g subsample.

An analysis of variance was performed to test the effect of genotype, N level, and their interaction in all traits measured. Rate and duration for seed components and seed biomass 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):

\[
\text{Seed weight (mg/seed)} = a + b \times d \text{ for } d < c \text{ linear function) } \tag{1}
\]

\[
\text{Seed weight (mg/seed)} = a + b \times c \text{ for } d > c \text{ plateau function) } \tag{2}
\]

where \(d\) are the days after R5, \(a\) is the y-intercept (mg/seed), \(b\) is the linear rate of dry mass or seed component accumulation (mg/seed d\(^{-1}\)), 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 2). For seed number, modern varieties showed greater values of seed number \((P < 0.001)\).

Differences between genotypes and N levels were highly significant for the final seed weight \((P < 0.001; \text{Table 2})\). Nitrogen application increased seed weight by extending the duration of the SFP, but without changing the seed growth rate.

**Seed Components Accumulation**

Large differences between genotypes were reported for oil content \((P < 0.001; \text{Table 3})\) and the rate \((P < 0.01)\). Oil content varied from 29.8 to 36.2 mg/seed showing the large range of genotypic diversity. As expected, different N levels did not affect the oil content, the rate and duration of this component. The protein content and the duration were primarily affected by N availability \((P < 0.05)\). Large differences between genotypes were observed for the genotypes tested and for the rate of protein deposition \((P < 0.05)\). However, despite genotypic variability the increase in N availability during the SFP managed to boost the protein content from 48.2 to 55.3 mg/seed.

**Conclusions**

- This study does not warrant application of N to soybeans, it only demonstrates that the crop can be limited for this nutrient at the end of the growing season. However, for the environment tested there was a positive and significant response in seed yield to N applications in soybean.
- Seed weight was significantly affected by N availability. Larger seed size was explained by changes on duration of the SFP.
- The range of values observed in oil content was due to the genotypic effect rather than higher N during the SFP.
- Nitrogen availability increased the protein content in seeds through longer duration of the SFP.

**Reference**

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
Table 1. Environmental conditions and treatments imposed in the experiment

| Location            | Rossville, Kansas |
|---------------------|-------------------|
| Planting Date       | May 12, 2016      |
| Temperature         | 73°F              |
| Precipitation       | 31 in.            |
| N Level (lb/a)      | 0, 500            |
| Varieties           | 80s P3981 9391    |
|                     | 90s 9392 93B82    |
|                     | 00s 93B67 93M90   |
|                     | 10s P35T58R       |
| Soil (ppm)          | N, P, K 3; 21; 153|

Table 2. Analysis of variance and means for seed yield (13.5% moisture), seed number, seed weight, and seed filling rate and duration for all genotypes and nitrogen (N) levels

| Genotype | Release year | N level | Seed yield (bu/a) | Seed number (seed/m²) | Seed weight (mg/seed) | SFP rate (mg/day/seed) | SFP duration (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 b                 | 34                  |
| High-N   |              | 54.5    | a                 | 2469                  | 154 a                 | 4.08 b                 | 40                  |

Genotype ns
N Level ns
Genotype × N level ns

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$. NS = non-significant. Different letters represent the least significant differences (LSD) between means at $P \leq 0.05$. 

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Table 3. Analysis of variance and means for oil and protein filling rate, duration and content for all genotypes and nitrogen (N) levels

| Genotype | Release year | N level | Oil Rate | Duration | Content | Protein Rate | Duration | Content |
|----------|--------------|---------|----------|----------|----------|-------------|----------|---------|
|          |              |         | mg/day   | days     | mg/seed  | mg/day      | days     | mg/seed |
| P3981    | 1980         | 0.93    | 40 a     | 32.2 b   | 1.45 bc  | 39          | 52.6 bc  |
| 9391     | 1987         | 0.94    | 36 b     | 29.8 bcd | 1.41 bc  | 38          | 50.1 cd  |
| 9392     | 1991         | 1.04    | 31 c     | 28.5 cd  | 1.55 ab  | 32          | 48.8 cd  |
| 93B82    | 1997         | 1.02    | 39 ab    | 36.2 a   | 1.66 a   | 38          | 59.8 a   |
| 93B67    | 2001         | 0.87    | 36 b     | 27.6 d   | 1.4 bc   | 37          | 49.6 cd  |
| 93M90    | 2003         | 0.98    | 37 ab    | 32 b     | 1.5 abc  | 39          | 55.1 b   |
| P35T58R  | 2013         | 0.97    | 36 b     | 30.6 bc  | 1.34 c   | 37          | 46.6 d   |
|          |              |         |          | Zero-N   | 0.92     | 35          | 29.3      | 1.43    | 35 b   | 48.2 b |
|          |              |         |          | High-N   | 1.01     | 38          | 32.7      | 1.51    | 39 a   | 55.3 a |

Genotype ns ** *** * ns ***
N Level ns ns ns ns * *
Genotype × N level ns ns ns ns ns ns

*Significant at P ≤ 0.05; *** Significant at P ≤ 0.001. NS = non-significant. Different letters represent the least significant differences (LSD) between means at P ≤ 0.05.