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Half-sib progeny evaluation and selection of potatoes resistant to the US8 genotype of *Phytophthora infestans* from crosses between resistant and susceptible parents

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Summary

The objectives of this study were to evaluate the use of potato (*Solanum tuberosum* L.) late blight (*Phytophthora infestans* (Mont.) de Bary) resistant parents in cultivar development and identify superior clones possessing moderate to high late blight resistance combined with acceptable maturity and tuber quality. Ninety-five crosses were made between eight unadapted parents with reported late blight resistance (B0718-3, Bertita, Bzura, Greta, Libertas, Stobrawa, Tollocan and Zarevo) and susceptible parents (cultivars or advanced breeding clones) adapted to North American growing conditions. A total of 408 field selected clones were assessed for late blight resistance in the greenhouse and in the field using a mixture of US8 *P. infestans* isolates (A2 mating type, metalaxyl resistant) that overcame all known R-genes except R8 and R9. Clones with $\leq$ 10% infected foliar area in the greenhouse test or $\leq$ 0.30 RAUDPC (relative area under the disease progress curve) value in the field in 1998 were re-tested in 1999. A total of 118 (29% of 408) putative late blight resistant clones were selected. The eight late blight resistant parents differed in both the ability to transmit late blight resistance and in the level of resistance transmitted to the progeny. The Tollocan and B0718-3 families (half-sib progeny) had the greatest degree of resistance and frequency of resistant clones. Scott-Knott cluster analysis ranked 79 clones (67% of 118) in the high and moderate late blight resistant groups. Among these 79 clones, 19 clones had vine maturity equal to or earlier than mid-season combined with acceptable tuber quality. Further selection in 2000 resulted in eight advanced selected clones (six from Tollocan and two from B0718-3 families) with the same level of resistance as the parent combined with vine maturity and tuber quality equivalent to Atlantic, a standard cultivar for chip processing in North America. The results indicate that this breeding approach can be used to select parents for late blight resistance breeding and to identify superior clones with high levels of late blight resistance and marketable vine maturity and tuber quality.

Introduction

Late blight, caused by the fungal-like oomycete *Phytophthora infestans* (Mont.) de Bary, is present in almost all potato (*Solanum tuberosum* L.) growing areas (Ross, 1986; Henfling, 1987; Kamoun et al., 1999). Late blight epidemics result from rapid asexual reproduction of the pathogen in potato tissue (Henfling, 1987). *Phytophthora infestans* can complete an asexual cycle from initial infection to production of sporangia in less than five days and sporangia can be washed from foliage into soil where the spores can infect tubers (Fry & Goodwin, 1997). Infected tubers may rot in storage or become a primary source of inoculum for the following season if used as seed. Yield losses in potato caused by *P. infestans* were estimated

to exceed $2 billion annually worldwide (Kamoun et al., 1999).

Challenges to the development of late blight resistant cultivars include the association between resistance and late maturity, durability of resistance and poor tuber quality in the resistant parents. Late blight resistant cultivars are more likely to have late maturity and indeed the most significant quantitative trait locus for foliar resistance across three environments was mapped in the same position as late maturity (Collins et al., 1999). Horizontal resistance is a form of durable resistance to *P. infestans* (Colon et al., 1995; Umaerus et al., 1983), is effective against a broad range of pathogen races, and should be pursued in breeding for late blight resistance (Umaerus & Umaerus, 1994). Recent breeding efforts have resulted in the identification of potato late blight resistant sources (Douches et al., 1997), evaluation of the resistant phenotype stability (Haynes et al., 1998) and in the release of late blight resistant germplasm (Goth & Haynes, 1997). However, the majority of the late blight resistance sources are not adapted to North American growing conditions, because of late maturity and marginal tuber characteristics (appearance, specific gravity, sugar level, defects, etc).

