

# **2007 POTATO BREEDING AND GENETICS RESEARCH REPORT**

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## **INTRODUCTION**

At Michigan State University we are breeding potatoes for the chip-processing and tablestock markets. The program is one of four integrated breeding programs in the North Central region. At MSU, we conduct a multi-disciplinary program for potato breeding and variety development that integrates traditional and biotechnological approaches. In Michigan, it requires that we primarily develop high yielding round white potatoes with excellent chip-processing from the field and/or storage. We conduct variety trials of advanced selections and field experiments at MSU research locations (Montcalm Research Farm, Lake City Experiment Station, Muck Soils Research Farm and MSU Soils Farm), we ship seed to other states and Canadian provinces for variety trials, and we cooperate with Chris Long on grower trials throughout Michigan. 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 and late blight resistance). 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 solids, insect resistance, disease resistance and nutritional enhancement. 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 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. We are also developing potato tuber moth resistant lines as a component of our international research project. If these goals can be met, we will be able to reduce the grower's reliance on chemical inputs such as insecticides, fungicides and sprout inhibitors, and improve overall agronomic performance with 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. These include the establishment and expansion of the scab nursery, the

development of the Muck Soils Research Farm for late blight testing, the incorporation of no-choice caged studies for Colorado potato beetle assessment, the Michigan Potato Industry Commission (MPIC)-funded construction of the B.F. (Burt) Cargill Demonstration Storage adjacent to the Montcalm Research Farm, new land at the Lake City Experiment Station along with a well for irrigation and expanded land at the Montcalm Research Farm.

## **PROCEDURE**

### **I. Varietal Development**

Each year, during the winter months, 500-1000 crosses are made using about 150 of the most promising cultivars and advanced breeding lines. The parents are chosen on the basis of yield potential, tuber shape and appearance, chip quality, specific gravity, disease resistance, adaptation, lack of internal and external defects, etc. These seeds are then used as the breeding base for the program. We also obtain seedling tubers or crosses from other breeding programs in the US. The seedlings are grown annually for visual evaluation (size, shape, set, internal defects) at the Montcalm and Lake City Research Farms as part of the first year selection process of this germplasm each fall. Each selection is then evaluated post harvest for specific gravity and chip processing. These selections each represent a potential variety. This system of generating new seedlings is the initial step in an 8-12 year process to develop new varieties. This step is followed by evaluation and selection at the 8-hill, 20-hill and 30-hill stages. The best selections out of the four-year process are then advanced for testing in replicated trials (Preliminary, Adaptation, Dates-of-Harvest, Grower-cooperator trials, North Central Regional Trials, Snack Food Association Trials, and other out-of-state trials) over time and locations. The agronomic evaluation of the advanced breeding lines in the replicated trials is in the annual Potato Variety Evaluation Report.

There is a need to find a russet table potato that will be profitable and produce quality russets for the eastern market. Currently the two most desirable potatoes for production and type in Michigan are Russet Norkotah and Silverton Russet. These potatoes suffer as symptomless carriers of PVY. Norkotah also has a weak vine and susceptibility to potato early die. We need a PVY resistant or PVY expressing Silverton Russet potato. We will be making more russet crosses in 2008 to support this new russet market.

### **II. Evaluation of Advanced Selections for Extended Storage**

With the Demonstration Storage facility adjacent to the Montcalm Research Farm we are positioned to evaluate advanced selections from the breeding program for chip-processing over the whole extended storage season (October-June). Tuber samples of our elite chip-processing selections are placed in the demonstration storage facility in October and are sampled monthly to determine their ability to chip-process from colder (42-48°F) and/or 50°F storage. In addition, Chris Long evaluates the more advanced selections in the 10 cwt box bins and manages the 500 cwt. storage bins which may have MSU-bred lines.

### **III. Germplasm Enhancement**

To supplement the genetic base of the varietal breeding program, we have a "diploid" (2x = 24 chromosomes) breeding program in an effort to simplify the genetic

system in potato (which normally has 4x chromosomes) and exploit more efficient selection of desirable traits. This added approach to breeding represents a large source of valuable germplasm, which can broaden the genetic base of the cultivated potato. The diploid breeding program germplasm base at MSU is a synthesis of seven species: *S. tuberosum* (adaptation, tuber appearance), *S. raphanifolium* (cold chipping), *S. phureja* (cold-chipping, specific gravity, PVY resistance, self-compatibility), *S. tarijense* and *S. berthaultii* (tuber appearance, insect resistance, late blight resistance, verticillium wilt resistance), *S. microdontum* (late blight resistance) and *S. chacoense* (specific gravity, low sugars, dormancy and leptine-based insect resistance). In general, diploid breeding utilizes haploids (half the chromosomes) from potato varieties, and diploid wild and cultivated tuber-bearing relatives of the potato. Even though these potatoes have only half the chromosomes of the varieties in the U.S., we can cross these potatoes to transfer the desirable genes by conventional crossing methods via 2n pollen.

#### **IV. Integration of Genetic Engineering with Potato Breeding**

Through transgenic approaches we have the opportunity to introduce new genes into our cultivated germplasm that otherwise would not be exploited. It has been used in potato as a tool to improve commercially acceptable cultivars for specific traits. Our laboratory has now 15 years experience in *Agrobacterium*-mediated transformation to introduce genes into important potato cultivars and advanced breeding lines. We are presently using genes in vector constructs that confer resistance to Colorado potato beetle and potato tuber moth (*Bt-cry3A*, *Bt-cryIIa1* and avidin), potato tuber moth, late blight resistance via the *RB* gene, drought resistance (*CBFI*) and vitamin E. Furthermore, we are investing our efforts in developing new vector constructs that use alternative selectable markers and give us the freedom to operate from an intellectual property rights perspective. In addition, we are exploring transformation techniques that eliminate the need for a selectable marker (antibiotic resistance) from the production of transgenic plants.

