**Developing Improved Soybean Lines for Seed Composition, Quality, and Heat Tolerance in Mississippi**
**Project No: 33-2019**

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## Background and Objectives

High heat in the Early Soybean Production System (ESPS) is a major environmental stress factor resulting in yield reduction and poor seed quality, lowering the level of seed composition constituents in heat sensitive soybeans, lowering market grade, and reducing the quality of soymeal. According to the Risk Management Agency, seed damage in the Southeast caused by heat was 40.4 % of the national total acreage with heat damage, and the monetary payouts were 36.1 % of the national payout total for heat damage. Concerning U.S. seed composition, historic data from 1986 to 2015 showed a decreasing trend of protein content in soybean. Therefore, among the objectives of the USB 2011-2016 Strategic Plan is to increase the value of US soymeal and oil in the value chain by changing seed composition.
To our knowledge, there are no commercial soybean cultivars that are heat-tolerant with high levels of seed composition (seed protein, oil, and minerals) and quality components (reduced seed damage including reduced green-seed, seed wrinkling, hard-seed, and diseased seed). Therefore, developing heat tolerant germplasm with high seed composition and quality is essential.

Improvement of soybean seed composition, including protein, oil, fatty acids, sugars, and minerals is important. Our previous research identified breeding lines with heat-tolerance and high seed quality, including seed protein and germination. Thus, this project focused on phenotyping seed quality traits, including seed protein, oil, fatty acids, sugars, minerals, and other seed quality traits in a previously developed recombinant inbred line (RIL) population segregating for heat tolerance. The first-year field trial (2019) was conducted in Stoneville, MS, using 200 heat-tolerant RILs with parental lines and heat-sensitive cultivars as controls with a total of 430 plots in replicated trial. Mature seeds, harvested shortly after full maturity (R8 growth stage), were phenotyped for seed composition constituents and mineral nutrition as well as seed quality traits. The experimental was non-irrigated to promote stress, and the design was a randomized complete block with two replicates across multiple years. The second-year funding will be used to repeat the experiment in 2020.

**OBJECTIVE(S):**

**Objective One:**
To phenotype seed composition constituents (protein, oil, fatty acids, sugars, and minerals) as well as seed quality traits, including Federal Grain Inspection Service (FGIS) seed damage ratings, including green-seed, hard-seed, and diseased seed. Identifying heat tolerant RILs with high seed compositional and nutritional qualities will be an essential component for soybean producers to compete nationally and internationally in the global market.

## Report of Progress/Activity

The first-year experiment was conducted at the USDA ARS's Jamie Whitten Delta States Research Center at Stoneville, MS. A recombinant inbred line population derived from the cross DS25-1 x DT97-4290 was planted in the field on April 22, 2019. Parents and cultivar checks were included in the planting. The experimental design was a randomized complete block with two replicates for a total of 430 plots. Mature seeds, harvested shortly after R8 (full-maturity growth stage), were collected and evaluated for seed composition and seed mineral analyses as well as measurements of seed quality traits. Seed analyses were performed using standard laboratory protocols, including near infra-red (NIR) for seed protein, oil, fatty acids, and sugars; inductively coupled plasma (ICP) spectrometry for minerals analysis; and C/N/S (carbon, nitrogen, and sulfur) Elemental Analyzer for C, N, and S. Harvest began the second week of August and lasted through the second week of October. All plots were harvested and seeds from each plot were analyzed for seed composition and seed minerals, and seed damage, including, green-seed, hard-seed, diseased seed, and grain damage estimates using the Federal Grain Inspection Service (FGIS).

Preliminary results of seed composition analysis and seed quality traits showed significant variability across RILs, parents, and cultivars for seed germination (accelerated aging) and hard-seed (Figure 1); green-seed and molded-seed (Figure 2); seed protein and oil (Figure 3); and sugars (Figure 4). Seed minerals showed also significant levels across RILS, parents, and cultivars; for example, in %, for Ca (0.33-0.56); Mg (0.2-0.4 ); K (0.9-2.58); B(20.11-40.34); Fe (37-98.8); and Zn (31.38-69.9). Seed damage evaluation using FGIS is still underway and will be reported in the first quarter report of this year (limitation of on-site presence due to corona virus policy slowed down the analysis for this component).

