**Kansas Soybean Commission 1st 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

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**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 (Carrera et al., 2011 a, b). 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 (1st Quarter report for FY2017)**

Plant materials: During the past three months, a diverse set of exotic soybean (Soybean Association Panel; 249 accessions) grown at Manhattan in 2016 (by Prof. Schapaugh and team) have been scanned with NIRS. Secondly, we have also scanned soybean entries tested in 2016 from private seed companies, certified growers, and agricultural experiment stations were grown in different locations (Cherokee, Manhattan, Ottawa and Parsons) in Kansas. The information on the local temperature variations during pod-filling stage (August to October) has been obtained for all locations (Cherokee, Manhattan, Ottawa and Parsons) from the Kansas Mesonet (<http://mesonet.k-state.edu/>). About 500 seeds of each entry and replicate were profiled for quality traits such as protein, oil, fatty acids (oleic acid, linoleic acid and linolenic) and a few major amino acids (lysine, methionine, threonine and tryptophan) using Near Infra-Red Spectroscopy (DA 7250 NIR analyzer, Perten Instruments) along with moisture content on percent dry basis. NIR spectra were collected using a black rotating cup measuring ~10.5 cm in diameter at room temperature. Each soybean seed set (500 seeds) was scanned for 6 seconds (15 spectra/sec) with a wavelength ranging between 950 and 1650 nm (optical resolution ~7 nm).

**Results**

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

The reproducibility of NIRS scanning was tested with two independent runs of the same set of 133 SAP accessions [oil (r=0.97), protein (r=0.98), and oleic acid (r=0.89)]. The key parameters were significantly (p<0.001) consistent, indicating the accuracy of the high throughput instrument in detecting quality compositions in soybeans (**Figure 1; for all figures see the attachment**). Further, we have examined the range of genetic variability in seed composition and their correlations using the mean of the two NIRS runs. The exotic accessions (n=133, excluding black and brown coated beans) showed a wide range of diversity in soybean seed composition (Figure 2A). Protein content in soybeans ranged from 37.8 to 46.4% (Figure 2A), oil content ranged from 18.1 to 23.6 % (Figure 2B), with a narrow range of moisture (8.8 to 9.6%; Figure 2C). Further, there were large genetic variability in fatty acids (oleic acid, linoleic acid and linolenic acid) and a few major amino acids (lysine, methionine and threonine) [Figure 2 D to I]. Oleic acid (% dry basis) varied from 16.6 to 29.1%, linoleic acid ranged from 47.3 to 57.7%, linolenic acid ranged from 5.5 to 9.9% on dry basis (Figure 2C-D). Similarly, we investigated some of the major soybeans amino acids such as lysine (2.51 to 2.96 %), methionine (0.51 to 0.61%) and threonine (1.44 to 1.7%), which displayed relatively narrow variation but sufficient quantitative distribution (Figure 2E-G). There was a strong negative correlation between protein and oil (r = -0.72, p< 0.001). All other relationships between specific fatty acids and amino acids are presented in Figure 2H. These relationships will be further strengthened.

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

Soybean entries grown in a wider range of climatic conditions were obtained from Kansas performance trials (from Prof. Schapaugh’s and Jane Lingenfelser team). The daily average maximum temperature (TMax; Figure 3A) during pod-filling stage (August to October) ranged from 27.1 (Cherokee) to 28.3 oC (Parsons). Further, large variation in pod yield (bushel/acre; Figure 3B), protein (Figure 3C), and oil (Figure 3D) was noticed, indicating the influence of growing environment across locations on yield and quality. Soybean grown in the Cherokee had the lowest pod yield (44.1 bushel/acre, average of 27 entries), but the protein level was highest (40.6%) compared to other locations (Figure 3A). While, soybean entries grown in the Ottawa regions recorded the highest pod yield (75.2 bushel/acre, averaged across 80 entries), while the protein level decreased by 2.1% compared to Cherokee. Soybeans collected from the Manhattan region contained 2.85% low protein compared to Cherokee. To understand the tradeoff and/or relationships between seed compositions and yield in soybean, correlation matrix is developed using all the entries grown across all the four locations (Figure 4E). A negative relationship between oil and both yield (r=-0.34, p<0.05) and protein (r = -0.42, p< 0.05) were noticed, suggesting that the soybean entries grown in these regions contained a higher level of oil by virtue of lower pod yield (bushels/acre). Meanwhile, the pod yield was significantly and positively correlated with amino acid such as tryptophan (r=0.67, p<0.001). As expected, protein showed significant positive correlations with most of the amino acids including lysine and methionine. Averaged daily maximum temperature showed a positive association with protein (R2=0.415) and a weak negative response with pod yield (R2=-0.875).

**On-going work:** The tradeoff between pod yield and soybean quality parameters among entries grown across four locations are being estimated. Further, based on the level of diversity (n=133) observed for the protein, oil and fatty acids, groups of contrasting samples will be chosen for the validation studies using gas chromatography mass spectrometry analysis. To capture the diversity in the brown and dark seeded beans (not been included in the above studies), we are in the process of grinding the beans, to improve our chances of estimating their composition.

**Knowledge dissemination:**

Raju BR, Shetty NJ, Lingenfelser JE, Schapaugh W, Jagadish SVK. 2017. Soybean yield and quality trade-offs. Soybean Breeders Workshop: Physiology & Agronomy, February 13-15, St. Louis, MO, USA (poster presented).

**Final Project Results**

Our preliminary results suggest considerable quality and yield tradeoffs. NIRS is a promising high-throughput platform that help in understanding the effect of microclimate on quality and also in selecting location specific soybeans. NIRS output does require more validation which is currently ongoing.

**Benefit to Soybean Farmers**

Enhance quality of soybeans grown to provide Kansas soybean growers an edge in domestic and international market and provide newer opportunities to enhance their revenue.



Figure 1: Reliability of the high-throughput platform (NIRS scanning) between scans (runs) on detecting soybean seed compositions (oil, protein and oleic acid) in a diverse collection (*n*=133) grown in the Manhattan during 2016.

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Figure 2: Histogram of seed composition traits variability and their relationships in soybean germplasm collection (*n*=133) grown in Manhattan during 2016.





Figure 3. Variations in maximum temperature (TMax, oC; A), yield (bushel/acre; B), protein (%; C), and oil (%; D), across Kansas soybean performance trials (*n*=215) and a correlation matrix between seed compositions and yield among soybean entries (E). \* P<0.05.