Although efforts have been made to develop late blight resistant cultivars, two-thirds of 147 North American cultivars and breeding clones were classified as very susceptible (Douches et al., 1997) and no cultivar currently grown has an adequate level of resistance (Helgeson et al., 1998). Late blight susceptible cultivars have early maturity, good tuber appearance and specific gravity (Douches et al., 1996), and good chip processing quality (Love et al., 1998). As late blight resistance is not considered a characteristic that confers enough advantage for a clone to become a successful cultivar (Umaerus et al., 1983), new cultivars must combine resistance with acceptable maturity and tuber quality characteristics for tablestock and processing markets.

Progeny evaluation has been suggested as a means to study the inheritance of quantitative traits and to identify superior parents for breeding (Bradshaw & Mackay, 1994). Progeny evaluation can also reduce the time for each cycle of recurrent selection if parents with good general combining ability are identified shortly after hybridization (Bradshaw et al., 1995). An extension of progeny evaluation is a multi-trait evaluation of half-sib progeny. The research reported here is a half-sib progeny evaluation of late blight resistant parents crossed with a set of susceptible parents possessing acceptable maturity and tuber quality. The objectives of this research were to evaluate the use of late blight resistant parents in cultivar development and to identify superior clones possessing moderate to high resistance combined with acceptable maturity and tuber quality.

Materials and methods

Crosses, segregating populations and evaluated clones

In this study, eight late blight resistant parents were crossed with susceptible parents to develop 95 segregating populations (Table 1). The parents B0718-3, Bertita, Bzura, Greta, Libertas, Stobrawa, Tollocan, and Zarevo have reported resistance to late blight (Goth & Haynes, 1997; Douches et al. 1997; Haynes et al., 1998), but were not well adapted to North American growing conditions. The susceptible parents were cultivars or advanced breeding clones evaluated in previous field trials (Douches et al., 1996) and greenhouse studies (Douches et al., 1997). All susceptible parents are adapted to North American growing conditions. For each cross, 50 seedlings (4,750 seedlings total) were transplanted at the Michigan State University Montcalm Experiment Station, Entrican, Michigan in 1997 with 75 cm within-row spacing between plants. At harvest, approximately 10% of the best clones from each cross were selected based on overall appearance and tuber number, shape, and internal defects. These selected clones were tested in greenhouse and field trials for late blight reaction in 1998 (Table 2). Clones with $\leq$ 10% infected foliar area in the greenhouse test or $\leq$ 0.30 relative area under the disease progress curve (RAUDPC) in the field in 1998 were re-tested in 1999 (Table 3). Advanced selected clones possessing late blight resistance combined with acceptable maturity and tuber quality were further evaluated in 2000. The value of each late blight resistant parent was determined by the performance of its half-sib progeny. For the remainder of this paper, family refers to half-sib progeny.

Characterization of P. Infestans *isolates and inoculum preparation*

All *P. infestans* isolates were obtained from late blight infected potato crops in Michigan and were characterized as US8/A2 mating type, the most common and aggressive genotype of *P. infestans* currently present

Table 1. Late blight resistant parents (top row) and susceptible adapted cultivars/advanced breeding clones (below) were crossed to generate segregating populations

B0718-3	Bertita	Bzura	Greta	Libertas	Stobrawa	Tollocan	Zarevo	
MSB107-1[1]	MSB110-3	MSC127-3	MSC127-3	MSA097-1	YMSC127-3	MSA091-1	MSA091-1	MS716-15
MSC122-1	MSC084-A	MSE234-7	MSE234-7	MSC127-3	MSE234-7	Allegany	MSA097-1	NorValley
MSC127-3	MSC108-2	MSF077-8	MSF077-8	MSC135-4	MSF134-1	Chaleur	MSA199-1	ND860-2
MSC148-1	MSE226-2	ND860-2	ND860-2	MSE230-3	ND860-2	Conestoga	MSB076-2	NY102
MSD001-3Y	MS702-80	Yukon Gold		MSD040-4RY	Yukon Gold	Andover	MSC010-1	NY84
MSE234-7	Reddale			MSF023-4		Krantz	MSC011-1	Onaway
MSE251-1	Spunta			MSF077-8		Lenape	MSC122-1	Pike
Andover	Steuben			Andover		MS716-15	MSC127-3	Rose Gold
NorValley	W877			Atlantic		NY88	MSD040-4RY	Saturna
NY101						Pike	Allegany	Snowden
Pike						Rose Gold	Andover	Spunta
Prestile						Snowden	Atlantic	W870
Shepody						St. Johns	Brador	W877
W870						Superior	B1254-1	Yukon Gold
Yukon Gold						W870	Conestoga	
						W877	Krantz	
						Sag. Gold	MS702-80	