**Impacts and Benefits to Mississippi Soybean Producers**

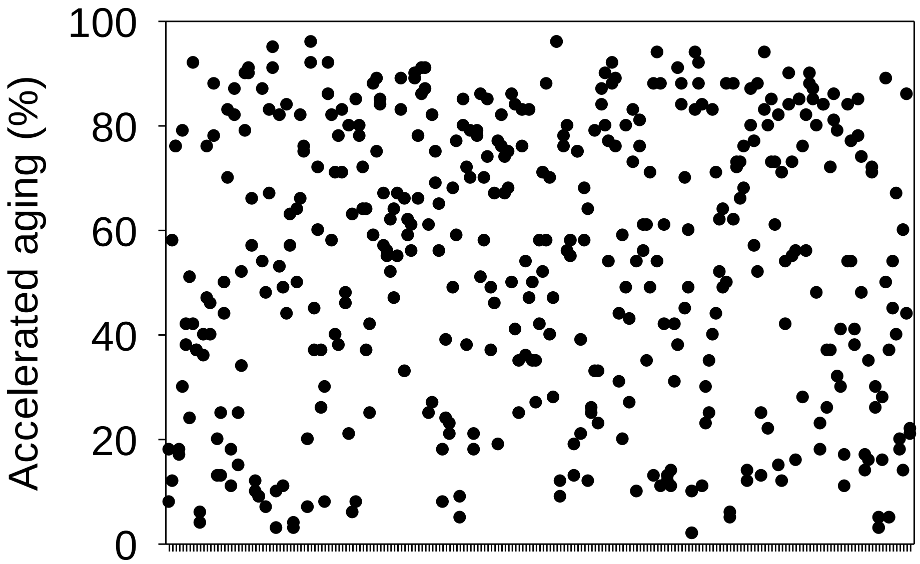
High heat in the Midsouth is a major environmental stress factor, leading to poor seed quality and influencing seed composition constituents, including protein, oil, fatty acids, sugars, mineral contents, and seed quality traits. These traits determine seed quality and are essential for human consumption and livestock nutrition. Protein content determines the soymeal quality. High seed oil is desirable for human consumption. High oleic and low linolenic acids contribute to oil stability. Raffinose and stachyose sugars are undesirable components because they have detrimental effects on the nutritive value of the meal and are indigestible by human and animals, often causing flatulence or diarrhea in non-ruminants. Sucrose sugar is desirable because it improves taste and flavor in tofu, soymilk, and natto. Higher mineral contents such as iron, zinc, magnesium, and potassium are desirable for human nutrition and animal rations. Reports from USB indicated that the trend of protein content in U.S. soybeans has been declining during the period of 1986 to 2015. Therefore, in order to maintain high seed compositional qualities, the continued evaluation of seed composition and mineral nutrition qualities is critical. Identifying heat tolerance varieties with high levels of seed composition, especially protein and minerals, and seed quality, will be essential for the Midsouth. Also, high levels of seed compositional qualities, especially protein and minerals will impact soybean producers to compete nationally and internationally in the global market.

We conducted a trail that involved 200 heat-tolerant RILs with parental lines and heat-sensitive cultivars as controls with a total of 430 plots in replicated trial. Preliminary results showed significant variability in seed quality traits as indicated above and in graphs below. We have already genotyped the RIL population and a molecular map is being constructed. The seed trait measurements obtained through the research outlined herein, can be used in conjunction with the molecular map to identify the genomic location of genes controlling/influencing the traits. Knowing these genomic locations would allow the development of molecular markers for specific traits which could increase the efficiency of selection thereby reducing development time for new germplasm releases.