### **RESULTS AND DISCUSSION**

#### **I. 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 2007 field season, progeny from over 500 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. In addition to crosses from the MSU breeding program, crosses were planted and evaluated from collaborative germplasm exchange from other breeding programs including North Dakota State University, University of Minnesota, and the USDA/ARS program at the University of Wisconsin as part of the Quad state cooperative effort. During the 2007 harvest, over 1200 selections were made from the 40,000 seedlings produced. All potential chip-processing selections will be tested in January or March 2008 directly out of 40°F and 45°F 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 process that have desirable agronomic characteristics and chip-processing potential. At the 8-hill and 20-hill evaluation

state, about 350 and 150 selections were made, respectively. Selection in the early generation stages has been enhanced by the incorporation of the Colorado potato beetle, scab and late blight evaluations of the early generation material.

### **Chip-Processing**

Over 80% of the single hill selections have a chip-processing parent in their pedigree. Based upon the pedigrees of the parents we have identified for breeding cold-chipping potato varieties, there is a diverse genetic base. We have at least eight cultivated sources of cold-chipping. Examination of pedigrees shows up to three different cold-chipping germplasm sources have been combined in these selections. Our promising chip-processing lines are MSJ147-1, MSJ036-A (scab resistant), MSH228-6 (moderate scab resistance), MSJ126-9Y (moderate scab resistance), MSJ316-A (moderate scab resistance), MSK061-4 (moderate scab resistance), MSK409-1 (scab resistant), MSN238-A (scab resistance), MSL007-B (scab resistance), MSM246-B, MSN191-2Y, MSL292-A, MSR061-1 (scab and PVY resistant) and MSQ070-1 (scab and late blight resistant). Other promising lines include MSQ089-1 (scab resistant), MSQ492-2 (scab and late blight resistant), MSP516-A (scab and late blight resistant), MSR036-5 (scab and late blight resistant), MSR102-3 (scab and late blight resistant), MSR127-2 (scab resistant), MSR041-5 (scab and late blight resistant) and MSR160-2Y (PVY, scab and late blight resistant).

### **Tablestock**

Efforts have been made to identify lines with good appearance, low internal defects, good cooking quality, high marketable yield and resistance to scab and late blight. 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 and yellow-fleshed lines. Potato lines with We have also been spinning off some pigmented skin and tuber flesh lines that may fit some specialty markets. 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 long, russet type and purple skin selections that carry many of the characteristics mentioned above. We are also selecting for a dual-purpose russet, round white, red-skin, and improved Yukon Gold-type yellow-fleshed potatoes. Some of the tablestock lines were tested in on-farm trials in 2007, while others were tested under replicated conditions at the Montcalm Research Farm. Promising tablestock lines include MSI005-20Y, MSN105-1 and MSM171-A. We have a number of tablestock selections with late blight resistance. These are MSL072-C and MSM171-A. MSL211-3 and MSN105-1 has late blight and scab resistance. MSA8254-2BRUS is a russet table selection that has scab resistance, while MSL794-BRUS has late blight resistance. Some newer lines with promise include MSQ176-5 (late blight resistant), MSN230-6RY (scab and late blight resistant), MSM182-1 (PVY and late blight resistant), MSQ440-2 (scab and late blight resistant and MSL268-D (late blight resistant). MSM288-2Y is a yellow flesh selection with scab resistance. Some new pigmented lines are MSS582-1 (purple splash) and Michigan Red and Purple Splash. MSQ558-2RR is a red fleshed chipper and MSQ432-2PP is a purple-fleshed chipper. MSL228-1 (purple splash) is being considered by Gurney's Seed for their home garden catalog (Garden's Alive).

### **Disease and Insect Resistance Breeding**

**Scab:** Disease screening for scab has been an on-going process since 1988. Results from the 2007 MSU scab nursery indicate that 62 of 166 lines evaluated had a scab rating of 1.4 or less (better or equivalent to Pike). The limitation of breeding for scab resistance is the reliance on the scab nursery. We found a moderate correlation between the field screening and greenhouse screening for scab. We expanded the scab nursery with an additional acre of land nearby. This expansion has allowed us to conduct early generation selection for scab resistance among our breeding material. In 2007, 90 of 279 early generation selections showed strong scab resistance. These data were incorporated into the early generation evaluation process at Lake City. We are seeing that this expanded effort is leading to more scab resistant lines advancing through the breeding program.

**Late Blight:** With support from GREEN, the Muck Soils Research Farm, Bath, Michigan has become an excellent North American site for late blight testing because of the humid microclimate and isolation from major commercial potato production. As a result, late blight infection has been consistently achieved each year making breeding efforts to select late blight resistant germplasm very efficient. In 2007 41 of 160 advanced breeding lines were classified as late blight resistant. Of the early generation material tested 49 of 190 lines were late blight resistant.

**Colorado potato beetle:** With support from GREEN, we also introduced an early generation Colorado potato beetle screen at the Montcalm Research Farm. In 2007, 10 of 69 breeding lines were at least moderately resistant to Colorado potato beetle at the Montcalm Research Farm Beetle Nursery. The beetle pressure was extremely high leading to complete defoliation in all susceptible check lines. Percent defoliation was visually estimated during the beetle infestation in June and July. This resistant material was selected for further advancement in the breeding program and also for use in the next round of crossing to develop beetle resistant cultivars. Some of these lines are beginning to enter the preliminary trials in the breeding program. Concurrently, a field cage (no-choice) experiment was conducted to evaluate 3 avidin transgenic lines. In 2007 beetle behavior and defoliation was evaluated in lines that expressed differing levels of avidin. The data from this experiment has not been analyzed yet.

It is a great challenge to achieve host plant resistance to insects in a commercially acceptable line. We have some promising advanced selections with partial resistance to Colorado potato beetle. In addition, we have *Bt-cry3A* transgenic lines that could be commercialized if the processors renewed their acceptance and regulatory environment was modified to reduce costs. I am on a national committee to help build infrastructure to so that transgenic specialty crops like potato can be deregulated in a more efficient and less costly manner. However, the national potato industry needs to be supportive of this technology before we can move forward.

