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10/30/2017

Nebraska Soybean Board
Year-End Research Findings Report

Please use this form to summarize the practical benefits of your research project and what has been accomplished. Your answers need to convey why the project is important and how the results impact soybean production.

Project Title: Integrating high throughput field phenomics into Nebraska soybean breeding

Contractor & Principal Investigator: Yufeng Ge

Please check/fill in appropriate box: ☐ ***Continuation research project***
☐ ***Year 2 of 3 research project (for example: Year 1 of 2)***

1. What was the focus of the research project or educational activity?

The focus of the research project is to develop high throughput field phenotyping system and to integrate the system into Nebraska soybean breeding program. The specific research aims of 2017 were to (1) Continue to collect field data using the existing field phenotyping platform from two soybean breeding yield trials in the 2017 growing season, and (2) develop effective pipelines for field phenotyping data processing and analysis. As can be seen in section 2, we successfully achieved these research aims.

2. What are the major findings of the research or impacts of the educational activity?

First, PI Ge's team (including Yufeng Ge, Postdoc Frank Bai, and graduate student Wenan Yuan) worked together with Co-PI Graef's team (including Dr. George Graef, graduate student Shawn Jenkins, and a few other students) to conduct the field data collection in the 2017 season.

Table 1 below summarizes the name of experiments, locations, dates and the number of plots scanned by the phenotyping cart in 2017. As can be seen, a total of 6760 plots were scanned. As in the last season, the sensor-based measurements from each plot included a RGB image, a VisNIR reflectance spectrum (400 to 900 nm), a canopy temperature reading, and a canopy height reading. One difference from the last reason was that the canopy height readings were measured from a LiDAR scanner sensor, which took many height measurements from the canopy and were supposed to be more accurate and representative than the ultrasonic sensor. In addition, three environmental variables: solar radiation, air temperature, and relative humidity were also measured at the time of each plot measurement.

Table 1. Summary of the field data collection activities with the high throughput field phenotyping system in 2017.

Experiments	Location	Early-season scan dates (V4-V5)	Late-season scan dates (R5)	Plot scanned (approximately)
Drought/yield study	Mead	Jun/19, Jun/20	Aug/11, Aug/12	2130
	Stevens Creek	Jul/1	Aug/14	2130
IDC study	Fremont	Jun/27 (V3)		1250
	North Bend	Jun/27 (V3)		1250

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Figure 1. Some field photos we took at Fremont and North Bend during our IDC experiments

In data analysis, we focused on the development of image processing algorithms to allow (1) remove weed pixels from soybean images; and (2) extract quantitative color information for soybean pixels that correlates with IDC visual rating. Figure 2 below summarized the new algorithms. As can be seen, inter-row weed became a significant problem in image processing and caused bias in the estimation of soybean green pixels (3 in Fig. 2). Therefore we developed a new algorithm that first realized crop line detection (4a in Fig. 2) and then weed exclusion (4b in Fig. 2). This new algorithm worked effectively for the soybean plot images in 2017. After that, a series of pixel color parameters were derived. They are PPC (plant pixel count), GC (Green-Cyan), G (Green), YG (Yellow-Green), Y (Yellow) and OY (Orange-Yellow).

It can be seen in Fig. 3 that among the six parameters extracted, PPC, G and Y exhibited clear correlation with the IDC visual rating. PPC and G showed strong negative associations with IDC ratings, whereas Y had a strong positive association.