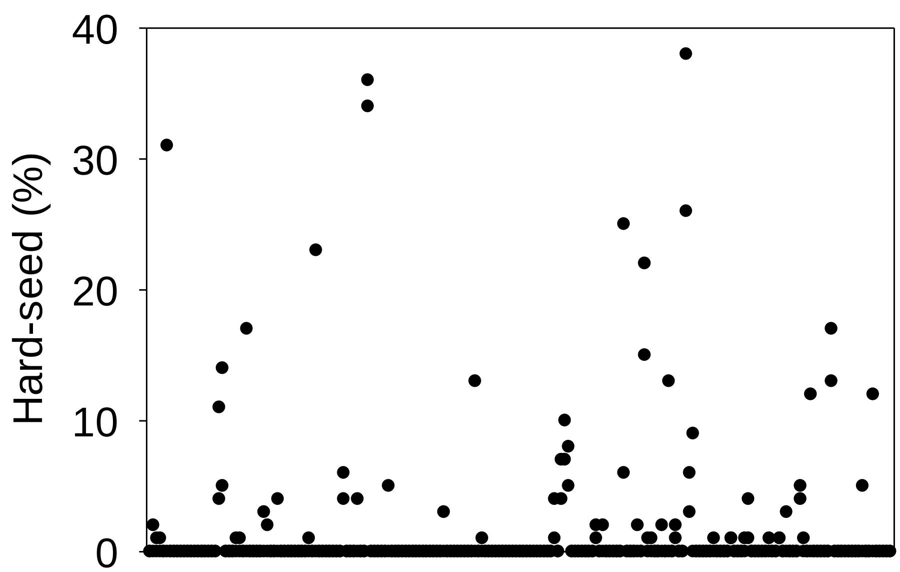
**End Products–Completed or Forthcoming**

In addition, this research will provide seed compositional and quality phenotypes for the future identification of genes associated with seed protein, oil, fatty acids, sugars, and minerals and will identify breeding lines with both heat-tolerance and high seed compositional qualities for release. These heat-tolerant breeding lines will be used by public and private breeders to develop improved cultivars which, when adopted by Mississippi producers, will be a valuable seed quality component, enabling the producers to more effectively compete nationally and internationally in soybean markets. The current results are preliminary, the second- and third-year results will be combined and prepared to be published in peer-reviewed journal articles, presented at scientific meetings and field days.