## **II. Sugar Profile Analysis of Early Generation Selections for Extended Storage: Chip-processing Results From the MPIC Demonstration Commercial Storage (October 2006 - June 2007)**

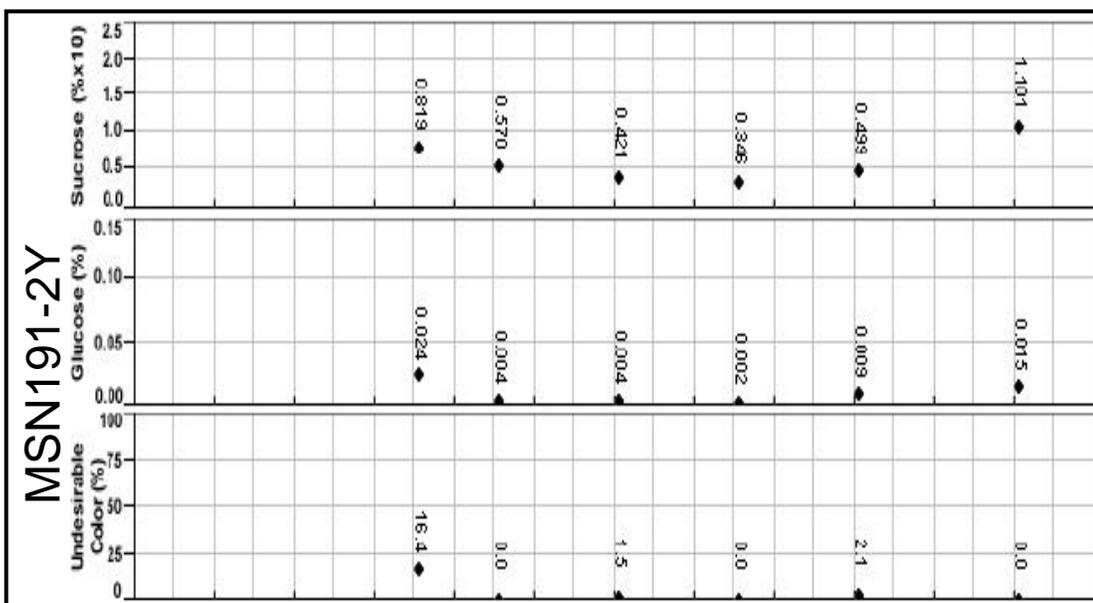
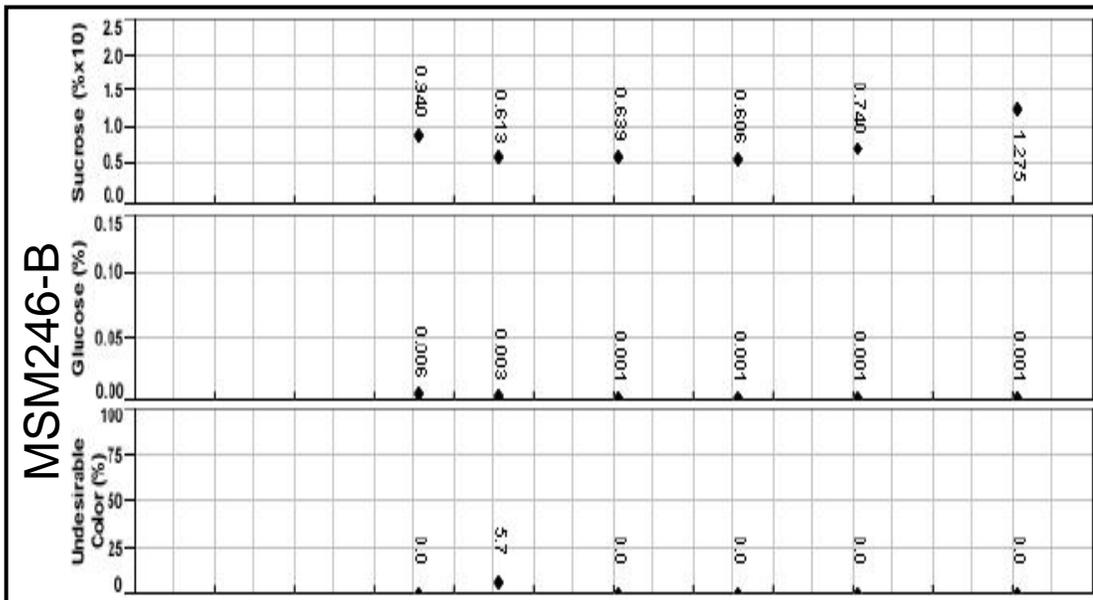
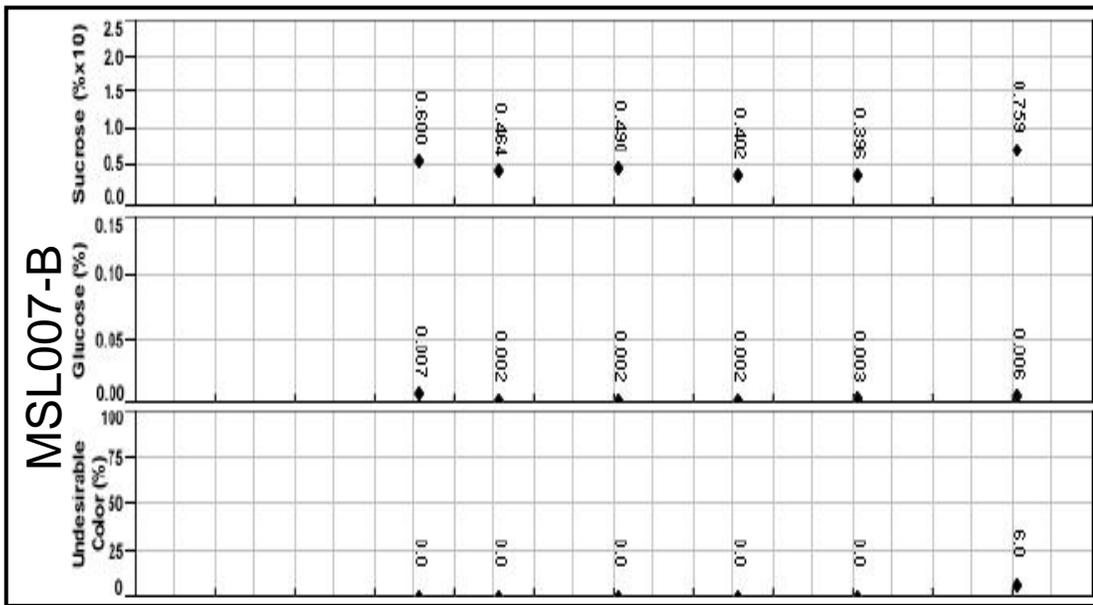
The MSU Potato Breeding Program has been conducting chip-processing evaluations each year on potato lines from the MSU breeding program and from other states. For 8 years we have been conducting a long-term storage study to evaluate advanced breeding lines with chip-processing potential in the Dr. B. F. (Burt) Cargill Potato Demonstration Storage facility directly adjacent to the MSU Montcalm Research Farm to identify extended storage chippers. We evaluated advanced selections from the MSU, Wisconsin and North Dakota breeding programs for chip-processing over the whole extended storage season (October-June). Tuber samples of our elite chip-processing selections were placed in the demonstration storage facility in October and were sampled 6 times to determine their ability to chip-process from storage. In October 2006, tuber samples from 12 MSU lines, 1 North Dakota line, and four Wisconsin lines from the Montcalm Research Farm and Lake City Experiment Station trials were placed in the bins. The first samples were sugar-profiled and chip-processed at TechMark in November and then five more times until June 4, 2007. Samples were evaluated for chip-processing color, defects and glucose and sucrose were measured. Because of the extremely high sugar levels of some samples in November and December, 5 lines were dropped from the sugar profiling study. These high sugar levels were attributed to the poor fall harvest conditions and will be retested in 2007-8.