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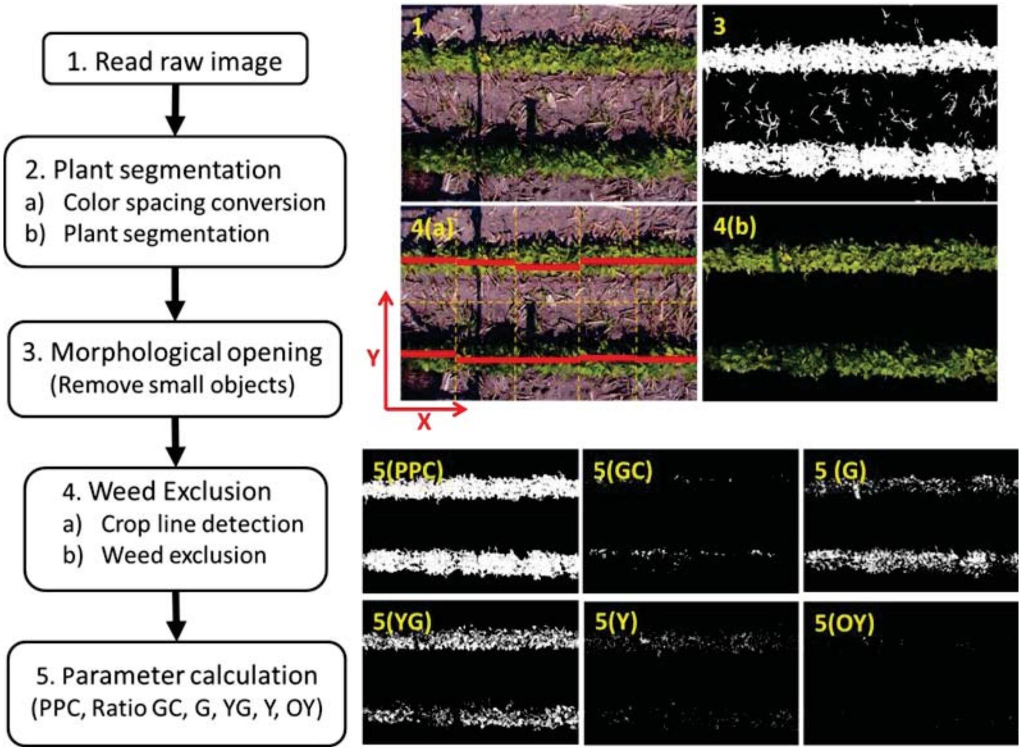

Figure 2. Summary of the new image processing algorithm developed for soybean plot images.

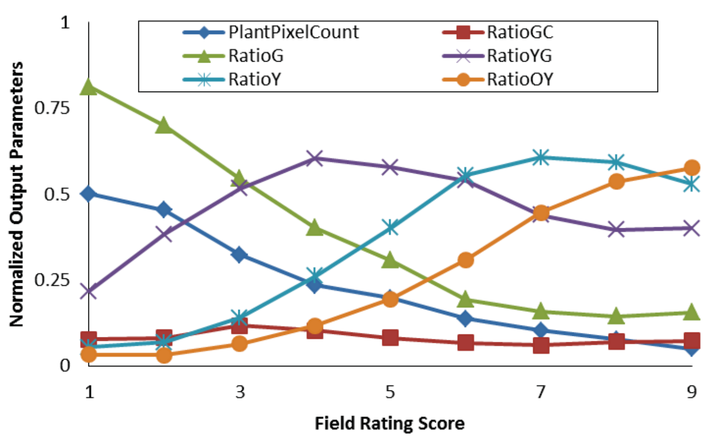

Figure 3. The relationship between the six color parameters extracted from the soybean plot images and their IDC visual rating in the field.

We further conducted an analysis to test how useful these six color parameters from the field phenotyping system can be used for automated IDC rating. To do this, we selected ~1500 two-row IDC plots from 2016 and 2017 seasons (each of them also has a visual rating). We fed all these images to our new algorithm and extracted these six color parameters for each plot. We then split the 1500 plots equally into two groups. The first group (or the training set) was used to develop a Linear Discriminant

Analysis (LDA) classifier. The LDA classifier was then applied to the second group (or the validation set); and the predicted IDC score was compared with the actual field rating. The result of this analysis was given in Figure 4 below.

If the classifier worked perfectly, then all the numbers would be diagonally (cells coded red) and everywhere else will be 0 (meaning no misclassification). As can be seen here, we have large numbers in those red cells diagonally, meaning the LDA classifier works good. If we further consider the cases that misclassification happen only by one category (yellow cells), then the LDA classifier works very good. The overall accuracy is 85%.

		Field Visual Rating								
		1	2	3	4	5	6	7	8	9
Machine Rating	1	283	41	26	12	3	1	0	1	0
	2	3	5	4	1	0	0	1	0	0
	3	5	15	23	9	11	2	0	0	0
	4	0	5	23	28	21	7	2	0	0
	5	0	1	1	6	5	10	3	0	1
	6	0	0	1	3	16	32	12	3	0
	7	1	0	1	3	3	19	26	16	2
	8	0	0	0	1	2	10	18	21	12
	9	0	0	0	0	0	0	2	0	2
	PCA	0.98	0.91	0.63	0.68	0.69	0.75	0.88	0.9	0.82

Figure 4. The comparison of automated IDC rating from soybean images and field visual rating.

