



## **Creating a New Paradigm for Potato Breeding Based on True Seed**

USDA NIFA SCRI Award No. 2019-51181-30021

**Year 4 Progress Report** (Sept. 1, 2022 – Aug. 31, 2023)

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

Progress toward understanding and exploiting the genetics of self-fertility has been made on several fronts. Until now, inbreeding efforts have relied on the *Sli* gene to overcome the gametophytic self-incompatibility that naturally exists in cultivated potato, but the effectiveness of this approach varies with genetic background. The wild species *S. verrucosum* does not exhibit gametophytic self-incompatibility, and an interspecific mapping population with cultivated potato was characterized in a greenhouse experiment in Year 4 to better understand the genetics of this trait; analysis of the results is underway. Another accomplishment was to complete a fine mapping experiment (N=376) for a gene involved in cytoplasmic male sterility, which manifests as shrunk or missing anthers. This trait could be exploited to facilitate the production of hybrid seed and male sterility in hybrids, which may enhance tuber yields by eliminating true seeds as competitive “sinks” for nutrients.

**Plans for Year 5:** Phenotyping and genetic analysis of the F2 mapping populations segregating for self-compatibility will be completed: pop 1 is segregating for *Sli* and the SRNase-knockout, and pop 2 is segregating for stylar self-compatibility from *S. verrucosum*. For the study of cytoplasmic male sterility (CMS), we will develop and characterize F2 populations segregating for the restorer gene in T cytoplasm, to determine if it shows expression of the CMS trait in addition to the already established P cytoplasm.

### **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. We have met our target of whole-genome sequencing for 100 dihaploids, generated from 60 different tetraploid clones spanning the major US germplasm groups (russet, chip, red). Confirming earlier studies, we found extreme genetic diversity in cultivated potato, with more than 20 variants per gene in the population on average. The sequencing data have been leveraged to design DNA markers for key traits, including maturity (*CDF1*) and tuber shape (*OFP20*). From the beginning, our goal has been to generate de novo, haplotype-resolved assemblies for 20 of the 100 dihaploids, which are now complete. Gene annotation is complete for 5 of the 20 so far, and we have identified 9,684 regions of perfect synteny between our dihaploids, the tetraploid Atlantic reference genome, and the DM reference genome. A multi-location (MN, MI, WI, ME) field trial of the dihaploids was conducted in 2023, to better characterize its phenotypic diversity.

**Plans for Year 5:** Several manuscripts are planned to disseminate results: (1) Description of the genetic and phenotypic diversity of the 100 dihaploids; (2) Population genetic study of selection, deleterious alleles, transposon frequency, and introgression in the dihaploids. (3) Investigation of whole-genome imputation accuracy from genotyping-by-sequencing and microarray technologies.

### **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. Elite diploids, selected at Michigan State University using similar techniques as a conventional tetraploid program, were evaluated in MI, NY, and WI in 2023. Total yields for several clones were comparable to elite tetraploids (although tuber appearance was not), providing further evidence that yield is not a limiting factor for diploids. The University of Maine and Oregon State University programs generated their first diploid breeding populations by crossing dihaploids with self-fertile diploids from the UW and MSU programs, bringing the total number of diploid breeding programs in the project to five (MI, WI, MN, ME, OR). Our first assessment of the accuracy of genomic selection for diploid potato was completed. Using a new genetic marker for maturity (*CDF1*), progress was made to develop diploid breeding populations fixed for different maturity variants, which is an essential step toward generating hybrid varieties with uniform maturity. Inbreeding experiments produced clones homozygous for the late blight resistance gene *RB/Rpi-blb1* and the potato virus Y resistance gene *Ry<sub>chc</sub>*.

**Plans for Year 5:** New breeding populations will be generated and evaluated in 5 states: ME, MI, MN, OR, and WI. A manuscript describing the genomic selection model will be published.

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

Our first agronomic experiment in 2023 was designed to study the influence of seed tuber size on yield components for a first-generation diploid hybrid. Seed tubers produced in 2022 from transplants were sorted into three size categories for the 2023 trial: small (~10 g), medium (~20 g) and large (~40 g). The large size is comparable to the size currently used for uncut tetraploid potato seed, while the small size is similar to hydroponically produced minitubers. Our key finding is that harvested tuber size was not affected by seed size, but the number of tubers and therefore total yield increased with seed size. A second field experiment was designed to validate hybrid maturity predictions based on the gene *CDF1*. Different hybrids were created with 0, 1, or 2 copies of the early variant *CDF1.3* (in a wild-type genetic background), which showed progressively earlier flowering time and senescence in the field.

**Plans for Year 5:** An experiment is planned to optimize greenhouse parameters for producing seedling transplants, including the timing of seed sowing and size of the plug tray. Our hypothesis is that the optimal stage of plant development has sufficient root mass to maintain mechanical integrity of the plug during transplanting but without triggering early tuberization due to container volume stress.