**Graphics/Tables**

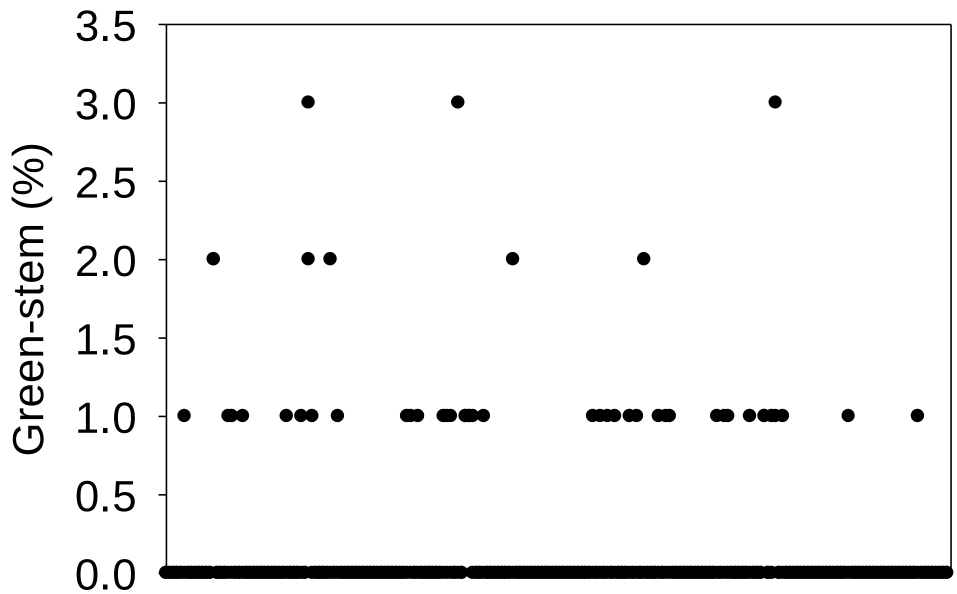



Distribution of Accelerated aging across all RILs, checks, and parents

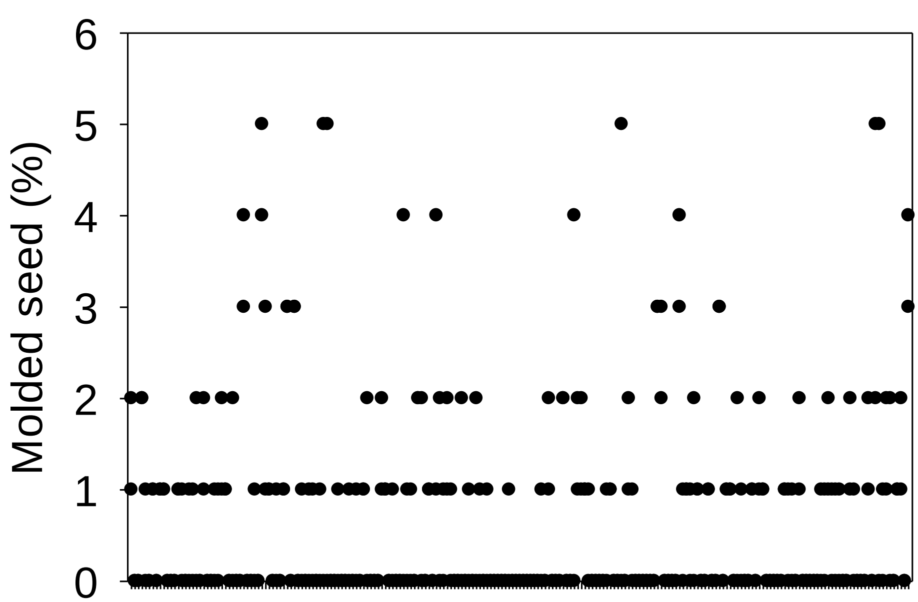



Distribution of hard-seed across all RILs, checks, and parents

Figure 1: Variability of accelerated aging (top graph) and hard-seed (bottom graph) across RILs, checks, and parents .