Analysis of the phenotyping data from the water stress experiment in both 2016 and 2017 seasons are ongoing now.

In addition to the above activities, the team also worked on UNL's new Spidercam field phenotyping system (Fig. 5). Half of the field under the Spidercam were planted soybean by Dr. George Graef in 2017; and we worked to collect field phenotyping data of soybean from Spidercam.

Figure 5. The Spidercam field phenotyping facility at UNL's Eastern Nebraska Research Extension Center.

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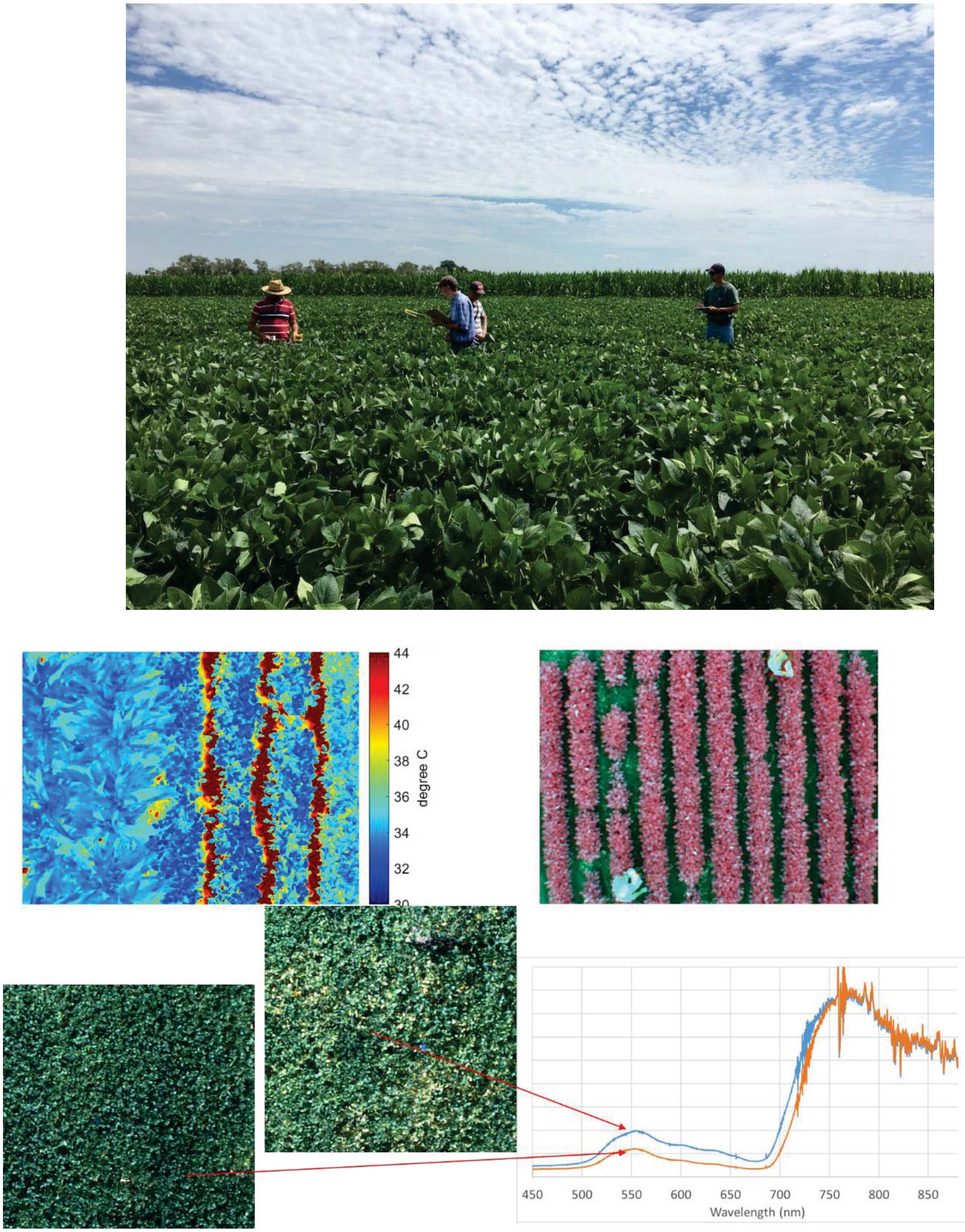

Figure 6. Picture showing the team working in the Spidercam field (soybean part) for data collection; and example soybean canopy images (thermal infrared and color-infrared) and spectral reflectance from the Spidercam instruments.

