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2005 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 program 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 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 11 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 and early die), 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 material 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 reported in the annual Potato Variety Evaluation Report.

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 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-compatability), *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 13 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-cry1Ia1* and avidin), potato tuber moth, late blight resistance via the *RB* gene, drought resistance (*CBF1*) 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 2005 field season, progeny from over 600 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 2005 harvest, over 1000 selections were made from the 45,000 seedlings produced. All potential chip-processing selections will be tested in January or March 2006 directly out of 40°F and 45°F storages. Atlantic (50°F chipper) and Snowden (45°F chipper) are chipped 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, 231 and 87 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

About 82% 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 MSH095-4, MSJ036-A (scab resistant), MSH228-6 (moderate scab resistance), MSJ147-1, MSJ126-9Y (moderate scab resistance), MSJ316-A (moderate scab resistance), MSK061-4 (moderate scab resistance), MSK409-1 (scab resistant), MSM051-3 (scab resistant) and late blight resistant chipper MSJ461-1.

Dr. Joe Sowokinos, Univ. of Minnesota, has conducted biochemical analyses of our best chipping lines and has discovered that our lines differ from older varieties in their proteins (UGPase) involved in chipping. Some of these lines are MSJ147-1, MSG227-2 and MSJ126-9Y. Moreover, MSJ147-1 and MSJ126-9Y have the desirable levels of acid invertase to chip process from colder storage. His analysis will also allow us target specific crosses to find improved chip-processing varieties that will allow processing from colder storage temperatures.

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. 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 2005, while others were tested under replicated conditions at the Montcalm Research Farm. Promising tablestock lines include MSE221-1 as a scab resistant tablestock, and MSN084-3, a round white with a smooth round shape and bright skin. We have a number of tablestock selections with late blight resistance. These are MSK128-A, MSL072-C and MSM171-A. MSL211-3 and MSN105-1 has late blight and scab resistance. MSE192-8RUS and MSA8254-2BRUS are two russet table selections that have scab resistance. MSI005-20Y is a yellow-fleshed line with smooth round appearance and high yield potential.

At the Great Lakes Expo the MPIC sponsored a booth where we helped promote the sale of Michigan Purple, Jacqueline Lee and Liberator seed to the roadside stand and farm market operations.

Disease and Insect Resistance Breeding

Scab: Disease screening for scab has been an on-going process since 1988. Results from the 2005 MSU scab nursery indicate that 95 of 201 lines evaluated demonstrated little to no infection to common scab. The limitation of breeding for scab resistance is the reliance on the scab nursery. The environmental conditions can influence the infection each year, thus multiple year data provides more reliable data. A laboratory-based screening process is currently under development that would use thaxtomin in tissue culture to expedite selection of material with potential scab resistance. In 2004, 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 2005, 156 of 464 early generation selections showed moderately to strong scab resistance. These data were incorporated into the early generation evaluation process at Lake City. We expect that this expanded effort will lead to more scab resistant lines advancing through the breeding program.

Late Blight: 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 2005 we evaluated over 100 advanced breeding lines for late blight resistance. Forty one lines were classified as resistant. Ninety eight of 801 early generation selections were resistant. The breeding program has been able to identify advanced breeding lines with strong foliar resistance to late blight. MSJ461-1, a round white chip-processor, is being considered for release and commercialization. MSJ461-1, the chip-processing selection, has the same late blight resistance source as Jacqueline Lee. Our other promising late blight resistant lines that have been tested in replicated agronomic trials (see Potato Variety Evaluation Report for agronomic data). In each of these lines, the resistance is based on a single resistance source. If we rely on a single source of resistance, the varieties developed from this strategy may be overcome by *P. infestans* at some future date that we cannot predict. Therefore, the most effective breeding strategy is to combine resistance from different pedigrees to build a more durable resistance. Our efforts are now focusing on pyramiding the different resistance sources. These lines are in the early generation phase of the breeding program.

The Muck Soils Research Farm is also used for early generation selection for late blight (801 lines evaluated), genetic studies involving late blight resistance, screening germplasm from other US breeding programs (USDA/ARS funded) and Dr. Kirk-led fungicide x variety management studies to determine schemes to reduce fungicide usage when late blight resistant cultivars are grown. In 2005 we also screened our *RB*-transgenic potatoes for their foliar resistance to late blight. This gene holds promise to introduce or increase late blight resistance in our advanced material.

