2018 MSU POTATO BREEDING AND GENETICS RESEARCH REPORT
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INTRODUCTION

At Michigan State University, we have been dedicated to developing improved potato varieties for the chip-processing and tablestock markets since 1988. The program is one of four integrated breeding programs in the North Central region supported through the USDA/NIFA Potato Special Grant. At MSU, we conduct a comprehensive multi-disciplinary program for potato breeding and variety development to meet Michigan's needs. Our program integrates traditional and biotechnological approaches to breed for disease and insect resistance that is positioned to respond to scientific and technology opportunities that emerge. We are also developing more efficient methods to breed improved potato varieties.

In Michigan, variety development requires that we primarily develop high yielding round white potatoes with excellent chip-processing from the field and/or storage. In addition, there is a need for table varieties (russet, red, yellow, and round white). We conduct variety trials of advanced selections and field experiments at MSU research locations (Montcalm Research Center, Lake City Experiment Station, Clarksville Research Center, and MSU Agronomy Farm), we ship seed to other states and Canadian provinces for variety trials, and we cooperate with Chris Long on grower trials throughout Michigan. This testing is crucial in determining the commercial potential of the lines. Through conventional crosses in the greenhouse, we develop new genetic combinations in the breeding program, and also screen and identify exotic germplasm that will enhance the varietal breeding efforts. With each cycle of crossing and selection we are seeing directed improvement towards improved varieties (e.g. combining chip-processing, scab resistance, PVY resistance, late blight resistance and higher specific gravity). I am happy to see the increase in scab, late blight and PVY resistance in the breeding material and selections. We need to continue to combine these traits in long-term storage chip-processing lines. Through the USDA/AFRI SolCAP project we developed a new set of DNA genetic markers (8,303) called SNPs that are located in the 39,000 genes of potato. We are now 10 years down the road and we are benefiting from this technology as we can now query 35,000 SNPs for the same cost. This SolCAP translational genomics project has finally giving us the opportunity to link genetic markers to important traits (reducing sugars, starch, scab resistance, etc.) in the cultivated potato lines and then breed them into elite germplasm. The

SNPs also allow us to accurately fingerprint the varieties (DNA ID database). In addition, our program has been utilizing genetic engineering as a tool to introduce new genes to improve varieties and advanced germplasm for traits such as insect resistance, late blight and PVY resistance, lower reducing sugar, nitrogen use efficiency and drought. In 2019, we will test invertase silencing on a larger scale and learn more about the potential for drought tolerance and late blight resistance. Furthermore, PotatoesUSA is supporting national early generation trials called the National Chip Processing Trial (NCPT) which will feed lines into the SNaC (SFA) trials and also fast track lines into commercial testing. This national cooperative testing is key! We are leveraging the NCPT Fast Track program to have seed increased for promising chip-processing lines. We also have funding to develop genome editing technologies that may not be classified as genetic engineering through a USDA/BRAG grant. This technology can be used to introduce lower sugars, bruising and asparagine as well a number of other traits in the future. We also have a USDA/AFRI diploid breeding grant to develop some foundational diploid breeding germplasm. In 2015, we were awarded the USAID Feed the Future grant to generate late blight resistance potatoes for Bangladesh and Indonesia. This project brings us into cutting edge GM work with Simplot and the International Potato Center. Lastly, we have NSF-funded grants to better understand the potato genome and study wound-healing in potato. We feel that these in-house capacities (both conventional and biotechnological) put us in a unique position to respond to and focus on the most promising directions for variety development and effectively integrate advanced technologies with the breeding of improved chip-processing and tablestock potatoes.

The breeding goals at MSU are based upon current and future needs of the Michigan potato industry. Traits of importance include yield potential, disease resistance (scab, late blight, early die, and PVY), insect (Colorado potato beetle) resistance, chipping (out-of-the-field, storage, and extended cold storage) and cooking quality, bruise resistance, storability, along with shape, internal quality, and appearance. If these goals can be met, we will be able to reduce production input costs, keep potato production profitable as well as reduce the reliance on chemical inputs such as insecticides, fungicides and sprout inhibitors, and improve overall agronomic performance through new potato varieties.