[1] MS identifies Michigan State University advanced breeding clones.

Table 2. Number of evaluated clones and foliar late blight reaction for family mean based upon greenhouse and field testing in 1998

Late blight families	Evaluated clones	Greenhouse testing (%)[1]		Field testing (RAUDPC)[2]	
		Range	Mean infection	Range	Mean infection
Tollocan	71	0–85	16.9 a[3]	0.004–0.234	0.101 a
B0718-3	59	0–97	38.3 d	0.024–0.322	0.156 b
Bzura	32	2–95	35.3 cd	0.101–0.267	0.181 bc
Bertita	40	4–95	36.6 d	0.051–0.275	0.192 cd
Greta	28	4–80	27.1 b	0.143–0.268	0.194 cd
Stobrawa	34	3–95	27.0 b	0.115–0.304	0.196 cd
Libertas	52	2–95	36.7 d	0.081–0.327	0.198 cd
Zarevo	92	1–95	27.9 bc	0.006–0.371	0.218 d
Average	51		30.7		0.180
$LSD_{0.05}$			8.1		0.026
Atlantic			41.9[4]		0.438

[1] Percent infected foliar area at seven days after inoculation.
[2] Relative area under the disease progress curve calculated from inoculation until most susceptible clones reached 100% infection (maximum RAUDPC = 1).
[3] Means in columns followed by the same letter are not significantly different using Fisher's LSD at $\alpha = 0.05$.
[4] Mean of all plants tested when three to four plants of the standard susceptible cultivar were included in each mist chamber.

Table 3. Number of evaluated clones and foliar late blight reaction for family mean and parents based upon greenhouse and field testing in 1999

Late blight families	Evaluated clones	Greenhouse testing (%)[1]		Field testing (RAUDPC)[2]		Parents RAUDPC[3]
		Range	Mean infection	Range	Mean infection	
Tollocan	45	0–85	32.9 b[4]	0.048–0.698	0.285 a	0.020 a
B0718-3	17	6–53	16.1 a	0.160–0.670	0.376 b	0.175 b
Bzura	–	–	–	–	–	0.253 b
Greta	7	31–56	37.7 bc	0.500–0.615	0.561 c	0.377 c
Libertas	6	22–46	40.2 bc	0.450–0.581	0.531 c	0.446 cd
Stobrawa	6	21–51	36.9 bc	0.388–0.695	0.554 c	0.466 cd
Bertita	4	42–73	57.2 d	0.551–0.629	0.542 c	0.473 cd
Zarevo	33	14–74	44.6 c	0.452–0.777	0.598 c	0.485 d
Average	16.8		37.9		0.492	0.335
$LSD_{0.05}$			10.0		0.058	0.106
Atlantic[5]			49.3		0.648	0.648 e

[1] Percent infected foliar area at seven days after inoculation.
[2] Relative area under the disease progress curve calculated from inoculation until most susceptible clones 100% infection (maximum RAUDPC = 1).
[3] RAUDPC values for the late blight resistant parents and the standard susceptible cultivar Atlantic in the field testing in 1999.
[4] Means in columns followed by the same letter are not significantly different using Fisher's LSD at $\alpha = 0.05$.
[5] Standard susceptible cultivar.

