Iowa State University
Iowa Soybean Research Center
Project Final Report

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ROOT AND MICROBIOME TRAITS TO TAILOR THE NEXT-GEN SOYBEAN CULTIVARS

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The Iowa Soybean Research Center (ISRC) works to leverage university and soybean checkoff funds with industry support to foster innovative soybean research at Iowa State University.

The center solicits research ideas from farmer advisors and from industry representatives. Also, ISU researchers propose cutting-edge soybean research topics. And "Think Tank" events are organized by the center to facilitate farmers, industry representatives, and ISU researchers to brainstorm together about research needs.

The project described in this report was identified as a novel research need at an ISRC Think Tank event held in March 2016.

The center provided financial support to Drs. Beattie and Singh to work together on this topic. The researchers received $400,000 from 2016 to 2019. The money was a blend of soybean checkoff funds from the Iowa Soybean Association and industry funds from Bayer, Cornelius Seeds, and Monsanto.

Following is a nontechnical summary of the work conducted in the project.

For more information on the Iowa Soybean Research Center, go to iowasoybeancenter.org.

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FINAL REPORT
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Outputs:

- Root system architecture (RSA) traits are of interest for breeding selection; however, measuring these highly variable traits is difficult and resource intensive. **We developed in-house a mobile, low-cost, high-resolution, root phenotyping system**. This system, composed of an imaging platform with computer vision and machine learning-based segmentation approach, provides a seamless end-to-end pipeline - from obtaining large quantities of root samples through image- based trait processing and analysis. This high-throughput phenotyping system can handle hundreds to thousands of plant root phenotyping samples. We used it to characterize the RSA traits (e.g., root shape, length, number, mass, and angle) of genetically diverse soybean accessions from a wide geographical distribution.
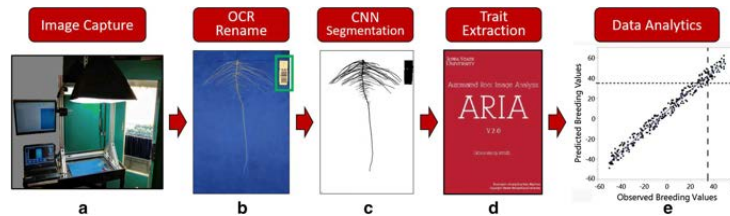
Description: This system integrates time series image capture coupled with automated image processing that uses optical character recognition (OCR) to identify seedlings via barcode, followed by a robust segmentation integrating a convolutional auto-encoder (CAE) method prior to feature extraction. The pipeline includes an updated and customized version of the Automatic Root Imaging Analysis (ARIA) root phenotyping software.

This cost effective, non-destructive methodology delivers biologically relevant time-series data on root growth and development for phenomics, genomics, and plant breeding applications. By quantifying root traits and ranking genotypes in a common environment, it enables the use of RSA traits for developing more robust and resilient crop cultivars. (Falk KG, T Jubery, SV Mirnezami, KA Parmley, S Sarkar, A Singh, B Ganapathysubramanian, AK Singh. 2019. Computer vision and machine learning enabled soybean root phenotyping pipeline. *BMC Plant Methods*, 16:5)

- We used this end-to-end phenotyping pipeline to examine the RSA traits in a large genotype set. We deciphered the genetic diversity of this set by combining 35,448 single nucleotide polymorphisms (SNPs) with an imaging phenotyping platform (292 accessions, replications = 14). We developed informative root (iRoot) categories using literature-based root morphology traits. These profiles indicated that **most genotypes of U.S. origin lack genetic diversity for RSA traits, and useful genetic variation for these traits could be infused from diverse accessions**. Shape-based clusters were created by integrating convolution neural net and Fourier transformation methods, enabling trait cataloging for breeding and research applications. Our results showed that RSA traits displayed genetic variability for root shape, length, number, mass, and angle, giving new tools for plant breeders to build the next generation of soybean varieties based on both above- and belowground traits. The combination of genetic and phenotypic analyses, machine learning, and mathematical models provides opportunities for breeding targeted root traits to maximize the beneficial diversity for future genetic gains. (Falk et al. 2020. *Science Plant Phenomics*, Accepted pending revisions).