Over the years, key infrastructure changes have been established for the breeding program to make sound assessments of the breeding selections moving through the program. In 2016, we constructed a greenhouse to expand our breeding and certified minituber seed production with a hydroponic nutrient film technology (NFT) system. This greenhouse is at the MSU Crops facility on south campus. Also in 2016, we began to upgrade the grading line and this was completed with funding from MPIC and AgBioResearch. Variable speed drives control; a new lift; custom built barrel washer; grading table; and Kerian speed sizer are all part of the set up. Incorporation of bar-coded labels and scales synchronized to computer hot keys have improved the speed, accuracy and efficiency of the grading process. All entities of the potato group: Potato Breeding and Genetics; Potato Outreach Program; pathologists and soil fertility researchers have access to this new equipment. Thank you!

Varietal Development

Breeding

The MSU potato breeding and genetics program is actively producing new germplasm and advanced seedlings that are improved for cold chipping, and resistance to scab, late blight, and Colorado potato beetle. For the 2018 field season, progeny from about 450 crosses were planted and evaluated. Of those, the majority were crosses to select for round whites (chip-processing and tablestock), with the remainder to select for yellow flesh, long/russet types, red skin, and novelty market classes. During the 2018 harvest, over 1,000 selections were made from the 45,000 seedlings produced. In addition, about 700 first year selections from elite chip-processing crosses segregating for PVY resistance were made. All potential chip-processing selections will be tested in January and April 2019 directly out of 45°F (7.2°C) and 50°F (10°C) storages. Atlantic, Pike (50°F chipper) and Snowden (45°F chipper) are chip-processed as check cultivars. Selections have been identified at each stage of the selection cycle that have desirable agronomic characteristics and chip-processing potential. At the 12-hill and 30-hill evaluation state, about 300 and 100 selections were made, respectively; based upon chip quality, specific gravity, scab resistance, late blight resistance and DNA markers for PVY and Golden nematode resistance. Selection in the early generation stages has been enhanced by the incorporation of the scab and late blight evaluations of the early generation material. *Unfortunately, in 2018 we were unable to get the late blight infection to spread enough to collect useful data.* We are pushing our early generation selections from the 30-hill stage into tissue culture to minimize PVY issues in our breeding and seed stock. We are now using a cryotherapy method as well as the traditional methods that was developed in our lab to remove viruses. We feel that this technique predictably as well as quickly remove virus from tissue culture stocks. Our results show that we are able to remove both PVY and PVS from lines, but PVS can be difficult to remove in certain lines. We tested the removal of PLRV and succeeded. Over 1500 lines are maintained in tissue culture for the breeding and genetics program.

Chip-Processing

Over 80% of the single hill selections have a chip-processing parent in their pedigree. Our most promising advanced chip-processing lines are Mackinaw (MSX540-4) (scab, late blight and PVY resistant), MSV030-4 (scab resistant), MSV313-2 (scab resistant), Huron Chipper (MSW485-2) (late blight resistant), MSW075-2 (scab resistant), MSZ222-19 (scab resistant), MSZ242-13 (scab resistant) and MSZ219-1, MSZ219-13 and MSZ219-14 (all three sibs are scab, late blight and PVY resistant). We have some newer lines to consider, but we are removing virus from those lines. We are using the NCPT trials to more effectively identify promising new selections with broad adaptability. Manistee was licensed to Canada and Chile. Saginaw Chipper (MSR061-1) and Mackinaw (MSX540-4) are being tested in Australia.