in United States (Fry & Goodwin, 1997). Genotype of these isolates was determined by restriction fragment length polymorphism using RG57 as the probe (Goodwin et al., 1992) and by the two allozyme loci glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) (Goodwin et al., 1995). Growing isolates of unknown mating type in the presence of known A1 and A2 mating types and monitoring oospore production determined the mating type (Galindo & Gallegly, 1960; Honour & Tsao, 1974). The presence of avirulence genes in each isolate was evaluated using detached-leaf assays on a series of R-gene potato differentials. The mixture of isolates (MS94-1, MS94-4, MS95-7 and MS97-2) overcame all known R-genes except R8 and R9 in detached-leaf assays. In the field, the isolates also overcome all differentials except R8 and R9, which were weakly pathogenic on Black's differential (Black et al., 1953).

Cultures of *P. infestans* were grown on rye agar plates in the dark at 15 °C and started about 20 days prior to each inoculation. Sporangia were harvested from Petri dishes by rinsing the mycelia/sporangia mat in cold (4 °C) sterile, distilled water and scraping the mycelia/sporangia mat from the agar surface with a rubber policeman. The mycelia/sporangia suspension was strained through four layers of cheesecloth and the concentration of sporangia was adjusted to about 1×10^6 sporangia ml^{-1} using a hemacytometer. The suspension was stored at 4 °C for four hours to stimulate zoospore release prior to inoculation.

Late blight reaction in greenhouse tests

Plants were grown from sprouted tuber pieces for about 6 weeks in the greenhouse with natural light supplemented by high-pressure sodium lamps (16h day length). Prior to flowering, plants were transferred to a mist chamber of approximately 3 m^3. The chamber was situated within a greenhouse and covered with 0.6 mm transparent polyethylethene plastic sheets. Relative humidity was maintained at 90% by misting the chamber atmosphere for 15 minutes every hour (6 liters of deionized water per 24-hour period) with gravity-fed humidifiers (Herrmidifier Series 500 – Trion, Stanford, NC). Plants were inoculated in the evening, by spraying the plants with 50 ml of inoculum per m^2 using a hand-held bottle sprayer. Temperature within the chamber was maintained between 18 °C and 25 °C. Infected foliar area was estimated based on a visual observation of the diseased area of stems and leaves at seven days after inoculation. The experimental unit was a single plant in one pot (16 cm diameter). In 1998, 408 clones were tested in 2 replications in a completely random design. In 1999, 118 clones were tested in 4 replications in a random-

ized complete block design. All tests were carried out from January to April of each year and the late blight susceptible cultivar Atlantic was used as standard.

Late blight reaction in field tests

The field tests were carried out at the Michigan State University Muck Soils Research Farm, Bath, Michigan in a randomized complete block design. No fungicides were applied on the plants. The 408 selected clones tested in the greenhouse in 1998 were planted in 2 replications as single-hill plots on June 15th and inoculated on July 22nd. The 118 advanced selected clones tested in the greenhouse in 1999 along with the 8 late blight resistant parents were planted in 3 replications of four-hill plots on May 27th and inoculated on July 22nd. In 2000 the evaluated clones were planted in 3 replications of four-hill plots on June 9th and inoculated on July 26th. Inoculation was done through a permanent sprinkle irrigation system in the early evening and high humidity was maintained in the canopy through periodic irrigations throughout the season. A visual estimation of the percentage of stem and leaf infected area was scored at three to five day intervals until the most susceptible clones reached 100% infection. The area under the disease progress curve (AUDPC) was calculated as described by Shaner & Finney (1977) and divided by the maximum AUDPC (e.g. 3300 for 33 days after inoculation) converting the value to relative AUDPC (RAUDPC). The RAUDPC was calculated from inoculation until most susceptible clones reached 100% infection, with 1.0 being the maximum RAUDPC value.