- We are creating **a software solution to identify, count and study root nodules**. This work leveraged phenotyping and machine learning-based algorithm development that can accurately perform nodulation quantification. In a parallel study, we studied six soybean genotypes to connect RSA trait diversity with temporal and spatial nodulation patterns. Two manuscripts will be submitted in 2020. (Clayton Carley, PhD dissertation)

- Plant health is intrinsically tied to the activity of root-associated microbes; however, our understanding of the composition, dynamics and functions of these microbes is still at an early stage. To help fill this knowledge gap, we generated high-resolution spatial and temporal maps of the microbiomes on soybean roots. **These maps are the most comprehensive and the highest resolution maps of root microbiomes generated for any plant species to-date.** We generated these maps using two cultivars that represent extremes in RSA based on Dr. Singh's phenotyping analysis. We developed a novel, highly detailed sampling strategy across the root network to capture microbiome assembly. Our methods surgically targeted 0.5- and 1-cm tissue segments representing distinct root system zones, including the tips, subterminal tips, and basal regions of tap roots, lateral roots and tertiary roots. We generated these maps using molecular fingerprints for 308 root tissue samples and 339 rhizosphere samples collected from plants grown in a natural, sandy agricultural soil under controlled conditions in the laboratory, with samples collected from seedling to pod set. In addition, we collected 104 root tissue samples and 120 rhizosphere samples from plants grown in the same agricultural soil under field conditions; these samples were employed as a ground truth.

  Our data indicated clear, consistent temporal and spatial shifts in the root microbiomes. These shifts were associated with soybean developmental phases and also with distinct root locations. Moreover, they were consistent across the two cultivars, despite distinct RSAs, and in the laboratory and the field, indicating that the observed successional patterns are robust and reproducible. **These reproducible successional shifts highlight targets for future investigations into functional shifts in root microbiomes during soybean growth.** (Manuscripts will be submitted for publication in 2020)

  We also discovered several novel organisms present in unexpectedly high abundance - these merit further investigation into their potential roles in plant fitness:

  - Bacteria of a single, poorly characterized genus, *Massilia,* dominated the communities in the root tips of young plants (through V1 growth phase); they comprised up to 87% of the communities. Few members of this genus have been cultured or studied. This discovery was possible because of our comprehensive surgical sampling and highlights the importance of examining if this genus, as a clear hallmark of root tip communities, influences root function.

  - Another novel group emerged in significant numbers in the root tips between the V1 and V3 stages and later in the basal root tissues. Members of this group (Phylum Patescibacteria), which have not been cultured or studied, have small genomes suggesting dependence on a host.

  - A third novel bacterial group constituted a significant portion (up to 14%) of the communities on the tertiary roots, which made up much of the root mass after the V3 stage. This bacterial group is sufficiently new as to evade identification even at the broadest level. The predominance of this group on this large root mass spotlights interest in a potential functional role.

- We generated **a collection of over 450 bacterial isolates from soybean roots** grown in the field soil used for microbiome mapping (above) with methods designed to capture diverse microbes. Molecular classification confirms that the collection includes diverse bacteria (>30 genera). Ongoing molecular and phenotypic characterization efforts are identifying isolates with traits associated with plant growth promotion, such as iron scavenging. This collection serves as a source of isolates for future functional tests probing bacterial impacts on soybean growth and health, with selection of the isolates based on their abundance in the spatial and temporal microbiome maps generated above.

Leveraging of ISRC funding:
- *ISRC funded activities increased competitiveness for:* Grant from USDA NIFA: Agricultural Microbiomes in Plant Systems Program. PIs: Beattie, Nettleton and Nikolau. Title: Mechanistic drivers shaping root microbiomes and Microbiome drivers of fitness benefits in drought-stressed plants. $749,590 for 1/1/2019 – 12/31/2021.

- *ISRC funded activities increased competitiveness for:* Plant Science Initiative Fellowship Award to AK Singh for $225,000 for 2018-2020.

- *ISRC funded activities were synergistic with:* Cooperative Research Project between ISU and the National Institute of Crop Science of the Rural Development Administration of the Republic of Korea. PIs: Beattie and Kang. Title: Exploring the role of the microbiome in microbial-mediated pathogen reduction on soybean cultivated in paddy fields. $150,000 for 03/01/2017 – 12/31/2019.

- *ISRC funded projected increased competitiveness for, and benefitted from, work performed by undergraduate:* NASA Iowa Space Grant Consortium, PI: Dankle. Maximizing plant growth in a drought stressed environment by exploiting the root microbiome. $7,000 for 8/1/2019-6/21/2020.

Additional proposals were submitted during the period of the award to the United Soybean Board and the Foundation for Food and Agriculture Research, but were not funded.