Tablestock

Efforts have been made to identify lines with good appearance with an attractive skin finish, low internal defects, excellent culinary quality, high marketable yield and resistance to scab, late blight and PVY. Our current tablestock development goals now are to continue to improve the frequency of scab resistant lines, incorporate resistance to late blight along with marketable maturity and excellent tuber quality, and select more russet

types, red-skinned, and yellow-fleshed lines. We have also been selecting some pigmented skin and tuber flesh lines for specialty markets. There is also interest in additional specialty mini-potatoes for the "Tasteful selections" market. We have interest from some western specialty potato growers to test and commercialize these lines. From our breeding efforts, we have identified mostly round white lines, but we also have a number of yellow-fleshed and red-skinned lines, as well as some purple skin selections that carry many of the characteristics mentioned above. We are also selecting for round white, red-skin, and improved Yukon Gold-type yellow-fleshed potatoes. Some of the tablestock lines were tested in on-farm trials in 2018, while others were tested under replicated conditions at the Montcalm Research Center. Promising tablestock lines include MSV093-1 (yellow, scab resistant), MST252-1Y (scab resistant), MSV179-1 (scab resistant), MSW343-2R, MSX569-1R (scab resistant) and MSX324-1P (scab resistant). MSZ109-8PP and MSZ109-10PP (Blackberry) are purple-fleshed chippers with deep purple flesh, round shape and attractive skin as well as scab resistance. Jacqueline Lee (late blight resistant) was licensed to Australia and is being grown in Central America for its late blight resistance. Spartan Splash, Blackberry and our PVY resistant Red Marker #2 potato are being marketed in the specialty markets.

Disease and Insect Resistance Breeding

Scab: In 2018, we had two locations to evaluate scab resistance: a commercial field with a history of severe scab infection and a highly infected site at the Montcalm Research Center. The commercial site and the Montcalm Research Center both gave us very high infection levels. The susceptible checks of Snowden and Atlantic were highly infected with pitted scab. Promising resistant selections were MSV313-2, MST252-1Y, MSV179-1, MSX324-1P, MSW474-01, MSZ219-1, MSZ219-13, MSZ219-14, MSZ222-19 as well as the MSZ-series selections from the commercial scab site. If you examine the Advanced Chip trial results, you will notice that almost all the lines are scab resistant. We need to continue in this direction of many selections with scab resistance so we can find the great scab resistant chipper. The high level of scab infection at the on-farm site with a history of scab infection and MRC has significantly helped with our discrimination of resistance and susceptibility of our lines. The MRC scab site was used for assessing scab susceptibility in our advanced breeding lines and early generation material and is summarized below (Figure 1). All susceptible check plots (Snowden and Atlantic) were scored as susceptible.

Based upon these results, common scab resistance is strong in the breeding program. We lead the nation in scab resistant lines as observed in the national NCPT scab disease trials. These data were also incorporated into the early generation selection evaluation process at Lake City. We are seeing that this expanded effort is leading to more scab resistant lines advancing through the breeding program. The ability to select under commercial settings on-farm is accelerating our ability to select for increased scab resistant varieties. MSZ219-1, MSZ219-13, MSZ219-14, MSZ022-07, MSZ222-19 and MSZ242-13 are some of the first scab resistant chippers to advance through this effort.

Fig. 1. Scab Disease Nursery Ratings from MRC trials

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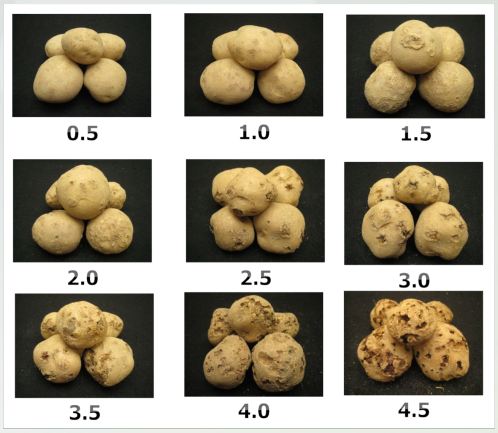
Breeding for Scab Resistance

	Scab Disease Rating (0-5)									
Trial	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	Total
Variety Trial	1	19	48	55	50	28	17	5	1	224
Early Generation	40	31	37	46	18	23	5	5	0	205
Diploid	39	38	38	58	21	7	3	2	0	206