Maturity and tuber quality evaluations

The selected clones evaluated in 1999 were also planted in non-replicated 20-hill plots at the Michigan State University Lake City Experiment Station, Lake City, Michigan for vine maturity and tuber quality evaluations. Advanced selected clones were planted in non-replicated 40-hill plots at the Michigan State University Montcalm Experiment Station, Entrican, Michigan in 2000. From this point on, tuber quality refers to a combination of tuber appearance, specific gravity and chip color. Vine maturity was evaluated in the field when the standard commercial cultivar Atlantic had a rating of 3 on a 1 to 5 scale (1 = early, as cultivar Superior and 5 = late, as cultivar Ontario). Tuber appearance was evaluated on a 1 to 5 scale of increasing defects (1 = excellent, as cultivar Atlantic; 2 = very good; 3 = acceptable; 4 = poor; and 5 = very poor). Chip color was evaluated on a 1 to 9 scale of increasing color darkness (1–2 = excellent; 3 = very good, as cultivars Atlantic and Snowden; 4 = acceptable; 5 = unacceptable; and 6–9 = poor). Specific gravity was measured on a minimum 2 kg sample using the formula [dry weight / (dry weight – wet weight)].

Statistical analysis

The percentage of infected foliar area in the greenhouse and RAUDPC in the field was analyzed using analysis of variance. Family means were calculated as the average of the clone values for each replication. Family means were compared by Fisher's least significance difference (LSD) at $\alpha = 0.05$ for greenhouse and field tests in 1998 and 1999. Fisher's LSD ($\alpha = 0.05$) was also used to compare the standard susceptible cultivar Atlantic and the eight late blight resistant parents in the 1999 field testing. For greenhouse and field 1999 data, Dunnett's T test ($\alpha = 0.05$) was used to compare clones with Atlantic. Pearson correlation analysis was done to compare greenhouse and field testing for 1999 data. Scott-Knott cluster analysis was used to rank clones, resistant parents and Atlantic in discrete groups differing in late blight reaction based on field testing in 1999 (Scott & Knott, 1974). Fisher's LSD ($\alpha = 0.05$) was used to compare Atlantic, Tollocan, B0718-3 and the advanced selected clones in the 2000 field testing. All analyses were done following the procedures of SAS (SAS Institute, 1995).

Results

Identification of superior parents for late blight resistance breeding

The greenhouse testing in 1998 showed a wide range of infection for all families and the susceptible cultivar Atlantic had a foliar infection of 42% at seven days after inoculation (Table 2). Clones with less infection than Atlantic were identified in all families. The Tollocan family had the lowest mean infection at 17%, which was significantly less than any other family mean. High mean infection levels (over 35%) were found in the B0718-3, Bertita, Libertas, and Bzura families.

Foliar infection in the field showed a large range of RAUDPC values for all families (Table 2). However, all evaluated clones had RAUDPC values lower than

Atlantic. Again, the Tollocan family had the lowest RAUDPC mean infection. The B0718-3 family had the second lowest RAUDPC mean infection, but was not significantly different from the Bzura family.

Of the 408 selected clones, those with $\leq$ 10% infected foliar area in the greenhouse test or $\leq$ 0.30 RAUDPC value in the field test in 1998 were re-tested in 1999, resulting in 118 advanced selected clones (29% of 408) with putative resistance to late blight. The Bertita family had the lowest (4) and the Tollocan family had the highest (45) number of evaluated clones in 1999 (Table 3). Tollocan (63%), Zarevo (36%) and B0718-3 (29%) families had the highest percentage of selected clones, based on greenhouse and field tests in 1998. The Bzura family was not represented in the 1999 testing, because there was only one selected clone.