Colorado potato beetle: With support from GREEEN, we also introduced an early generation Colorado potato beetle screen at the Montcalm Research Farm. In 2005, 69 breeding lines from the MSU potato breeding program that had Colorado potato beetle resistant germplasm in their pedigree were evaluated 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. The lines were then sorted into four categories: susceptible, reduced susceptibility, moderately resistant and resistant. The majority of the lines were susceptible, but 6 and 4 lines were classified as moderately resistant and resistant, respectively. The majority of the lines that were moderately resistant or resistant can be attributed to the expression of the *Bt-cry3A* gene or glycoalkaloid/leptine based mechanisms. The most 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. Concurrently, a field cage (no-choice) experiment was conducted to evaluate 6 lines. In 2005 beetle behavior was evaluated in lines that expressed differing levels of Bt genes. The data from this experiment has not been analyzed yet. The study will be repeated in 2006**.**

It is a great challenge to achieve host plant resistance 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.

II. Evaluation of Advanced Selections for Extended Storage: MSU Potato Breeding Chip-processing Results From the MPIC Demonstration Commercial Storage (October 2004 - June 2005)

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 6 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 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 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 lines.

In October 2004, tuber samples from 10 MSU lines, three Frito Lay lines, B0776-3, along with Pike and Atlantic from the Montcalm Research Farm trials were placed in the bin to be cooled to 52°F. The bin temperature in November was 58°F and reached a low of 55.8°F in March. Tubers from 5 other MSU lines and Snowden were placed in the bin that was to be cooled to 48°F. The bin was at 54°F in November and held at 49.2°F. The first samples were chip-processed at MSU in October and then, each month until May 2005. Samples were evaluated for chip-processing color and quality.

Table 1 summarizes the chip-processing color of select lines over the 8-month storage season. In the standard temperature bin, Atlantic and Pike were the check varieties. From November to March all lines chip-processed acceptably. The storage test was terminated in mid-May. The tubers were physiologically stressed and many of the tubers had a black necrosis in the flesh and resultant chips. Only Beacon Chipper (UEC) and FL1922 were free of the black necrosis. Based upon chip color and defects the most promising chip-processing lines for long term storage are Beacon Chipper, MSM051-3, MSJ036-A, and FL1922. MSM051-3, MSJ036-A and FL1922 offer scab resistance in addition to their chip-processing ability. MSJ461-1 also has strong foliar resistance to late blight.

In the bin for colder temperature storage, Snowden was used as check variety and chip-processed acceptably through May, but had some black necrosis in the May chip sample. MSG227-2, MSH095-4, MSJ080-1, MSJ126-9Y and MSJ147-1 produced acceptable chips throughout the storage season. Unlike Snowden, none of the MSU lines had black necrosis in the tuber or chip samples. MSJ080-1 is being dropped because of the low specific gravity. MSG227-2 and MSJ126-9Y offer scab resistance along with their ability to chip-process. MSG227-2, Beacon Chipper, MSJ461-1 and Liberator were also in the 500 cwt storage bins. See Chris Long's storage report for those results and results from the box bins.

III. Germplasm Enhancement

In 2005, less than 5% of the populations evaluated as single hills were diploid. 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 GREEEN 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 at the Michigan State University Horticulture Farm in 2006. 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 will evaluated these lines are the Muck Farm in 2006.

IV. Integration of Genetic Engineering with Potato Breeding

Combining engineered and natural host plant resistance to *Phytophthora infestans* in cultivated potato

General susceptibility of potato cultivars to *Phytophthora infestans* (Mont.) de Bary is a major concern for potato production. The major resistance gene *RB* was cloned from *Solanum bulbocastanum* Dun.a diploid (2n=2x=24) Mexican species that is highly resistant to all known races of *P. infestans*. The objective of this work is to combine conventionally bred sources of resistance with the *RB* gene via *Agrobacterium* transformation. Our hypothesis is that by pyramiding engineered resistance with natural plant resistance we expect to obtain stronger and more durable resistance to potato late blight. Therefore, this study was undertaken to test the effectiveness of the *RB* gene on its own by transforming late blight-susceptible clones (Atlantic, and the breeding line MSE149-5Y), and to test the effectiveness of the gene in combination with natural late blight resistance by transforming resistant clones (Stirling, and the advanced breeding line MSJ461-1). In 2005 we identified 5 lines with RB-based late blight resistance (MSE149-5Y, Spunta and MSG227-2) at the Muck Soils Research Farm trials. The Spunta and MSE149-5Y lines were used in crosses to transfer the RB-based resistance to other genetic backgrounds in the breeding program.

Insecticidal activity of avidin against Colorado potato beetle larvae, *Leptinotarsa decemlineata* (Say)

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the most destructive insect pest of potato, *Solanum tuberosum* (L.) in eastern North America. The insect has adapted to every insecticide used to manage it. Avidin is a protein found in chicken egg whites that has demonstrated insecticidal properties against a number of Lepidopteran and Coleopteran pests. This protein protects the chicken embryo by sequestering biotin from disease causing organisms. Biotin is an essential co-enzyme required for all organisms, including insects. Biotin is a cofactor of a carboxylase which is required for many important processes like lipogenesis, gluconeogensis, fatty acid and amino acid catabolism. Without this co-enzyme, an insect's growth is severely stunted, eventually leading to death. The gene for avidin production has been cloned and inserted into a few crops, including maize, tobacco and potato and has demonstrated resistance to a wide spectrum of insect pests. We have expressed avidin in two potato lines: MSE149-5Y, a susceptible potato line, and ND5873-15, a high glycolakaloid line. Detached leaf bioassays were performed on transgenic and non-transgenic clones of MSE149-5Y and ND5873-15 using Colorado potato beetle neonates and third instars. Survivorship and consumption were measured every 2d over a 12d period for neonates and avidin was effective in reducing growth and increasing larval morality. These tests were repeated in 2005 with similar results. We are excited that avidin is protein that we can use to complement the natural or Bt-based resistances.