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3. Briefly summarize, in lay terms, the impact your findings have had, or will have, on improving the productivity of soybeans in Nebraska and the U.S.

Breeding programs are now relying more and more on genomic data to make selection decisions to improve genetic gain and potentially shorten breeding cycles. However, the potential of genomic data cannot be fully realized until meaningful plant phenotypic data can be generated, analyzed, and used. It is believed that integrating high-throughput phenomics will enable better utilization of both phenotype and genotype information and greatly improve the breeding efficiency, whether the goals are for yield enhancement, quality improvement, water/nitrogen use efficiency, or resistance to specific pests or diseases in targeted Nebraska environments. Therefore, this project will benefit all Nebraska soybean growers, by establishing more efficient soybean breeding programs to deliver superior varieties that meet growers' specific needs, or through shortening the time needed for cultivar development. In the long run, this research would enhance growers' income and their overall competitiveness in the national and international markets.

4. Describe how your findings have been (or soon will be) distributed to (a) farmers and (b) public researchers. List specific publications, websites, press releases. etc.

Peer-reviewed journal publication:

Pandey, P., Ge, Y., Stoerger, V., Schnable, J.C., 2017. High throughput in vivo analysis of plant leaf chemical properties using hyperspectral imaging. Frontiers in Plant Science 8. DOI: 10.3389/fpls.2017.01348.

Presentations:

Bai, G., Ge, Y., Jenkins, S., Graef, G., 2017. Field phenotyping for soybean iron deficiency chlorosis. ASABE Annual International Meeting, July 16-19, 2017, Spokane, Washington, USA.

Yufeng Ge. Advanced imaging for phenotyping water related crop traits. Presented at 2017 Water for Food Global Conference. April-10-12. Lincoln, NE

Yufeng Ge. High throughput plant phenotyping in greenhouse and field – Translational pipelines from gene discovery to crop improvement. R.F. Baker Plant Breeding Symposium. Mar-3-2017. Iowa State University, Ames, Iowa. (Invited talk, audience are mainly graduate students and industry people in plant breeding).

Yufeng Ge. High throughput plant phenotyping research at University of Nebraska-Lincoln. Nebraska Agricultural Technologies Association. Feb-2-2017. Lincoln, Nebraska. (Also Nebraska Extension Workshop on Agricultural Technologies).

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Website report – the field phenotyping cart we built attracted interest from a number of sensor companies and they reported our system on their website:

https://www.apogeeinstruments.com/high-throughput-field-phenotyping-multi-sensor-system/

https://senix.com/senix-toughsonic-sensors-help-farmers-reduce-risk-and-increase-profits/

News media report about our activity on the Spidercam field phenotyping facility:

http://journalstar.com/news/local/education/dangling-above-the-action-spidercam-covers-corn-not-cornhuskers/article_467b4586-e9dc-5808-942b-046175a04833.html (by Lincoln Journal Star)

http://www.nebraskafarmer.com/technology/field-phenotyping-center-speed-breeding-research (by Nebraska Farmer)

5. Did the NE soybean checkoff funding support for your project leverage any additional state or Federal funding support? (Please list sources and dollars approved.)

The NE soybean checkoff funding support allowed us to generate preliminary data and important concepts and design/development of the field phenotyping platform that lead to the following additional federal funding:

(1) Yufeng Ge, James Schnable, Santosh Pitla. PAPM Eager: Transitioning to the next generation plant phenotyping robots. 11/15/2016-11/14/2018. USDA-NIFA-AFRI (Joint NSF-USDA PAPM EAGER program). $285,000.
(2) Yeyin Shi, Yufeng Ge, P. Stephen Baenziger, Gary Hein, V. Belamkar, Reka Howard, Juan Diego Hernandez Jarquin. Decision-support tools based on aerial and ground sensing systems for Nebraska small grains research and production: A transdisciplinary study. 7/1/2017-6/30/2020. UNL-ARD Small Grain Foundation program. $222,818.

There is also one pending grant:

(1) Yufeng Ge, John Hay, Sibel Irmak, Jenny Keshwani, James Schnable, Jinliang Yang. Integrating advanced genomics and phenomics to improve energy sorghum for Nebraska and beyond. USDA-NIFA. $998,775.

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