**Table 1** summarizes the chip-processing color and scab rating of 12 lines over the 7-month storage season. From November to April all lines chip-processed acceptably with a 1.0 color score. This is not surprising since the best chip-processing lines were selected for this study. In some cases, SED or hollow heart was observed in a few chips, but no patterns emerged. In June the color darkened for 9 of the 12 lines and the storage test was terminated. Based upon the data, many of these lines have potential to be further tested in storage tests. Six lines had a good combination of color, low defects and desirable sugar profiles. **Figure 1** shows the TechMark sugar profiles of sucrose, glucose and undesirable color for three MSU lines (MSL007-B, MSM246-B and MSN191-2Y) that performed well in the study. MSL007-B has scab resistance. These and other lines will be tested further in 2007-8.

**Table 1. 2006-2007 Early Generation Sugar Profiling Study**  
SFA Chip Scores for seven months storage at the MPIC Demonstration Storage

Line	Female	Male	Date						2006 Scab
			11/6/06	12/4/06	1/15/07	2/26/07	4/11/07	6/4/07	
MSJ461-1 <sup>LBR</sup>	Tollocan	NY88	1.0	1.0	1.0	1.0	1.0	1.5	1.8
MSL007-B	MSA105-1	MSG227-2	1.0	1.0	1.0	1.0	1.0	1.5	0.8
MSM102-A	MSI111-A	MSG227-2	1.5	1.0	1.0	1.0	1.0	2.5	1.5
MSM246-B	MSE274-A	NY115	1.0	1.0	1.0	1.0	1.0	1.5	2.5
MSN099-B	MSG015-C	MSI111-A	1.0	1.0	1.0	1.0	1.0	2.5	1.3
MSN191-2Y	MSI234-6Y	MSH098-2	1.5	1.5	1.0	1.0	1.0	1.5	2.5
MSP292-7	MSF015-1	MSJ212-2	1.0	1.0	1.0	1.0	1.0	2.0	1.8
ND5775-3	unknown	unknown	1.0	1.0	1.0	1.0	1.5	1.5	2.0
W2133-1	Snowden	RHL167	1.0	1.0	1.0	1.0	1.0	1.0	2.0
W2310-3	unknown	unknown	1.0	1.0	1.0	1.0	1.0	1.0	1.8
W2324-1	Snowden	RHL166	1.0	1.0	1.0	1.0	1.0	1.5	2.6
W2717-5	unknown	unknown	1.0	1.0	1.0	1.0	1.0	1.0	2.5
Average			1.1	1.0	1.0	1.0	1.0	1.6	1.9

\*Note: These five lines were chipped at MSU beginning 1/15/07  
SFA Chip Score 1-5 scale: 1=excellent; 5=poor (>= 2.5 is considered unacceptable).  
Scab Rating: 0: No Infection; 1: Low Infection <5%; 3: Intermediate; 5: Highly Susceptible.



### **III. Germplasm Enhancement**

In 2007, only a few diploid populations were evaluated as single hills. From this breeding cycle, we plan to screen the selections chip-processing from storage. In addition, selections were made from over progeny that was obtained from the USDA/ARS at the University of Wisconsin. These families 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. Through GREEN funding, we were able to initiate a breeding effort to introgress leptine-based insect resistance. From previous research we determined that the leptine-based resistance is effective against Colorado potato beetle. We will continue conducting extensive field screening for resistance to Colorado potato beetle at the Montcalm Research Farm and in cages at the Michigan State University Horticulture Farm in 2008. In 2004 we made crosses with late blight resistant diploid lines derived from *Solanum microdontum* to our tetraploid lines. This *S. microdontum*-based resistance is unique and very effective against the US-8 strains. These progeny are being grown in the greenhouse and now we have used DNA marker analysis to identify which lines have the late blight resistance. We have conducted lab-based detached leaf bioassays and have identified resistant lines. We will field test these in 2008.