Reference Variety	Scab Rating
Pike	1.8
Lamoka	2.0
Snowden	3.0
Atlantic	3.0



Late Blight: One of our core objectives is to breed improved cultivars for the industry that have foliar and tuber resistance to late blight using a combination of conventional breeding, marker-assisted strategies and transgenic approaches. Through conventional breeding approaches, the MSU potato breeding and genetics program has developed a series of late blight resistant advanced breeding lines and cultivars that have diverse sources of resistance to late blight. In 2018, we conducted late blight trials at the MSU campus. We inoculated with the US23 genotype but lacked good disease progression due to the 2018 climate. We are not reporting late blight trial results this year. We did conduct detached leaf bioassays in 2018 to study late blight resistance in a mapping study. US23 gave us good infection in the detached leaf tests, so we know that our cultures are virulent. We will continue with late blight trials in 2019 on the MSU campus.

PVY: We have focused on incorporating PVY resistance in our germplasm for years, with an increased emphasis in recent years to increase the frequency of PVY resistance in our advanced selections. We are using PCR-based DNA markers to select potatoes resistant to PVY. The gene is located on Chromosome 11. In 2013, we generated over 7,000 progeny segregating for PVY resistance. Each year since 2013 we are making new crosses, making selections and expanding the germplasm base that has PVY resistance (Fig. 3). We are also

using DNA markers to also screen for PVX resistance, PLRV resistance, late blight resistance and Golden nematode resistance. As a result of this work, Mackinaw has PVY resistance as well as MSZ219-1, -13 and -14 (in addition to scab and late blight resistances). More PVY resistant advanced selections are in the queue.

Fig. 2 PVY resistant selections in the breeding program

Year	Family	PVY Resistance
YR0	MSGG	332 Families
YR1	MSFF	535 Selections to DNA screen
YR2	MSEE	473 Selections
YR3	MSDD	36 Selections
YR4	MSCC	11 Selections
YR5	MSBB	23 Selections

MSU Lines with Commercial Tracking

Manistee (MSL292-A)

Parentage: Snowden x MSH098-2
Developers: Michigan State University and the Michigan Agricultural Experiment Station
Plant Variety Protection: Applied for.

Strengths: Manistee is a chip-processing potato with an attractive round appearance with shallow eyes. Manistee has a full-sized vine and an early to mid-season maturity. Manistee has above average yield potential and specific gravity similar to Snowden. This variety has excellent chip-processing long-term storage characteristics and a similar to better tolerance to blackspot bruise than Snowden.

Incentives for production: Excellent chip-processing quality with long-term storage characteristics, above average yield, specific gravity similar to Snowden, and good tuber type.

Saginaw Chipper (MSR061-1)

Parentage: Pike x NY121
Developers: Michigan State University and the Michigan Agricultural Experiment Station
Plant Variety Protection: Trademark

Strengths: MSR061-1 is a chip-processing potato with resistance to common scab (*Streptomyces scabies*) and moderate foliar late blight (*Phytophthora infestans*) resistance. This variety has medium yield similar to Pike and a 1.079 (average) specific gravity and an attractive, uniform, round appearance. MSR061-1 has a medium vine and an early to mid-season maturity.

Incentives for production: Chip-processing quality with common scab resistance similar to Pike, moderate foliar late blight resistance (US8 genotype), and uniform, round tuber type.

MSV093-1Y

Parentage: McBride x MSP408-14Y
Developers: Michigan State University and the MSU AgBioResearch.
Plant Variety Protection: To Be Applied For

Strengths: MSV093-1Y is a high yield potential yellow-flesh breeding line with an attractive, round tuber shape. This line has demonstrated excellent high yield potential in replicated trials at the MSU Montcalm Research Center and on grower field trials throughout Michigan. This yellow flesh line has excellent internal quality (few defects) and a low incidence of blackspot bruise. MSV093-1Y also has moderate scab tolerance. MSV093-1Y has a strong vine and a mid-early season maturity.

