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

Year 2 Progress Report (Sept. 1, 2020 – Aug. 31, 2021)

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

Self-fertility is critical to developing inbred lines, and one of the requirements for self-fertility is inactivation of the gametophytic self-incompatibility (SI) system present in many potato varieties and genetic resources. It had been hypothesized that a gene on chromosome 12, known as *Sli*, can inactivate SI in potato and confer self-compatibility (SC). This hypothesis was confirmed by Clot *et al.* (2020) in diploid breeding populations from the Netherlands, and although the molecular identity of *Sli* was not determined, they published a set of KASP DNA markers linked to the gene.

During Year 2, we tested a subset of these KASP markers and confirmed their utility for breeding in North American germplasm. Michigan State University recently completed the fourth cycle of phenotypic recurrent selection for self-fertility, maturity, and tuber quality, and clones from each cycle had been maintained to enable a retrospective analysis (Kaiser et al. 2021). The results indicate a steady increase in the frequency of clones homozygous for the marker allele linked to *Sli*, and all clones with this genotype were SC. In contrast, having only one copy of *Sli* (i.e., the heterozygous genotype) was not always associated with SC, and similar observations of incomplete dominance were made in breeding populations at the University of Wisconsin.

Plans for Year 3: Besides the naturally occurring allele at *Sli*, alleles for SC have been created with gene editing techniques at the genes for S-RNase and HT-B, which are stylar proteins involved in SI. Our focus for Year 3 will be to create and characterize an F2 population segregating at all 3 loci, to study the relative influence and interaction of these genes on self-fertility.

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

Diploid individuals called dihaploids are being created by haploid induction, which involves pollinating tetraploid varieties and breeding lines with certain Group Phureja clones. Research indicates the haploid inducer chromosomes are eliminated in the early stages of embryo development, so only the maternal genome is present in the offspring.

All of the breeding programs participating in this project are creating dihaploids from their germplasm and nominating the best individuals for genome sequencing, which occurs in two stages or tiers. Tier 1 sequencing occurs at 20X coverage using Illumina short reads, with a goal of at least 100 dihaploids before the end of the project. We are nearly halfway to that goal, having sequenced 21 chip, 18 russet, 7 red, and 2 specialty clones. The goal of Tier 2 sequencing is to create a *de novo* haplotype-resolved assembly by combining 100X Illumina short reads with long reads (either ONT or PacBio HiFi) and proximity-by-ligation (HiC) libraries. Both leaf and tuber

transcriptomes are also being sequenced in Tier 2. We have planned for 20 dihaploids in Tier 2 and made progress on 8 thus far, including completed assemblies for 2 dihaploids.

Plans for Year 3: An additional 26 dihaploids will be sequenced in Tier 1 in the coming year, as well as 12 more dihaploids in Tier 2. Tier 1 sequencing data will be analyzed to assess genetic diversity, identify introgressions, and predict deleterious alleles. The first 8 genomes from Tier 2 will be completed.

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

Inbred lines are needed to generate hybrid varieties with homogeneous true potato seed (TPS), but vigorous inbreds are difficult to create from *S. tuberosum* dihaploids because of the large number of deleterious alleles in the genome. We are making slow but steady progress toward this goal by selfing or sib-mating for several generations, followed by intermating with other inbreds or "backcrossing" to other dihaploids, to begin the next breeding cycle. Haplotype analysis is being used to track identical-by-descent segments during inbreeding at key loci. For example, a number of F2 individuals with early maturity have been created that are homozygous at the *CDF1* locus, and we are in the process of sequencing *CDF1* amplicons to identify which alleles are present.

Field trials of outbred F1 generations are being conducted in Wisconsin and Michigan to inform selection decisions, and the entries in the 2020 (N = 480) and 2021 (N = 432) trials were genotyped with the potato SNP array to enable genetic mapping and genomic prediction. Genome-wide association analysis of the data from uncrewed aerial surveys (UAS) of the 2020 field trial (i.e., using NDVI or other vegetation indices) identified a QTL on chromosome 2 for early vigor at 50 days after planting (DAP), which may be related to tuber dormancy. We also observed the effect of *CDF1* during vine senescence at 100 DAP. Tuber total glycoalkaloid (TGA) levels (which ranged from 1 to 60 mg/g DW for the top 15 clones selected based on agronomic performance in Wisconsin in 2020) are being used for selection to eliminate unfavorable alleles inherited from the *S. chacoense* founders of our diploid breeding program.

Plans for Year 3: Dihaploids with the Ry_{adg} gene for PVY resistance have been created, and we expect to create diploids homozygous for this gene in Year 3. Genetic mapping and genomic prediction studies of the 2021 field trial will be performed, and a coordinated field trial between the MSU and UW breeding programs will be planted in 2022.

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

TPS will enable a new type of production system in potato, based on seedling transplants. A field trial was planted in Wisconsin in 2021 to study the impact of seed source (seedling transplant vs. greenhouse minituber vs. field tuber) on plant development, tuber number, and yield. Three diploid F1 populations are being used for this experiment, with two replicates per population per seed source and a plot size of 20 hills. Demonstration experiments were also planted to compare transplanting by hand vs. machine and the use of plugs vs. bare root.

The prospect of diploid TPS varieties has already stimulated significant new investment in the potato breeding sector, and major changes are likely to occur in the future. To inform economic models of how the breeding and seed industries may reorganize, we have conducted interviews with breeders, minituber producers, and seed growers across the US. A conceptual framework for evaluating the distribution of social benefits among stakeholders is under development.

Plans for Year 3: The 2021 seed source experiment will be completed and repeated in 2022. The economics of field production based on seedling transplants will be investigated. Economic analysis of the organization of the breeding and seed sectors will continue and expand to include international markets.

Outreach

Project activities and goals have been communicated to stakeholders and the general public in several ways. Twitter and YouTube accounts (SCRI2xPotato) were created to promote the project, and new content was added to the website created in Year 1. A video about dihaploid extraction was produced for our YouTube channel, and the project was also featured in a research update video produced by Potatoes USA.

Multiple presentations about this project and diploid breeding more generally were made to a national audience at the January 2021 Potato Expo. PI Bethke spoke as part of a live-streamed panel discussion, and poster presentations were made by PDs Endelman and Douches. Outreach continued with presentations at the state and regional meetings, including Wisconsin, Michigan, Minnesota, Pacific Northwest, and Northeast. Members of our project team both organized and presented at the symposium "Diploid Potatoes as a Catalyst for Change in the Potato Industry", which occurred at the July 2021 Potato Association of America meeting.

2020 harvest, diploid variety trial