### **IV. Integration of Genetic Engineering with Potato Breeding**

#### **Segregation of Transgenes and Selectable Markers in Tetraploid Potato Crosses**

The insertion of transgenes into cultivated potato offers the opportunity to introduce novel genes/traits into the cultivated germplasm. If a transgenic event is commercialized in the US, the progeny generated from the transgenic lines are also approved for growing. Thus, transgenic lines can be valuable parents in a breeding program. We have generated transgenic lines and made crosses to examine segregation of transgenes and the *nptII* selectable marker. In one set of crosses we examined the segregation of the Bt-cryIIa1 gene and the *nptII* gene. We also tested a subset of the progeny for potato tuber moth resistance. We learned that the Bt gene is inherited in a simple genetic manner and all the progeny that carried the Bt gene were resistant.

In another set of crosses we examined the segregation of the *RB* gene and the *nptII* genes. In some of these crosses the *RB* gene was co-integrated with the *nptII* gene in the transgenic line. In another transgenic line the *RB* gene was independently integrated from the *nptII* gene so that *RB* and *nptII* would segregate independently in the progeny. Segregation data showed that we were able to select progeny that carried the *RB* gene, but not the *nptII* gene. Hence the progeny do not have the antibiotic resistance gene. Separating out the *nptII* gene makes transgenic potatoes have greater public perception. In addition, we have been able to combine the *RB* gene for late blight resistance with the conventionally bred resistance genes. We will further test these progeny in 2008.

#### **Commercialization of Potato Tuberworm Resistant Potatoes in South Africa**

The potato tuberworm (*Phthorimaea operculella* Zeller) is a primary pest problem facing potato farmers in developing countries. Currently, the primary means to control the potato

tuberworm and avoid major crop losses is the use of chemical pesticides. Michigan State University (MSU), funded by the U.S. Agency for International Development (USAID), initiated biotechnology research on the development of potato tuberworm resistant varieties in 1992. A *Bacillus thuringiensis* (Bt)-*cryIIa1* gene, was successfully introduced into several potato varieties and shown to be highly resistant to potato tuberworm in the Spunta-G2 line (both tuber and foliage). This Bt potato will be one of the first public sector developed products to reach farmers in developing countries and will serve as a model for the public sector deployment of insect resistant transgenic crops. The commercialization project includes six components: Product Development, Regulatory File Development, Obtaining Freedom to Operate and Establishing Licensing Relationships, Marketing and Technology Delivery, Documentation of Socio-Economic Benefits, and Public Communication. This technology would also have benefits in controlling PTM in the US and reducing the need for insecticide-based protection. In 2007 we focused on collecting the regulatory data that has to be submitted to the review agency. We also evaluated 15 Bt-progeny in replicated trials at the Montcalm Research Farm. All these lines are potato tuber moth resistant and many are also late blight resistant. Based upon the agronomic data this year, we have reduced the number of progeny to further advance to 7.

#### **V. Variety Release**

We are planning to release MSJ036-A as Kalkaska in 2008. We are continuing to promote the seed production and testing of Beacon Chipper, a 2005 release. In addition, we are continuing to promote Michigan Purple, Jacqueline Lee for the tablestock markets. Boulder is being commercially grown in Quebec. Commercial seed production has been initiated for MSN105-1, a round white potato for the tablestock market. Lastly, commercial seed of MSH228-6, MSJ147-1, MSK061-4 and MSJ126-9Y are being produced and we will continue to seek commercial testing of these lines. We have also initiated a focused ribavirin-based virus eradication system to generate virus-free tissue culture lines for the industry. Thirty lines are in ribavirin treatment at this time to remove PVS and PVY from lines. This year 74 new breeding lines are being put into tissue culture.

#### **MSU Variety releases**

##### **Michigan Purple**

**Parentage:** W870 x Maris Piper

**Developers:** Michigan State University and the Michigan Agricultural Experiment Station

**Plant Variety Protection:** Yes

**Strengths:** Michigan Purple is a purple-skinned tablestock variety with brilliant white flesh with low incidence of internal defects. The tubers have an attractive round-ovoid shape and a strong iridescent purple skin. Yield is high under irrigated conditions, and also performs well under dryland conditions.

**Weaknesses:** Susceptibility to common scab.

**Incentives for production:** The purple-skinned, white-fleshed tubers of Michigan Purple offer a unique type that could lend itself to the specialty variety market, such as gourmet restaurants and food stores, as well as farm and road-side markets.



### **MSJ036-A**

**Parentage:** B1254-1 X S440

**Developers:** Michigan State University and the Michigan Agricultural Experiment Station.

**Plant Variety Protection:** In preparation

**Strengths:** MSJ036-A is a high yielding, round white potato with an attractive round appearance with shallow eyes. MSJ036-A has a strong vine and a full season maturity. This variety has resistance to *Streptomyces scabies* (common scab) stronger than Pike. MSJ036-A also has chip-processing storage characteristics and better tolerance to blackspot bruise than Snowden.

**Weaknesses:** Sugar levels have to be watched at harvest during cold temperatures.

**Incentives for production:** High yield and good tuber type combined with scab resistance.



### **MSJ147-1**

**Parentage:** NorValley X S440

**Developers:** Michigan State University and the Michigan Agricultural Experiment Station

**Plant Variety Protection:** Will be considered.

**Strengths:** MSJ147-1 is a round white chip-processing potato that has a bright skin, white flesh and round shape. In addition, it has been determined to store at temperatures below 50F and maintain low reducing sugar levels into May or June.

**Weaknesses:** Small vine, slow to emerge.

**Incentives for production:** MSJ147-1 produces many A-size tubers that are low in defects. Potatoes maintain low reducing sugar content for chip-processing out of the field and from storage.



### MSJ461-1

**Parentage:** Tollocan X NY88

**Developers:** Michigan State University and the Michigan Agricultural Experiment Station, Michigan Potato Industry Commission

**Plant Variety Protection:** Plant Variety Protection is being considered for this variety.

**Strengths:** MSJ461-1 is a round white chip-processing variety with an attractive round shape and bright skin. The primary strength of this variety is its strong foliar resistance to late blight (*Phytophthora infestans*) combined with chip-processing quality. MSJ461-1 can also be marketed as tablestock because of its good culinary quality. The tubers will chip process out-of-the-field and from 10°C (50°F) storage. MSJ461-1 performed well in Michigan on-farm trials and regional testing. Under irrigated conditions, the yield is similar to Snowden. MSJ461-1 is being considered for release, although no name has yet been chosen for this line.