Incentives for production: High yield potential with an attractive tuber shape with good yellow flesh with excellent internal quality.

Mackinaw (MSX540-4)

Parentage: Saginaw Chipper x Lamoka
Developers: Michigan State University and the MSU AgBioResearch.
Plant Variety Protection: To Be Applied For.

Strengths: MSX540-4 is a chip-processing potato with resistance to potato virus Y (PVY), late blight (*Phytophthora infestans*), tolerance to common scab (*Streptomyces scabies*), and demonstrated tolerance to *Verticillium* wilt. This variety has average yield with a high specific gravity, and a high percentage of A-size tubers with an attractive, uniform shape. MSX540-4 has a strong vine and a mid- to late-season maturity, and has demonstrated excellent long-term storage chip-processing quality. MSX540-4 has performed well in multiple locations in the PotatoesUSA National Chip Processing Trials (NCPT).

Incentives for production: Long-term chip-processing quality with resistance to PVY and late blight, and tolerance to common scab.

Morphological Characteristics:
Plant: Medium height vine, semi-erect with a balance between stems and foliage visible, and flowers.
Tubers: Round tubers with lightly netted, tan colored skin. Tubers have a creamy-white flesh with a low incidence of internal defects.

Agronomic Characteristics:
Vine Maturity: Mid- to late-season maturity.
Tubers: Smooth shaped tubers with lightly netted, tan colored skin and a creamy-white flesh.
Yield: Average yield under irrigated conditions, with uniform A-size tubers.
Specific Gravity: Averages similar to above Snowden in Michigan.
Culinary Quality: Chip-processes from short to long-term storage.
Diseases: Resistant to PVY and late blight (*Phytophthora infestans*), tolerant to common scab (*Streptomyces scabies*).

Huron Chipper (MSW485-2)

Parentage: MSQ070-1 x MSR156-7
Developers: Michigan State University and the MSU AgBioResearch.
Plant Variety Protection: To Be Applied For.

Strengths: MSW485 is a chip-processing potato with resistance to and late blight (*Phytophthora infestans*), and stronger tolerance to common scab (*Streptomyces scabies*) than Atlantic. This variety has high yield and good specific gravity, with attractive, uniformly round tubers. MSW485-2 has a strong vine and a mid-season maturity, and has demonstrated excellent long-term storage chip-processing quality. MSW485-2 has performed well in multiple locations in the PotatoesUSA National Chip Processing Trials (NCPT) and national SFA (SNaC) trials.

Incentives for production: Excellent chip-processing quality out of the field and long-term chip quality with resistance to late blight and a good size profile.

Morphological Characteristics:
Plant: Medium height vine, semi-erect with a balance between stems and foliage visible, and flowers.
Tubers: Uniform, smooth, round tubers with lightly netted, tan colored skin. Tubers have a white flesh with a low incidence of internal defects.

Agronomic Characteristics:
Vine Maturity: Mid-season maturity.
Tubers: Smooth, round tubers with lightly netted, tan colored skin and a white flesh.
Yield: Above average yield under irrigated conditions, with uniform tubers.
Specific Gravity: Averages similar to above Atlantic and Snowden.
Culinary Quality: Chip-processes from short to long-term storage.
Diseases: Resistant to late blight (*Phytophthora infestans*) and tolerant to common scab (*Streptomyces scabies*).

MSX569-1R (Ilse Royale)

Parentage: MSS002-2R x MSS544-1R
Developers: Michigan State University and the MSU AgBioResearch.
Plant Variety Protection: To Be Applied For.

Strengths: MSX569-1R is a fresh market variety with an attractive red skin, bright white flesh, excellent round tuber shape, and tolerance to common scab (*Streptomyces scabies*). This variety has average yield with a high percentage of A-size tubers with an attractive, uniform shape. The bright red skin is highly desirable in the fresh market, and also maintains good red color in storage. This line has demonstrated good marketable yield potential in replicated trials at the MSU Montcalm Research Center, on grower field trials throughout Michigan, as well as in North Central Regional Trials, and trials in Florida and North Carolina. MSX569-1R has excellent internal quality (few defects) and a low incidence of blackspot bruise.