## Publications

Agha HI, Shannon LM, Morell P (2023) Unloading potatoes: Potato breeding moves forward with only half the genome. *Cell Genomics* 3:100343. <https://doi.org/10.1016/j.xgen.2023.100343>

Agha HI, Schroeder L, Eikholt D, Schmitz Carley CA, Cavender-Bares J, Shannon LM (2023) Assessing the Effectiveness of Reflectance Spectroscopy Analysis to Determine Ploidy in Potato. *American Journal of Potato Research* 100: 135-141. <https://doi.org/10.1007/s12230-022-09899-8>

Bethke PC, Halterman DH, Francis DM, Jiang J, Douches DS, Charkowski AO, Parsons J (2022) Diploid potatoes as a catalyst for change in the potato industry. *American Journal of Potato Research* 99: 337–357. <https://doi.org/10.1007/s12230-022-09888-x>.

Bethke P, Hamernik A, Endelman J (2023) A new beginning - Producing diploid potato seed. *The Badger Common Tater* 75 (9):59-63. [https://issuu.com/bctater/docs/standard\\_923/59](https://issuu.com/bctater/docs/standard_923/59)

Behling WL, Douches DS (2023) The effect of self-incompatibility factors on interspecific compatibility in *Solanum* Section *Petota*. *Plants* 12:1709. <https://doi.org/10.3390/plants12081709>

Lee S, Enciso-Rodriguez FE, Behling W, Jayakody T, Panicucci K, Zarka D, Nadakuduti SS, Buell CR, Manrique-Carpintero NC, Douches DS (2023) HT-B and S-RNase CRISPR-Cas9 double knockouts show enhanced self-fertility in diploid *Solanum tuberosum*. *Frontiers in Plant Science* 14:1151347. <https://doi.org/10.3389/fpls.2023.1151347>

Song L, Endelman JB (2023) Using haplotype and QTL analysis to fix favorable alleles in diploid potato breeding. *Plant Genome* e20339. <https://doi.org/10.1002/tpg2.20339>

Sorensen PL, Christensen G, Karki HS, Endelman JB (2023) A KASP Marker for the Potato Late Blight Resistance Gene *RB/Rpi-blb1*. *American Journal of Potato Research* 100:240–246. <https://doi.org/10.1007/s12230-023-09914-6>

## Year 4 in Pictures



Transplanting seedlings



Outreach at the Potato Expo



# Diploid Field Day



# AGBT-Ag Conference

**POTATO 2.0 Haplotype-resolved genome assembly of potato diploids capturing North American allelic diversity of tetraploid parents**  
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**Abstract**  
 Potato (*Solanum tuberosum*) is the third most important food crop in the world with a value of over \$3.9 billion in the United States. Cultivated potato, *Solanum tuberosum*, is a highly heterozygous tetraploid ( $2n=4x=48$ ) and as a consequence, breeding is primarily phenotypic based with the breeder selecting an F1 from a cross of two tetraploid parents. Diploid ( $2n=2x=24$ ) breeding of introgressed hybrids has the potential to revolutionize potato breeding due to the simpler genetics and the ability to use true seed instead of seed tubers. Primary diploids for this project were created by crossing a tetraploid female parent with pollen from haploid tuberos male parent (HTM) or (HTM) (*Solanum tuberosum* group Phureja). A set of 91 female fertile diploids were checked for aneuploidy using Illumina HiSeq 2500 sequencing data. Based on aneuploidy results, vigor, and diversity, a set of 20 female fertile diploids were selected for chromosome-scale long read assembly using Pacific HiFi reads, each with a corresponding Illumina HiSeq 2500 gene sequence and Ragtag with the DM6.1 reference genome. Scaffolds were sorted into 24 chromosomes. Version 2 assemblies will be released in 2024. This project will provide high quality contiguous assemblies, diversity of female diploid North American breeding to a diploid/2 hybrid approach.

**Abstract 2.0 Project**  
 This project is part of the overall USDA-SCRI funded "Determine the genetic basis and environmental..."  
 • Determine the genetic basis and environmental...  
 • Develop self-fertile, diploid genotypes that can...  
 • Create inbred lines that are food (homologous)...  
 • Develop agronomic and economic breeder-ready...  
 production systems and assessing its results.  
<https://potato2.github.io/>

**Figure 1. BUSCO scores of diploid assemblies before filtering.** Values across the light blue bars indicate the single copy BUSCO score.

**Figure 2. K-mer spectrum of haploid Haplotype 1 (A) and 2 (B) of Haplotype 2 (B) using gDNA Illumina sequencing.** Data and inspection inform assembly. Blue = 0 copies, red = 1 copy, purple = 2 copies.

**Figure 3. D-Gene plot of Haplotype 1 (A) and Haplotype 2 (B) assemblies against DM6.1 reference genome.**

**Table 2. Diploid assembly statistics before filtering.**

Diploid	Reads	Number of Assemblies	Length (bp)	GC (%)
HTM-1-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-2-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-3-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-4-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-5-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-6-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-7-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-8-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-9-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-10-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-11-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-12-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-13-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-14-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-15-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-16-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-17-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-18-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-19-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-20-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112

**Table 3. Diploid assembly statistics after filtering.**

Diploid	Reads	Number of Assemblies	Length (bp)	GC (%)
HTM-1-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-2-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-3-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-4-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-5-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-6-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-7-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-8-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-9-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-10-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-11-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-12-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-13-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-14-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-15-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-16-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-17-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-18-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-19-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112
HTM-20-2021	1,107,112,112	1,107,112,112	1,107,112,112	1,107,112,112