Creating a New Paradigm for Potato Breeding Based on True Seed
USDA NIFA SCRI Award No. 2019-51181-30021 (9/1/19 – 8/31/23)

Year 1 Technical Report for Advisory Board Meeting on July 21, 2020

Objective 1. Determine the genetic basis and environmental stability of self-fertility in potato.

Progress: The self-compatible (SC) diploid *Solanum chacoense* line M6 has been widely used to introduce SC into diploid potato. The self-incompatibility inhibitor gene *Sli* on chromosome 12 is believed to provide SC in M6, although the mechanism of *Sli* remains to be determined. We are currently testing the effectiveness of DNA markers linked to *Sli* in Dutch germplasm for their ability to predict SC in M6 and North American germplasm. We have found that *Sli* in M6 does not completely inactivate the self incompatibly reaction and that robust SC in diploid potato is complex, influenced by the environment, plant physiology, and multiple genetic factors. We are using a combination of genetic mapping and genome editing to investigate additional genetic factors involved in SC. Diploid SC lines have been created at MSU by knocking out S-RNase and other candidate genes involved in the SC interaction. Using these lines and SC lines derived from crosses with M6, we have created 13 populations in which to map genes associated with SC and self-fertility traits. Screening of SC and self-fertility in a subset of each population is underway and will identify the most informative population(s) to pursue.

Challenges: Phenotyping segregating populations for self-compatibility is not straightforward, as many factors influence whether seeds are produced when plants are self-pollinated. In addition to examining pollen viability to avoid the confounding effects of male sterility, we are quantifying fruit and seed set as well as pollen tube growth in the style to accurately identify all the genetic components of SC.

Plans for Year 2: The selected population(s) will be genotyped using genotyping-by-sequencing (GBS), phenotyped for SC in replicated greenhouse trials at MSU and UW, and additional regions associated with SC in these populations will be identified. After verifying the function of the candidate genes in these regions, we will develop DNA markers for use in potato breeding programs.

Objective 2. Generate diploids that capture the genetic diversity of elite tetraploid potato for the chip processing, russet and red markets.

Progress: Dihaploid extraction has thus far focused on 20 chip, 15 russet, and 10 red cultivars and elite clones. Ploidy of putative dihaploids has been confirmed in various ways, including chloroplasts per guard cell, genotyping, flow cytometry, and seed production following crosses to diploids. Confirmed dihaploids have been evaluated in the greenhouse for flower production and fertility, and some have been grown in the field to evaluate agronomic performance. We are using a two-tier strategy for genomic characterization of dihaploids, with a budgeted goal of 100 dihaploids in Tier 1 and 20 in Tier 2 by the end of the project. In Tier 1, all clones that meet a set of criteria (unique, confirmed diploid, produces tubers and seeds) are sequenced using a short-read Illumina platform and aligned to the DM reference genome. Illumina libraries are currently being prepared for our first 11 dihaploids, derived from Kalkaska, Missaukee, MSR127-2, Lelah, W14NYQ9-2, W9968-5, W14NYQ29-5, NY121, Caribou Russet, Modoc, and Red Norland.

Tier 2 sequencing involves de-novo genome assembly using long reads from the Oxford Nanopore Technologies (ONT) platform plus error correction with Illumina short reads. Clones are selected for Tier 2 based on the uniqueness of the haplotypes identified in Tier 1 and confirmation of eudiploidy. We have already prepared high molecular weight DNA from four, well-characterized dihaploids for Tier 2 submission after their Tier 1 sequencing is complete.

Challenges: Large numbers of pollinations are required to create a handful of putative dihaploids. Low proportions of spotless (putative dihaploid) seeds, low seed germination, poor seedling vigor, no flower or tuber production, and low female fertility all constrain efforts to generate dihaploids useful for breeding.