Incentives for production: Fresh market variety with a bright red skin, attractive tuber size and shape, excellent internal quality, and tolerance to common scab.

***Morphological Characteristics**:*
Plant: Medium height vine, semi-erect with a balance between stems and foliage visible, and flowers.
Tubers: Round tubers with a smooth, bright red colored skin. Tubers have an attractive white flesh with a low incidence of internal defects.

***Agronomic Characteristics**:*
Maturity: Mid-season maturity.
Tubers: Round tubers with a red skin and an attractive white flesh.
Yield: Average yield under irrigated conditions, similar or better than Red Norland.
Specific Gravity: Good fresh market specific gravity (1.055 in Michigan).
Culinary Quality: Excellent culinary quality.
Diseases: Tolerance to common scab (*Streptomyces scabies*).

Blackberry
(MSZ109-10PP)

Parentage: COMN07-W112BG1 x MSU200-5PP
Developers: Michigan State University and the MSU AgBioResearch
Plant Variety Protection: To Be Applied For.

Strengths: Blackberry is a tablestock variety with unique purple skin and a deep purple flesh. The tubers have an attractive, uniform, round shape and a purple flesh with common scab resistance and low incidence of internal defects. Yield can be high under irrigated conditions. Blackberry will also chip-process out of the field.

Incentives for production: The unique purple skin and purple flesh of the tubers of Blackberry offer a unique potato that could lend itself to the specialty variety market, such as gourmet restaurants and food stores, as well as farm and road-side markets. The primary market for this clone will be farm market and direct retail sale growers, and home gardeners. This variety is also used as a gourmet chip processing variety.

Morphological Characteristics:
Plant: Full-sized vine, semi-erect with a balance between stems and foliage visible, and flowers.
Tubers: Round tubers with a smooth skin and unique purple skin and purple flesh color. Tubers have a deep purple flesh with a low incidence of internal defects.

Agronomic Characteristics:
Maturity: Mid-season.
Tubers: Round tubers with unique purple skin and deep purple flesh.
Yield: Average to above average yield.
Specific Gravity: Averages 1.070 in Michigan.
Culinary Quality: Gourmet specialty with deep purple flesh and also chip-processes.
Foliage: Full-sized, semi-erect vine.
Diseases: Good common scab resistance.

Application of Molecular Markers in MSU Potato Breeding

With the development of molecular markers for potato breeding, marker-assisted selection has been incorporated into our routine breeding practice and greatly facilitates the selection process. Some of the main markers that are used at MSU include: *RYSC3* and M6, *Potato virus Y* (PVY) resistance markers; *RxSP*, a *Potato virus X* (PVX) resistance marker; *TG689*, a Golden Nematode resistance marker; RB and R8, Late Blight resistance markers. PVY markers have been the most frequently used tools to assist selection in our program due to the importance of PVY resistance. According to the pedigrees, selections from our single-hill trial (1st year of field selection) are screened for PVY markers every year. This allowed for a prioritization of the space in the field, and for earlier, more informed decisions in variety selection.

The trait mapping populations have been a major research focus for us over the previous four years as we try to correlate the field data with genetic markers. We now have DNA SNP markers linked to late blight resistance, scab resistance, chip color, tuber asparagine and specific gravity. We will now start using this linkage information to assist us in breeding. Our first SNP marker is linked to a gene for late blight resistance on Chr. 9 and the second is located on Chr. 10 with new ones recently identified on Chr. 4 and Chr. 5. The ability to use the DNA markers to stack a set of late blight resistance genes will lead to durable late blight resistance. We are now bringing in late blight resistance genes from germplasm from Europe and China.

