**Kansas Soybean Commission 3rd Quarter report for FY2017**

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**Project Title**: “High-throughput Platform to Enhance Quality of Beans and Add Value to Kansas Soybean Breeding Program”

**Checkoff Organization:** Kansas Soybean Commission

**Categories:** Breeding & genetics

**NCSRP, USB, QSSB Project Code:** 1778

**Amount of funding:** $35,000.00

**Project Year:** 2017

**Department Head:** Gary Pierzynski

**Project Summary**

Soybean breeding programs are successful in increasing yield potential but progress in breeding for optimizing seed quality composition such as protein, oil, fatty acids, amino acids has not received similar attention. This is primarily because of the lack of a rapid screening tool to capture the complex tradeoffs between yield and quality parameters. Hence there is a real need to develop a simple, robust and high-throughput platform for quantifying quality parameters and the seed compositional changes in response to a range of environmental conditions. The major quality determining components i.e. amino acids, protein, oil and fatty acids compositions are often negatively impacted by harsh environmental conditions (high temperatures and low or erratic rainfall) during pod filling stage. Enhancing the quality of the beans is emerging as a major priority that needs to be addressed, to obtain an edge in the domestic and international market. Till date, assessing the diversity in protein, oil, fatty acids, amino acids, and other quality composition in soybean grown in Kansas has not been systematically attempted. Thus, with the establishment of Near-infrared spectroscopy (NIRS)- a rapid and high-throughput tool in the Agronomy Department will strengthen Kansas soybean breeding program to develop beans with higher quality. Progress achieved in the project will increase additional income generation potential among Kansas growers.

**Project Objectives**

1. Develop and standardize a high-throughput approach to quantify genetic diversity in beans protein, amino acids, oil, oleic acid etc. from germplasm generated by the Kansas Soybean Breeding Program
2. Estimate the spatial and temporal impact of Kansas climatic variability on soybean quality with emphasis on essential amino acids, oleic acid etc.
3. Integrate the technology into Kansas soybean breeding program to enhance breeding efficiency towards developing high quality beans

 **Project Deliverables**

1. Genetic variability in protein, amino acids, oil, oleic acid compositions of native, exotic and advanced soybeans breeding lines profiled
2. Location specific climatic impact on soybean seed quality quantified
3. A high throughput platform to determine trade-offs between yield and quality parameters established
4. NIRS spectral curves developed and standardized for supporting the soybean breeding program during and beyond the time-frame of the proposed project

**Progress of Work (3rd Quarter report for FY2017)**

For the 3rd quarter report, we mainly focused on our first objective (to standardize the high-throughput technique for estimating beans quality composition). Based on the level of diversity observed for composition traits, representative genotypes capturing most of the diversity were chosen to validate NIRS estimated data using laboratory-based methods. Validation of the Perten calibration curves will allow us to determine soybean composition traits on larger sets of breeding lines with greater confidence.

**Plant materials:**

For the validation experiment of protein and oil, a representative set of soybean genotypes were selected from a diverse set of exotic soybeans (Soybean Association Panel; 133 accessions with replicates-total of 246 samples) grown in Manhattan in 2016 (by Prof. Schapaugh and the team). For oil and protein validation, approximately five samples were selected from each NIRS predicted integer percentage group to obtain an equally distributed test population. This enabled us to test the predictability of the calibration more or less equally across the whole constituent range. For moisture validation, seeds samples including freshly harvested seeds were received from Prof. Schapaugh and the team.

Secondly, we are in the process of scanning soybean entries grown in 2017 at different locations in Kansas (Cherokee, Manhattan, Ottawa, and Parsons) by private seed companies, certified growers, and agricultural experiment stations (received from Jane Lingenfelser and the team) to estimate the spatial and temporal impact of Kansas climatic variability (temperature and rainfall). Seeds grown in 2016 were already scanned (see quarter reports 1 and 2).

**NIRS scanning and laboratory-based analysis**

Selected seeds were ground into a fine powder using a SPEX mixer/mill (SPEX industries, Inc. Metuchen, NJ, USA) for laboratory-based analysis of protein and oil. Powdered samples were scanned using Near Infra-Red Spectroscopy (DA 7250 NIR analyzer, Perten Instruments), using the small plastic cup (25 mL). For moisture validation, whole soybean seeds were scanned using a small black rotating cup (volume of 125 mL). Each sample was scanned for 6 seconds (15 spectra/sec) with a wavelength ranging from 950 to 1650 nm (optical resolution ~7 nm).