**Weaknesses:** The specific gravity of MSJ461-1 is lower than Snowden in Michigan.

**Incentives for production:** High yield with uniform tuber size combined with strong foliar resistance to late blight, GN resistance and tolerance to verticillium wilt. Can be used for both chip-processing and table use.



### **Jacqueline Lee (MSG274-3)**

**Parentage:** Tollocan x Chaleur

**Developers:** Michigan State University and the Michigan Agricultural Experiment Station

**Plant Variety Protection:** Yes

**Strengths:** Jacqueline Lee has a bright golden skin, yellow flesh, attractive oval shape and excellent cooking qualities that make it suitable for tablestock use. In addition, it has been determined to have a high level of foliar resistance to the US-8 genotype of *Phytophthora infestans* under Michigan field and greenhouse conditions.

**Weaknesses:** Susceptibility to common scab.

**Incentives for production:** The tubers have an attractive tuber type, with bright and smooth skin and a yellow flesh, typical of many European varieties. The plants yield a heavy set of medium-sized (3-6 oz.), uniform tubers. The foliage has a high level of resistance to the US-8 genotype of *Phytophthora infestans*. These medium-sized, uniform, oval-shaped tubers of Jacqueline Lee offer a unique type that could lend itself to the specialty variety market, such as farm and road-side markets.



**Boulder (MSF373-8)**

**Parentage:** MS702-80 x NY88

**Developers:** Michigan State University and the Michigan Agricultural Experiment Station

**Plant Variety Protection:** In application

**Strengths:** Boulder is a round white selection with medium specific gravity that can be used in both the tablestock and chip-processing markets. The tubers of Boulder are large in size with a low incidence of internal defects. Boulder yields well under both irrigated and dryland conditions.

**Weaknesses:** Over-size tubers may have deep eyes similar to Red Pontiac.

**Incentives for production:** High percentage of A-size tubers and excellent culinary quality.



## **Map-based cloning of a Major Late Blight Resistant QTL in *Solanum microdontum* (GREEN Project)**

**Problem Statement:** Potato (*Solanum tuberosum* L.) is the fourth most important food crop in the world and the top vegetable crop in the United States. In Michigan the potato is approximately \$120 million value crop of which the majority is processed as chips, making Michigan the premier state for supplying this market. Late blight caused by *Phytophthora infestans* (Mont.) de Bary is a significant constraint to potato production in the US and worldwide. One of the major goals of our potato breeding program is to introduce new market-quality cultivars with late blight resistance. Quantitative trait loci analysis (QTL) of a diploid mapping population identified closely linked markers associated with foliar resistance to late blight that explained about 70% of the disease reaction (Bisognin et al. 2004) and is resistant to all Michigan *P. infestans* isolates. Based upon the pedigree and resistance reaction of the *S. microdontum* germplasm, the resistance in this material is a unique and it needs to be utilized and combined with other late blight resistance genes. Recently other late blight resistance genes have been mapped and cloned from *S. bulbocastanum* (Song et al. 2003; van der Vossen et al. 2003), *S. mochiquense* (Smilde et al. 2004), and a complex genomic hybrid (Park et al. 2005). The resistance genes from these *Solanum* species offers race non-specific resistance unlike those previously utilized from *S. demissum*. Access to specific resistance genes combined with the ability to pyramid transgenes is a powerful strategy to study the interaction of *P. infestans* and host plant resistance and breed cultivars with durable host plant resistance. The purpose of this research is to initiate the map-based cloning of a major late blight QTL from *S. microdontum*.

The ability to transform major late blight resistance genes into potato provides a unique opportunity to pyramid late blight resistance genes in an analytic manner. In this way we could study the interaction of *P. infestans* and single and combined gene-based host plant resistance. Moreover, the pyramided resistance genes in a single genotype should be a better strategy to deploy late blight resistant potato varieties (Dangl and Jones 2001).

### **Objectives:**

1. Construct a high resolution map of the late blight QTL region in *S. microdontum* to identify tightly linked flanking markers.
2. Conduct late blight assessment of mapping population.
3. Create a BAC library from *S. microdontum*.

### **Accomplishments:**

**1. Construct a high resolution map of the late blight QTL region in *S. microdontum* to identify tightly linked flanking markers.**

A mapping population, based upon SSRs and AFLPs, was established between a late blight resistant *S. microdontum* selection (TF75-5) and a susceptible diploid clone (MSA133-57). SSR marker, STM0020, was identified to be tightly linked to a major

QTL affecting late blight resistance (Bisognin et al. 2004). We currently have 5 markers (including STM0020) linked to the late blight resistance QTL.

The genetic marker STM0020 was placed on Chr. 4. A second marker, STM1002 (aka. S9101 and X67511), had earlier been placed in the same linkage group as STM0020 and it too has since been mapped to Chr.4, thus confirming the placement of our QTL on Chr. 4. Since that time, over 40 Chr. 4 specific markers (Table 1) have been screened using a subset of a mapping population to identify polymorphisms between late blight resistant and sensitive individuals. To date, none of these markers has resulted in a difference that allowed distinct identification of the resistance phenotype. Additional markers will need to be tested in order to develop a high resolution map of the region surrounding the late blight QTL. With this QTL being located on Chr. 4, it may be associated with the R-gene hotspot that already includes R2, R2-like and Rpi-blb3 genes for late blight resistance.

An alternative PCR based approach for isolating resistance genes analogs (RGAs) has also begun. This approach uses conserved regions found in most of the cloned R genes to design primers which are then used to amplify RGAs from genomic DNA. This approach has been used by others to map resistance genes in a number of different plants. In our situation, if any RGAs are found that map to Chr. 4, they will be considered candidates genes from which additional markers can be generated. To date, this protocol has yielded a few classes of amplification products (Fig. 1) which will be sequenced and subsequently compared to databases to confirm they contain putative RGA DNA.