USAID-funded International project to Develop Potato Tuber Moth Resistant Potatoes

Potato tuber moth, *Phthorimaea operculella* (Zeller), is the most serious insect pest of potatoes worldwide. The introduction of the *Bacillus thuringiensis* (Bt) toxin gene via genetic engineering offers host plant resistance for the management of potato tuber moth. The primary insect pest in Egyptian potato production, like many other countries in the Middle East, is the potato tuber moth. Recently it has emerged as a pest in Washington State and has also been a serious problem in Mexico.

Two transgenic 'Spunta' clones, G2 and G3, have been identified that produced high control levels of mortality in first instars of potato tuber moth in laboratory tuber tests (100% mortality), and field trials in Egypt (99-100% undamaged tubers). Reduced feeding by Colorado potato beetle first instars was also observed in detached-leaf bioassays (80-90% reduction). Field trials in the U.S. demonstrated that the agronomic performance of the two transgenic lines was comparable to 'Spunta'. In 2004 and 2005 the Spunta lines were resistant to the potato tuber moth and the Colorado potato beetle in Washington State. We are currently working with USAID, Syngenta and South Africa to commercialize the Spunta-G2 line. We have also transformed Ranger Russet, Jacqueline Lee and some South African varieties with the *Bt-cry1Ia1*.

V. Variety Release

No lines are planned for release in 2006, but we are continuing to promote the seed production and testing of Beacon Chipper, a 2005 release. In addition, we are continuing to promote Liberator, Michigan Purple and Jacqueline Lee for the tablestock markets. Lastly, commercial seed of MSJ461-1, MSE192-8RUS, MSH228-6, MSJ147-1 and MSJ036-A are being produced and we will continue to seek commercial testing of these lines.

Table 1.

2004-2005 Demonstration Storage Chip Results
Michigan State University Potato Breeding and Genetics
Montcalm Research Farm
Chip Scores: SFA Scale†

		Sample Dates:					
	Date:	11/3/2004	12/1/2004	1/12/2005	2/9/2005	3/9/2005	5/19/2005
Line	**Temp:**	58 °F	58 °F	58 °F	58 °F	56 °F	55 °F
ATLANTIC		**1.5**	**1.0**	**1.0**	**1.0**	**1.0**	**2.5**
Beacon Chipper (UEC)		1.0	1.0	1.0	1.0	1.0	1.0
B0766-3		1.5	1.0	1.0	1.5	1.0	1.0
FL1833		1.0	1.0	1.0	1.0	1.0	1.0
FL1879		1.5	1.0	1.0	1.0	1.0	2.0
FL1922		1.0	1.0	1.0	1.0	1.0	1.0
LIBERATOR		1.0	1.0	1.0	1.0	1.0	2.0
MSH228-6		1.0	1.0	1.0	1.5	1.0	2.0
MSJ036-A		1.0	1.0	1.0	1.0	1.0	1.0
MSJ461-1		1.0	1.0	1.0	1.5	1.0	2.0
MSK061-4		1.5	1.0	1.0	1.0	1.0	1.5
MSK128-A		1.0	1.0	1.0	1.0	1.0	2.5
MSK136-2		1.5	1.0	1.0	1.5	1.0	2.5
MSL007-B		1.0	1.0	1.0	1.0	1.0	1.0
MSM051-3		1.5	1.0	1.0	1.0	1.0	1.0
PIKE		**1.0**	**1.0**	**1.0**	**1.0**	**1.0**	**2.0**
	Temp:	54 °F	54 °F	51 °F	49 °F	49 °F	55 °F
MSG227-2		1.0	1.0	1.0	1.0	1.0	1.0
MSH095-4		1.0	1.0	1.0	1.0	1.0	1.0
MSJ080-1		1.0	1.0	1.5	1.0	1.0	1.5
MSJ126-9Y		1.0	1.0	1.5	1.0	1.0	ND
MSJ147-1		1.5	1.0	1.0	1.0	1.0	1.0
SNOWDEN		**1.0**	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**

†Snack Food Association Chip Score

Ratings: 1 - 5
1: Excellent
5: Poor

Chip scores were from two-slice samples from five tubers of each line collected at each sample date.