###### Laboratory-based analysis includes;

* Protein: Dry combustion (LECO C/N analyzer). To determine % dry basis protein, ground samples were dried at 130 °C for 1h.
* Oil: Extraction of oil with petroleum ether (Soxhlet apparatus)
* Moisture: Drying of whole soybean seeds at 103°C for 72 h
* Fatty acids (FA): GC-MS (Agilent Technologies 6890N network GC)

**Results**

**Develop and standardize a high-throughput technique for estimating beans quality composition**

The reproducibility of NIRS scanning was tested with two independent scans of the same set of 133 SAP accessions, including both whole and ground beans. These results indicated the accuracy and reliability of the high-throughput approach in capturing the genetic variability of composition traits (see quarter report Q2 and Q3).

Protein: NIRS predicted protein content and laboratory-estimated protein content showed a significant strong correlation (Fig. 1A and 1B) with both % dry basis (R2=0.98; n=49) and % as is basis (R2=0.97; n=49).

Oil: NIRS predicted oil content and laboratory-estimated oil content (% as is basis) showed a significant correlation (Fig. 2) with each other (R2=0.81; n=21).

Moisture: NIRS predicted moisture content and laboratory-estimated moisture content showed a significant correlation (Fig. 2) with each other (R2=0.95; n=38).

# Fatty acids: The GC-MS located at the Dr. Jagadish’s lab was tested for its ability to provide reliable results on FA analysis. Initially, a standard fatty acid methyl ester mix with 37 component mix (Supelco® 37 Component FAME Mix) was tested on a DB-5MS column (30 m in length and 0.25 µm in diameter). For the quantification of fatty acids, we need a column capable of separating soybean FAs (linoleic acid, linolenic acid, oleic acid, stearic acid, and palmitic acid). But the DB-5MS column showed a co-elution of linolenic, oleic, and linoleladic fatty acids at 33.92 min, confirming its unsuitability for quantification of soybean FAs.

Secondly, we tried with HP-INNOWax column (30 m in length and 0.25 µm in diameter) which showed a better resolution of the FAME 37 component mix. Next, the extraction of fatty acids from soybean seeds will be carried out using the method described in Obour et al. (2017) and the quantification will be conducted using HP-INNOWax column.

**The spatial impact of Kansas climatic variables (temperature) on soybean quality**

We are in the process of scanning soybean entries grown in 2017 at different locations in Kansas (data not shown). We already received some soybean seed sets and we will be receiving more seed sets from Jane Lingenfelser (Production agronomist at KSU) and the team. Our long-term objective is to estimate the spatial and temporal impact of Kansas climatic variability (temperature and rainfall) on soybean composition traits and seed yield.

###### **On-going work:**

###### Seeds representing a variety of breeding trials (received from Prof. Schapaugh and team) involving 100’s of lines are being analyzed using NIRS, enabling the breeders to select best genotypes for the next breeding cycle and to make more NIRS based efficient selections.

Two sets of seeds (BREC 2015 and DC 2016) received from Dr. Henry T. Nguyen and team (University of Missouri) will be scanned and data will be provided for a genetic mapping study.

Validation of the soybean fatty acids will be continued using GC-MS.

**Final Project Results**

Our preliminary results suggested considerable quality and yield tradeoffs across both whole and fine powdered soybeans. NIRS is a promising high-throughput platform that helps in understanding the effect of microclimate on quality and also in selecting location specific soybeans. Protein, oil, and moisture validation experiments showed the robustness of the existing calibration curves, developed by Perten Instruments.

**Benefit to soybean farmers**

Enhancement of soybean quality provided to Kansas soybean growers will offer newer opportunities to improve their revenue in domestic and international market.

**Figures:** 

**B**

**A**

**Figure 1: Correlation between NIRS predicted protein content and laboratory estimated protein content in % dry basis (A) and % as is basis (B).**



**Figure 2: Correlation between NIRS predicted oil content and laboratory estimated oil content.**

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**R2=0.95; n=38**

**Figure 3: Correlation between NIRS predicted moisture content and laboratory estimated moisture content.**