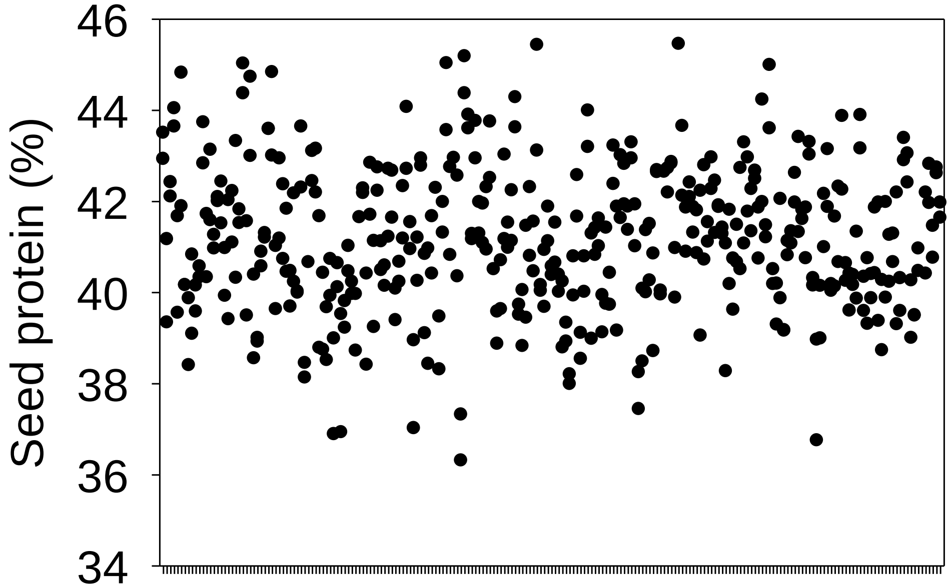



Distribution of green-stem across all RILs, checks, and parents

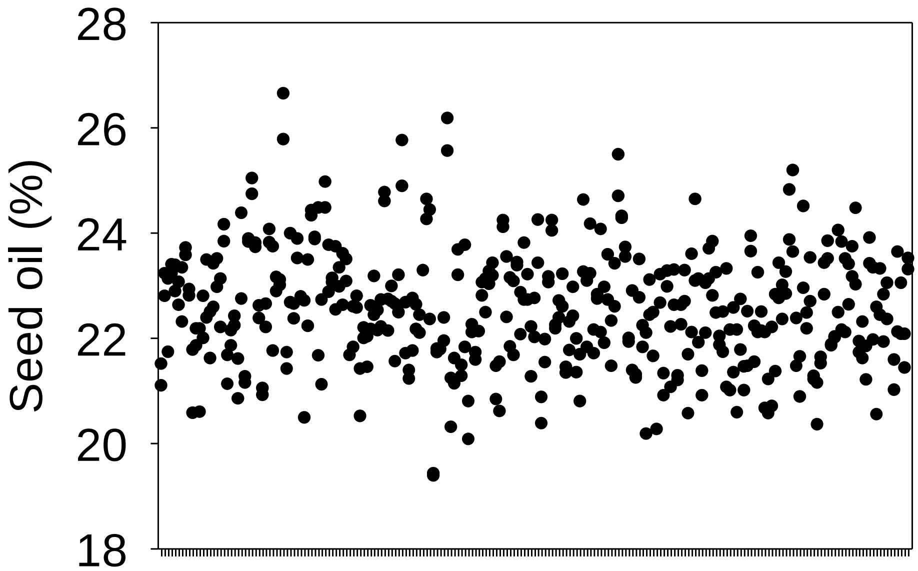



Distribution of molded seed across all RILs, checks, and parents

Figure 2: Variability of green-seed (top graph) and molded-seed (bottom graph) across RILs, checks, and parents.

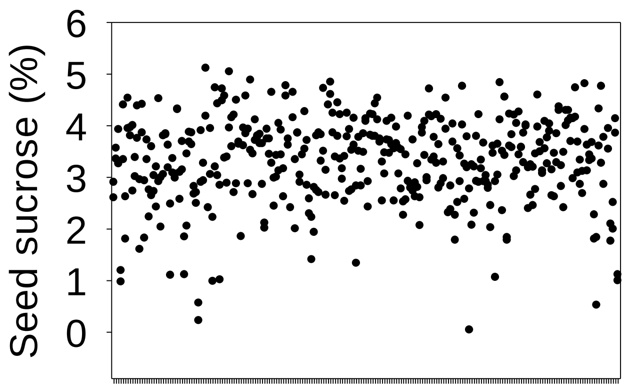



Distribution of seed protein across all RILs, checks, and parents

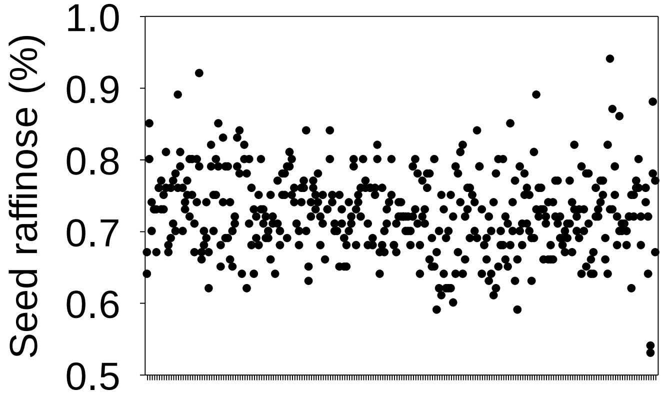



Distribution of seed oil across all RILs, checks, and parents

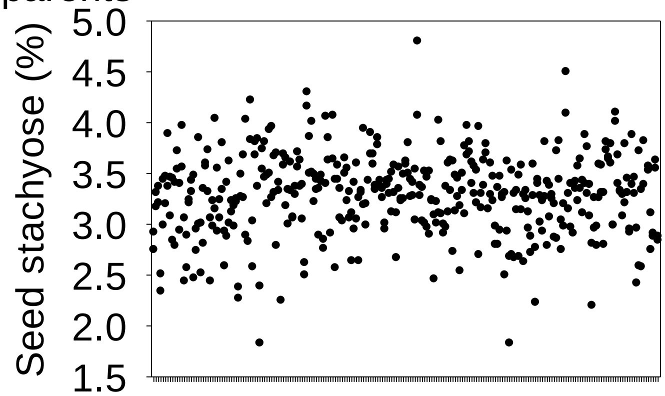
Figure 3: Significant variability of seed protein (top graph) and oil (bottom graph) levels across RILs, checks, and parents.




Distribution of seed sucrose across all RILs, checks, and parents




Distribution of seed raffinose across all RILs, checks, and parents




Distribution of seed stachyose across all RILs, checks, and parents

Figure 4: Significant variability of seed sucrose (top graph), raffinose (middle graph), and stachyose (bottom graph) levels across RILs, checks, and parents.