Plans for Year 2: We will continue to extract new dihaploids, with the goal of 20 more submitted for Tier 1 sequencing by the midpoint of Year 2. More emphasis will be placed on identifying dihaploids with major genes for disease resistance that can be fixed in a homozygous condition via molecular markers in Objective 3. Tier 1 sequence data will be used to select individuals for Tier 2 sequencing and to inform the selection of new tetraploid parents for which dihaploid extraction is predicted to yield new haplotypes. Finally, we will compare dihaploid sequences to GBS data from breeding programs to determine how rapidly diversity is decreasing in our populations and to identify deleterious haplotypes based on selection mapping techniques.

Objective 3. Develop improved inbreds through recurrent selection on tuber traits and true seed production.

Progress: Although the new dihaploids developed in Objective 2 are just starting to be used for breeding, some populations were already available at the start of the project from prior research. At UW, 300 F_1 diploid clones are being evaluated in the field as 8-hill plots planted from seed tubers. Canopy development is being monitored with biweekly flights of an unmanned aerial vehicle (UAV) carrying a multi-spectral camera. At MSU, 180 diploid clones are being evaluated in the field as 10-hill plots based on transplants. These populations are segregating for the key traits we intend to fix during the project, including resistance to PVY, late blight, and golden nematode. Leaf tissue has been collected and submitted for genotyping-by-sequencing (GBS) to generate genome-wide markers. Hundreds of clones have been self-pollinated in the greenhouse to enable selection on both self-fertility and tuber traits.

Challenges: Significant inbreeding depression and inconsistent transmission of self-fertility to offspring currently limit the number of inbred pollen donors available for breeding. For example, at UW only one F2 clone (pedigree = LelahDH12/((US.W4/chc39.7)-36)-22-45) has thus far emerged as a reliable male parent with good tuber traits.

Plans for Year 2: Field trials will be harvested in October 2020 and evaluated for agronomic (yield, tuber shape and size) and processing (specific gravity, fry color) traits. The GBS marker and phenotype data will be combined to make breeding value predictions for selection. Breeding germplasm will be genotyped with a new marker for the *Sli* self-compatibility gene, and the results will be used to guide inbreeding efforts in the greenhouse. At UW, an 8-hill field trial with 500 F_1 clones planted from seed tubers is planned for 2021. At MSU, a 10-hill field trial with 300 new F1 clones is planned for 2021.

Objective 4. Create agronomic and economic insights to guide the introduction of true seed into the commercial seed system.

Progress: In WI, 48 diploid seedling families have been planted in the field and are being scored for plant development and uniformity. The same families are being grown in a greenhouse to produce tubers for a field trial in 2021 to compare plant development in genetically similar material that starts from seed tubers versus transplants. Conversations with economists have been started to develop a list of questions to understand the current potato industry and potential changes with true potato seed (TPS).

Challenges: We do not yet have uniform diploid hybrid TPS for studies of seed production systems.

Plans for Year 2: The field trial described above will be carried out. Quasi-structured interviews will be carried out with growers and other industry representatives. A White Paper will be prepared describing the US seed potato industry structure and thoughts on how it might change with advent of TPS.

Outreach

Progress: We have developed a project website to share information about project goals and progress. Several trade journal articles about diploid breeding have been published and are available through the website. At the 2020 Potato Expo, we had a display about the project within the Potatoes USA booth and a poster presentation at the PAA-sponsored session. Shelley Jansky and Paul Bethke gave a presentation entitled "Are Hybrid Potatoes in Your Future?" on the Innovation Stage at the Expo. We have been working with Potatoes USA to communicate our goals to the potato industry. An interview discussing diploid potatoes was created and shared electronically with 1198 growers and industry reps. We designed a logo to improve visibility for the project and visual consistency during presentations by team members:

Challenges: The COVID-19 pandemic has limited opportunities for face-to-face interactions with members of the potato industry.

Plans for Year 2: A Q&A podcast is being produced about the project, and we will participate in several events at the 2021 Potato Expo.