The greenhouse testing in 1999 showed a wide range of foliar infection only for the Tollocan family (Table 3). In general, clones with less infection than Atlantic were identified in all families, but only the Tollocan and B0718-3 families had clones with less than 10% infection. The B0718-3 family had the lowest family mean infection with 16%, and the Tollocan family had the second lowest family mean infection (33%), but the Tollocan family was not significantly different from the Stobrawa, Greta, and Libertas families.

In the 1999 field testing, the B0718-3 and Tollocan families showed a wide range of RAUDPC values (Table 3). In a comparison among family means, Fisher's LSD test differentiated the eight families into three groups. The Tollocan family had the lowest RAUDPC value, the B0718-3 family had intermediate and the Greta, Libertas, Stobrawa, Bertita and Zarevo families had the highest mean RAUDPC values. There was a positive association between family means and parents, since the best parents produced the best family means (lower RAUDPC). Tollocan was the best parent with the best family mean. Also, the most resistant clone in each family tended to have an RAUDPC similar to its resistant parent. There were significant differences among late blight resistant parents and all resistant parents had a significantly lower RAUDPC than Atlantic. Also, there was a positive significant correlation (r = 0.56, $p < 0.001$) between greenhouse and field tests in 1999.

Grouping and selection of recombinant progeny

The Scott-Knott cluster analysis based on the field test in 1999 ranked the advanced selected clones, the resistant parents and Atlantic in three groups differing in late blight reaction (Table 4). A total of 24 clones were ranked in the resistant group (RAUDPC from 0.020 to 0.183), 63 clones in the moderately resistant group (RAUDPC from 0.222 to 0.560), and 40 clones in the susceptible group (RAUDPC from 0.565 to 0.777). The late blight resistant parents Tollocan and B0718-3 were ranked in the resistant group, while Bzura, Greta, Libertas, Stobrawa, Bertita, and Zarevo were ranked in the moderately resistant group. Atlantic was ranked in the susceptible group. Assuming the moderately resistant group as a threshold, 79 advanced selected clones (67% of 118) could be further advanced in a breeding program. These selections represent seven of the eight families.

Maturity and tuber quality evaluations in 1999 showed that 19 of the 79 advanced selected clones possessing high or moderate late blight resistance had a maturity rating as early mid-season or as mid-season. Of these advanced selected clones with marketable maturity, 5 were from the resistant and 14 were from the moderately resistant group (Table 4). The resistance of these 19 advanced selected clones came from 5 parents (12 from Tollocan, 3 from B0718-3, 2 from Stobrawa, 1 from Libertas, and 1 from Zarevo). Moreover, these 19 advanced selected clones also had acceptable tuber quality [(5 had chip processing quality (chip color $\leq$ 4, tuber appearance $\leq$ 3 and specific gravity $\geq$ 1.080) and 14 had tablestock quality (tuber appearance $\leq$ 3) (data not shown)]. These advanced selections possessing late blight resistance combined with acceptable maturity and tuber quality were further evaluated in 2000. The 2000 field evaluation showed that there were advanced selected clones (eight) with the same level of late blight resistance as their resistant parent that also combined maturity and tuber quality equivalent to Atlantic, a standard cultivar for chip processing in North America (Table 5). Only two families were represented in these new advanced selected clones; Tollocan with six and B0718-3 with two clones.

Discussion

The eight late blight resistant parents differed in the transmission rate and in the level of late blight resistance transmitted to the offspring. Based on the

Table 4. Scott-Knott cluster groups of clones and resistant parents differing in late blight resistance in the 1999 field testing and the respective range of relative area under the disease progress curve (RAUDPC)

