Creating a New Paradigm for Potato Breeding Based on True Seed
USDA NIFA SCRI Award No. 2019-51181-30021

Year 3 Progress Report (Sept. 1, 2021 – Aug. 31, 2022)

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

Self-fertility is critical to developing inbred lines, but gametophytic self-incompatibility (SI) has historically been a barrier to achieving this goal in diploid potato. Natural or engineered variation at three genes has previously been shown to weaken or eliminate SI in potato: S-RNase, HT-B, and Sli. During Year 3, steps were taken to create a mapping population in which all three genes are segregating, for the purpose of comparing their effects and possible interactions in the same genetic background. Of the 40 F1 candidates, 19 inherited Sli and set fruit upon selfing in a greenhouse experiment. These 19 are being screened for the presence of the S-RNAse and HT-B knockouts, and F2 seeds from individuals with all three genes can then be used for the mapping study. The second major accomplishment in Year 3 was a screening study of wild potato germplasm to identify other potential sources of self-compatibility. Self-compatible accessions were identified in four wild species: *S. pinnaseticum*, *S. polyadenium*, *S. kurtzianum*, and *S. verrucosum*.

Plans for Year 4: The F2 population segregating for S-RNase, HT-B, and Sli will be evaluated for self-fertility to compare the effects of these three genes and possible interactions. Mapping populations derived from the self-compatible wild species will be evaluated to potentially discover new loci affecting fertility traits.

Objective 2. Generate and sequence dihaploids to capture the genetic diversity of North American germplasm.

The development and genomic characterization of dihaploids (diploid haploids) from elite tetraploid germplasm creates a foundation for efficient diploid breeding. By the end of Year 3, we have completed 20X whole-genome sequencing of 91 dihaploids, only 9 short of the original goal of 100. Confirming earlier studies, we have found extreme genetic diversity in cultivated potato, with approximately 1% heterozygous nucleotide frequency in each dihaploid and more than 10 alleles per gene in the population, on average. For 20 dihaploids selected for de novo, haplotype-resolved genome assembly, we have completed HiC and PacBio HiFi sequencing, as well as Oxford Nanopore sequencing of cDNA transcripts from leaf and tuber tissue. The first five assemblies (comprising 10 haplotypes) have excellent statistics, with complete BUSCO scores of 96.6–98.6% and N50 values of 60.5–67.1 Mb.

Plans for Year 4: Sequencing will be completed for all 100 dihaploids, as well as haplotype-resolved and annotated assemblies for the 20 selected dihaploids. A practical haplotype graph will be generated from all available sequencing data and evaluated for its ability to impute haplotypes from skim sequencing.

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

Dihaploids from Objective 2 have been used as founders for recurrent selection, to improve tuber and fertility traits. Two main approaches are being used: 1. outbred recurrent selection, and 2. the development of partially inbred lines. Using similar field breeding techniques as a conventional tetraploid program, approach #1 is yielding dramatic results. In 2021 field trials at Michigan State University, many diploids outyielded the tetraploid checks in small plot (10-20 plant) trials. For approach #2, we have developed multiple lines with 40-50% homozygosity, including at key loci for maturity (CDF1.3 allele) and self-compatibility (Sli). A number of dihaploids have been identified with valuable disease resistance genes: 10 fertile dihaploids contain the extreme resistance gene Ryadg for potato virus Y (PVY), and three different R genes for late blight are distributed across 5 female-fertile dihaploids. Breeding populations have been enriched for these R genes using genetic markers.

Plans for Year 4: Diploid field trials will be conducted at four locations in 2023 (Maine, Michigan, Minnesota, Wisconsin), and genomic selection models will be created from SNP array data for traits such as yield and specific gravity. Multi-parental genetic mapping techniques will be used to characterize the effects of founder haplotypes for tuber shape and skin color.

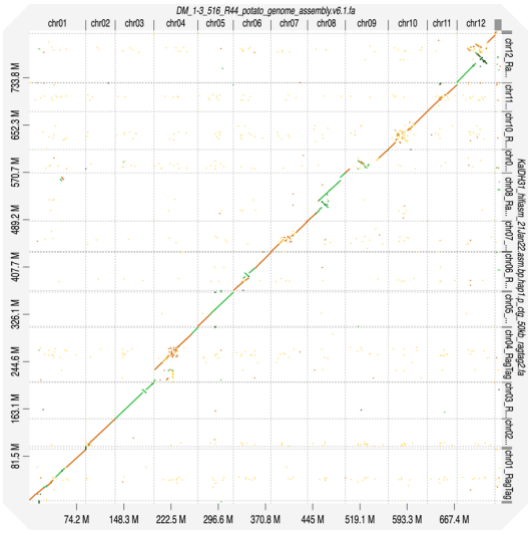
Objective 4. Conduct agronomic and economic studies about the introduction of true seed into the commercial seed system.

New economic analysis has led to more specific predictions about how true potato seed (TPS) hybrid varieties may affect the seed supply chain for potato production. At present, the “seeds” for the first field year (FY1) of potato production are greenhouse-grown minitubers, for which a typical cost is $0.55/minituber. The availability of TPS would enable the use of seedling transplants instead of greenhouse minitubers as the planting stock for FY1. Seedling transplants are widely used in processing tomato production, and one published cost from UC Davis Extension is $0.20/seedling. Around half of this cost is the price of seed, and the other half is the cost of producing the transplant in a greenhouse. If potato yields and production costs from a transplant-based production system are similar to the use of minitubers, the cost of FY1 seed would be reduced by over 50% under these assumptions. Based on a replicated field trial (plot size 20 plants) in 2021 with three diploid F1 families, yields were indeed similar for seedling transplant vs. greenhouse minituber planting stock. In addition, different transplant methods (e.g., plugs vs. bare root) were evaluated.

Plans for Year 4: A trial demonstrating the power of TPS for rapid seed multiplication will be completed. Starting with 8 plants each for 2 partially inbred lines, 12,000 seedlings were transplanted to fill a one-acre field in 2022. Yield, tuber number, and size distribution will be measured at harvest.

Year 3 in Pictures

Objective 1. Evaluating wild species for self-compatibility.



Objective 2. Haplotype 1 of the haplotype-resolved assembly Kalkaska-DH31 vs. the reference genome, DM.

Objective 3. F_3 line with 45% homozygosity, including early maturity and Sli.

Objective 4. Mechanical transplanting of F_1 hybrid seedlings.