Germplasm Enhancement

The diploid genetic material represent material from South American potato species and other countries around the world that are potential sources of resistance to Colorado potato beetle, late blight, potato early die, and ability to cold-chip process. We are now placing more emphasis on the diploid breeding effort because of the advantages the breeding system brings when we introduce the ability to self-pollinate a line. Features of diploid breeding include 1) a simpler genetic system than current breeding methods, 2) tremendous genetic diversity for economic traits, 3) minimal crossing barriers to cultivated potato, 4) the ability to reduce genetic load (or poor combinations) through selfing and 5) the ability to create true breeding lines like wheat, soybeans and dry beans. We are also using some inbred lines of *S. chacoense* that have fertility and vigor (also a source of *Verticillium* wilt resistance to initiate our efforts to develop inbred lines with our own diploid germplasm. We have over 40 populations that we are cycling to make selections and we also selected diploid progeny from Atlantic, Superior, Manistee, MSZ219-14, Kalkaska, MSR127-2, MSS576-5SPL and others to cross to the self compatible material so we can develop inbred chip-processing diploid lines. This new diploid potato breeding project is expanding to develop promising lines to use as parents in the future as well as to think about F1 hybrid varieties analogous to what the corn breeders release.

Decoding *S. chacoense*-derived Colorado potato beetle resistance

Introgression of Colorado potato beetle resistance from *S. chacoense*-derived diploid recombinant inbred lines into cultivated backgrounds is being conducted through GREEEN funding. Subsequent marker assisted selection will yield diploid breeding lines with beetle resistance and desirable tuber traits. The spatio-temporal durability of this glycoalkaloid-based host plant resistance will be assessed using Colorado potato beetle

populations from potato growing regions across the nation and examining 10 successive generations of beetles grown on host plant resistant material. Furthermore, the development of cross-resistance by beetles grown on host plant resistant material to commercial insecticides will be examined to inform the most sustainable deployment of this germplasm.

Overcoming self-incompatibility in diploid potato using CRISPR-Cas9

The aim of this project was to generate a targeted knock-out (KO) of the *S-RNase* gene, involved in gametophytic self-incompatibility in diploid potatoes, using CRISPR/Cas9 technology in an effort to avoid self-pollen degradation. We identified *S-RNase* alleles with flower-specific expression in two diploid self-incompatible lines using genome resequencing data. *S-RNase* gene mapped to chromosome 1 within a low recombination region. *S-RNase* KO lines were obtained causing premature stop codons. Fruits were set in selected KO and produced viable T1 seeds, and a Cas9-free KO line. Our results suggest that creating *S-RNase* KO can contribute to generation of self-compatible lines as a first step for the generation of commercial diploid cultivars.

Gene editing in diploid potato

MSU's breeding program has developed diploid germplasm with important agronomic qualities. These lines can be further characterized on traits for the use of gene editing. The first objective of this project is to characterize the MSU diploid germplasm for important molecular and morphological traits such as regeneration capability. The second major objective is to use gene editing, namely, CRISPR-Cas9 to knockout vacuolar invertase (*VInv*) in select diploid lines. The overall goal is to further advance the diploid breeding program by introducing economically important traits and proving the utility of gene editing in potato.

Dihaploid Potato Production at Michigan State University

The benefits of developing a broad germplasm of dihaploid potatoes brings the industry ever-closer to the expansive changes that would come with diploid potato breeding. Many of the challenges associated with tetraploid potatoes would be greatly reduced if the potato had a lower, and therefore less complicated, ploidy. Our goal is to develop a broad-based dihaploid germplasm that can be used in diploid potato breeding. We started by crossing successful varieties and advanced breeding lines from MSU tetraploid germplasm with a haploid inducer, *S. phureja* IVP 101. Tetraploid parent lines were selected based on traits such as high yield, disease resistance, and good chip quality, among others. Resulting seeds were inspected for a purple embryo spot and grown in tissue culture before transplanting in the greenhouse. Chloroplast numbers in guard cells were collected to determine ploidy level. Plants that we determined to be diploid were also SNP genotyped with the Infinium 22K Potato SNP array for ploidy confirmation. These dihaploids were then tested for disease resistance markers: RYSC3+ (Potato Virus Y extreme resistance), GN (Golden Nematode) resistance, and PVX resistance. Those with a Tollocan lineage were also tested for presence of R8 late blight resistance via a SNP KASP assay. Confirmed dihaploids were crossed with a diploid self-compatible inbred line of *S. chacoense*, M6 to introgress self-compatibility. Of the hundreds of seeds produced in the past 5 years from these diphaploid crosses with 18 breeding lines or