## **2. Conduct late blight assessment of mapping population.**

The greenhouse grown mapping population was subjected to detached leaf assays with three replications. Fully expanded and healthy leaflets were collected and placed in Petri dishes in which high moisture is maintained. Each leaflet was sprayed with a suspension of *P. infestans* US-8 isolate (Pi02-007, the most aggressive isolate). The leaflets will be evaluated for percent infection at 6, 9 and 12 days according to Kuhl et al. (2001).

Based upon the 12 day percent infection the population had a continuous distribution for reaction to late blight. Some progeny were more resistant than the original *S. microdontum* source of resistance (TF75-5). This mapping population will be useful for fine-mapping studies

## **3. Create a BAC library from *S. microdontum***

Clemson University Genomics Institute (CUGI) was chosen to produce a BAC library because of their extensive experience in creating custom large insert BAC libraries with an average insert size generally ranging between 120 and 160kb. CUGI has produced numerous plant, animal and microbial BAC libraries encompassing many different taxa in the tree of life. The *S. microdontum* BAC library was constructed using *HindIII* digested genomic DNA. Greater than 36,000 colonies/clones were picked, each containing inserts of at least 120kb of sequence which in total represents about 5 times coverage of the entire genome. The library was completed in June 2007 and we have plates containing each individual clone as well as arrayed filters for screening.

### Impacts:

We are making forward progress in cloning a putative R-gene from *S. microdontum* for late blight resistance. The ability to transform major late blight resistance genes into potato provides a unique opportunity to pyramid late blight resistance genes in an analytic manner. In this way we will be able to study the interaction of *P. infestans* and single and combined gene-based host plant resistance and extend our knowledge of fundamental host-pathogen interactions. Moreover, the pyramided resistance genes in a single genotype should be a more durable strategy to deploy late blight resistant potato varieties (i.e., should be more difficult to overcome multiple new R genes than one R gene at a time). The use of late blight resistant varieties will lessen the need for weekly protectant fungicides programs. As a result, production costs can be lowered and environmental impact of the fungicides will be lessened.

### Photos, charts, graphs:

Table 1. Screened Markers

SSR Markers	SCAR Markers	CAPS Markers	Others
STM0020	Th21	TG506R	E35M54.G
STM1002	TG339	TGH2	E32M48.b
STM3016	CT229	cLPT5B19	E35R50.a
STM5112	At4g09010	AF411807L	E32M61.b
STM5140	At3g16150	RGH3	GP511
STM5156	At1g42990	TG370F	GP180-a
STG008	At1g76080	B57R	
STG018	B1b22T	54I8L	
STG020	rpib1b3-1	139K15L	
STI001	rpib1b3-2	B10L	
STI012	rpib1b3-3	B1b22S	
STI020		B1b25S	
STI026		B1b25T	
STI055			

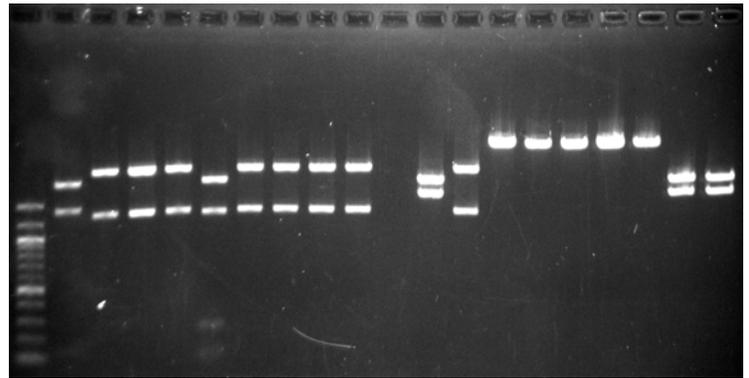


Figure 1. Restriction Digest of Putative RGA DNA. PCR amplified DNA using degenerate primers was cloned into pGEM T-Easy (Promega), transformed into *E.coli*, cultures grown and plasmid isolated. The plasmid was digested with *RsaI* and run on a 1% agarose gel. Lane M is a 100bp DNA marker. Lanes 1-1 through 1-9 and 2-1 through 2-9 represent individual clones originating from different sets of primer pairs respectively. X is an empty lane. This image shows at least four distinct classes of insert DNA based on banding pattern. Lanes 2-3 through 2-7 insert DNA was not cut by *RsaI*.

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## **What has that Potato Breeding Program been doing?**

This year marks a time of 20 years at Michigan State University and I have been continuously involved in potato breeding and genetics research for 25 years. This spring will begin the 20<sup>th</sup> year of the breeding program since I made my first crosses in Michigan and it seemed like a time for reflection. I fondly remember meeting with Dick Chase, Ben Kudwa and a few growers during the interview process and learning what the industry needs. There was a need for a better storage chipper than Atlantic and varieties with improved internal quality, scab resistance, bruise resistance and with high specific gravity. Potato early die was also a concern. In the table market there was a need for a better Yukon Gold and Onaway market type. With an emphasis on round white potatoes, I set off to work towards these objectives. Ray Hammerschmidt and I teamed up on the scab resistance efforts and with George Bird on the potato early die research.

I learned that the genetics of scab resistance is not well understood and characterizing scab resistance has a strong environmental interaction. There were also few lines that had scab resistance that were not russets. In these first four years we were trying to learn what parents could transfer the desired traits we needed. During that time we made and evaluated a few thousand crosses and this helped focus further crossing schemes to improve our chances of finding desirable varieties. Early on there was a common pattern: if the scab resistance was in a line, the chip quality was usually poor. The reverse was also common. Combining scab resistance with chip-processing quality was a challenge.

We also had to establish a tissue culture lab so for line maintenance and for providing the industry with plantlets in seed increase. Today we are now using ribavirin to remove virus from the advanced breeding lines that are brought into tissue culture. In 1992 we moved the breeding program to Lake City where we had better disease and insect isolation. This helped reduce the leaf roll and PVY infection. Also Colorado potato beetle was developing resistance and we began looking into Bt genes and natural resistance from the wild species as a long-term effort to breed beetle resistant potatoes. We established research collaborations with Ed Grafius and Walter Pett. Through USAID we were able to obtain funding to use genetic engineering techniques to introduce genes for insect resistance. This line of funding continues through 2008.