varieties, about 80 progeny have been confirmed as diploid (2x). These dihaploids are the foundation of our diploid breeding program for round white potatoes for the chip and table markets.

Introgressing Self-compatibility to *Solanum tuberosum* Dihaploids for Diploid Variety Development

Dihaploids of cultivated potato (*Solanum tuberosum* L.) have been produced for over 50 years to reduce the breeding and genetic challenges of autopolyploidy. Most dihaploids are male sterile (MS) that reduces the benefit of lower ploidy level of cultivated tetraploid potato. In this study, we used three self-compatibility (SC) donors to introgress SC into a wide range of dihaploid germplasm through a series of crosses to dihaploids which we refer to as *S. tuberosum* backcrossing. The SC increased from 11% in the F_1 generation to 33% in the BC_2 generations. Over 6,000 genome-wide SNPs were used to characterize the germplasm diversity, heterozygosity, and structure in two backcrossing generations. The BC_2 generation was significantly improved regarding maturity, scab resistance, average tuber number, however, the yield in BC_2 was not greater than the F_1 and BC_1 generations.

Certified NFT Minituber Production at Michigan State University

For two years, the MSU Potato Breeding program has operated a certified NFT minituber production greenhouse. The ability to produce certified seed allows faster introduction of early generation material to the potato industry with commercial certified seed growers. It also helps position the program for participation in international trials, since the minitubers meet Phytosanitary requirements. We offer this service of small volume certified seed minituber production to other breeders and industry partners.

Integration of Genetic Engineering with Potato Breeding

MSU conducts genetic engineering research to introgress and test economically important traits into potato. We have a USAID-funded project to create and commercialize 3-R-gene potato varieties in Bangladesh and Indonesia. This project is a partnership with Simplot Plant Sciences. Simplot has been creating the plants for the target countries. Greenhouse trials show that a high level of resistance to late blight has been achieved in events that have no backbone and are single inserts. Trials are planned for the fall of 2019.

Furthermore, regarding late blight resistance, we have many lines with the RB gene for late blight resistance transformed into MSU lines. The addition of the RB gene allows us to test the effect of multiple resistance genes on the strength of resistance. Our data supports the need to pyramid the late blight resistance R-genes to achieve the best levels of resistance. The RB gene is in Jacqueline Lee and MSL268-D. We now have generated some lines with 3-R-genes stacked with one transformation.

We have also generated and evaluated many lines with different genes for water use efficiency. The XERICO gene is showing the most promise. In 2018, we conducted a preliminary trial at MRC with tissue culture transplants of Ranger Russet events. These results are indicating that we are not seeing a yield hit from the XERICO gene. The

XERICO events also had a higher specific gravity than Ranger Russet. Meanwhile, the greenhouse experiments are showing an ability to refrain from wilting under sub-optimal soil water levels. In 2019, we will have a seed-tuber based field trial at MRC. Lastly, we have generated and selected a Kalkaska invertase silencing line (Kal91.03) that has resistance to accumulating reducing sugars in 40F storage. We tested the agronomic characteristics of Kal91.03 from 2016-2018. The initial results are suggesting that the invertase silencing line has good tuber type, size and similar specific gravity. This suggests that we can correct sugar issues in a chip processing lines with this genetic engineering strategy. We will conduct a larger block planting in 2019 to look more closely at this line under commercial setting.

Chipped directly after 3 months at 40F