In the second four years, Dr. Kaz Jastrzebski joined us from Poland and we identified many lines with excellent chip quality from storage. During this period we created the foundation for the chip processing quality in our breeding material. Scab resistance was elusive. We learned that the scab evaluation trials in the scab nursery required multiple years to confidently identify resistant lines, whereas other traits were more quickly characterized.

Then in the mid-1990s came the re-emergence of late blight, but it was a more aggressive strain than what was observed in the past. All breeding programs across North America began to add late blight resistance to the breeding objectives. There was a lucky convergence in Michigan. Willie Kirk joined MSU and the Muck Soils Research Farm

was the best site in the US for testing for late blight resistance. Adding late blight resistance to our breeding objectives created new challenges. Identifying resistance and learning the breeding value of the resistance sources required extra effort and also led to another set of observations. The late blight resistant sources were very late maturing, scab susceptible and poor chippers. It was a challenge to combine chip-processing quality, scab resistance and late blight resistance. With the Muck Soils Farm Research farm being such a reliable late blight testing site. We proposed that that we could use early generation selection for late blight resistance. This effort increased our breeding efficiency. We were able to select numerous late blight resistant lines and began to find lines that combined chip-processing or scab resistance with late blight resistance.

Comparing our breeding progress in late blight resistance to our progress in scab resistance, scab resistance breeding was not as efficient. We attributed this to the difficulty of accurately identifying resistant lines. We made the decision to expand the scab nursery, create higher levels of scab infection in the soil and attempt early generation selection. It took us five years to create a new scab nursery with high infection levels on the MSU campus and we then began to expand our scab evaluation to early generation selections in the breeding program.

We now screen all our early generation selections (8, 20, 30 and 50-hill selections) for late blight resistance, scab resistance, specific gravity and chip processing each year and incorporate this data into the selection process in the field. We also have established a Colorado potato beetle nursery at the Montcalm Research Farm for screening the breeding material that has been bred for insect resistance. In the lab we use DNA analysis to identify the Golden Nematode and PVY resistant lines. Advanced breeding lines are evaluated for all these traits above along with vine maturity, blackspot bruise resistance, yield, internal defects and chip-processing from storage. Select lines are screened in the potato early die trial at Montcalm and advanced chip-processing lines are evaluated in the Demonstration Storage. Chris Long takes the promising lines from the program and moves them through on-farm trials that complement the data from the Montcalm Research Farm. The most promising chip-processing lines for commercialization are tested in the 10 and 500 cwt bins and also sugar profiled.

Along side the development of the breeding program in the past two decades, there has been the concurrent development of the commercial seed production of MSU lines, commercial testing, construction of the Demonstration storage building, variety release mechanisms, plant variety protection, licensing of the varieties and determination of royalty structure. These steps, despite not being research oriented are critical to the successful commercialization and release of an MSU variety.

In 2007 we have two MSU lines in the Demonstration storage bins. We see MSJ036-A (Kalkaska), a cross from 1997, being released by MSU and commercialized. It is a scab resistant chipper. We have a series of other scab resistant chipping lines that will be evaluated on commercial scale in the future (MSH228-6, MSK061-4, MSJ126-9Y and MSK409-1). Interestingly, the scab resistance in these chippers comes from different sources. This gives us the opportunity to make improvements in scab resistance.

Moreover, the pedigrees also indicate that chip-processing (low sugars) in these lines are from different sources. So we also have the ability to make further genetic improvements in chipping.

Also in the storage is MSJ147-1. This line is an excellent long term storage chipper that can process from colder storage temperatures. If you study the pedigree you will see that multiple sources of chip-processing quality including two wild species from South America. On the female side ND860-2 (cold chipper derived from *S. phureja*) is crossed to Norchip. On the male side the cold chipping is introduced from S440 which has *S. tarijense* as a unique cold chipping source.

A line that has been very important in our breeding program is MSJ461-1. This round white potato has high yield, uniform round white potatoes with low incidence of internal defects, strong late blight resistance, Golden nematode resistance along with Verticillium wilt resistance. It has become an important parent to combine with our scab resistant chipping potatoes. We are now a number of 'next generation' advanced breeding lines that combine late blight and scab resistance with chip -processing. One clone, MSQ070-1, is being sugar profiled this storage season. With a desirable sugar profile, we will consider fast tracking this line. Three of the 'next generation' lines also are PVY resistant and many are Golden nematode resistant. We are very excited about the future!

When I look at the breeding material and varieties in our program up to this time, we have an excellent genetic base. We have incorporated 15 different sources of scab resistance into our advanced lines and varieties, 9 sources of late blight resistance and 20 sources of chip-processing. We have also broadened the genetic base by using 6 different wild potato species to bring in traits such as scab resistance, late blight resistance, chip-processing from cold storage, PVY resistance, Verticillium wilt resistance, high specific gravity and Colorado potato beetle resistance. With this material we will be able to continue to make genetic improvements. When we combine this with our genetic engineering efforts, we are in for an exciting next 20 years!

Lastly, for all this to work, the human capital is very important. The MSU breeding and genetics program will not work without the great work of Joe Coombs, Kelly Zarka, Jay Estelle, Devan Berry, Donna Kells, Dick Crawford (at Montcalm Research Farm) and many of the past technical staff over the years. In the industry, having great cooperators in the seed industry (Jeff Axford, Kruegers, Sklarzycks, Iotts), commercial growers (Sandyland, Lennards, Walthers, Sackett Potatoes, Sackett Acres, Crooks, Leep) have been valuable in moving the MSU advanced selections through the breeding program and along the commercialization path.

I feel much honored to be hired by Michigan State University to run the potato breeding and genetics program. The opportunity to run my own potato breeding program and release varieties important to the industry is an ideal career and I am still working hard to achieve my goal of breeding improved varieties.