Cluster groups	RAUDPC[1]	Clones and parents[2,3,4]	Total
Resistant	0.020–0.183	B0718-3[3], Tollocan[3], J306-5[3], J307-1[3], J453-2[4], J453-4[3], J456-4[3], *J457-2*[3], J457-4[3], J458-1[3], *J458-2*[3], J459-1[3], J459-2[3], *J459-3*[3], *J459-4*[3], J459-5[3], J460-3[3], *J461-1*[4], J461-2[3], J462-2[3], J464-5[4], J466-4[3], J468-2[4], J468-5[3]	24
Moderate	0.222–0.560	Bertita, Bzura[3], Greta[3], Libertas, Stobrawa, Zarevo, J306-3[3], J307-2[3], J309-6[3], *J310-3*, J314-3, *J317-1*[3], J317-5[3], *J319-1*[3], J319-7[4], J319-9[3], J320-1, J320-2[4], J324-2, J332-1, J332-6, J364-1, J365-2,J365-8, J366-4, J395-1, J395-10, *J399-1*, J404-5, J448-1[3], J449-5, *J452-3*, *J452-4*, J453-3[3], *J455-4*[3], *J456-2*[3], J458-3[3], J462-1[3], J462-3[3], *J464-1*[3], *J464-4*[3], J464-6, J465-1[3], J466-2, *J466-3*[3], J467-2[3], J467-3[3], *J467-6*, *J468-1*[3], J469-2[3], J471-5, J476-1, J481-1, J488-2, *J488-4*, J491-3, J492-2, J496-2, J497-1, J499-2, J501-6, J502-1, J503-1	63
Susceptible	0.565–0.777	Atlantic, J314-1, J315-1, J315-5, J326-5, J364-5, J365-10, J365-6, J400-3, J405-1, J450-5, J451-3, J455-1, J456-1, J456-3, J462-5, J463-1, J464-3, J465-3, J468-4, J476-5, J482-1, J482-2, J483-1, J484-2, J487-1, J487-3, J487-5, J489-1, J492-1, J492-4, J492-6, J493-2, J494-1, J494-4, J495-2, J496-1, J497-4, J501-1, J501-5	40

[1] Respective range between the lowest and the highest RAUDPC for each group.
[2] Italicized progeny had vine maturity rating ≤ 3 on a scale 1 to 5 (1 = early as cv. Superior and 5 = late as cv. Ontario).
[3,4] Foliar infection significantly different from Atlantic in the field[3] and in the greenhouse[4] based on Dunnett's T tests for $\alpha = 0.05$.

Table 5. Foliar late blight reaction, maturity and tuber quality performance of advanced selected clones in the 2000 field evaluation

Advanced selected clones	RAUDPC[1]	Maturity[2]	Tuber appearance[3]	Specific gravity[4]	Chip color[5]	Pedigree
MSJ457-2	0.009 a[6]	2	2.0	1.091	2	Andover × Tollocan
MSJ459-4	0.009 a	2	3.0	1.072	4	Lenape × Tollocan
MSJ461-1	0.016 ab	3	2.0	1.079	2	NY88 × Tollocan
MSJ459-3	0.019 ab	3	2.0	1.079	3	Lenape × Tollocan
MSJ319-1	0.031 ab	3	2.0	1.086	4	B0718-3 × W870
MSJ458-2	0.044 b	2	1.0	1.077	2	Krantz × Tollocan
MSJ456-2Y	0.045 b	3	2.0	1.082	5	Conestoga × Tollocan
MSJ317-1	0.050 b	3	1.5	1.072	4	Prestile × B0718-3
Tollocan	0.027 ab	5	5.0	1.075	7	
B0718-3	0.097 c	5	1.5	1.074	3	
Atlantic	0.298 d	3	1.0	1.088	3	

[1] Relative area under the disease progress curve calculated from inoculation until most susceptible clones reached 100% infection (maximum RAUDPC = 1).
[2] Scale 1 to 5 (1 = early as cv. Superior and 5 = late as cv. Ontario).
[3] Scale 1 to 5 of increasing defects (1 = excellent as in the cv. Atlantic, 2 = very good, 3 = acceptable, 4 = poor, and 5 = very poor).
[4] Specific gravity calculate as [dry weight / (dry weight–wet weight)].
[5] Scale 1 to 9 increasing color darkness (1–2 = excellent, 3 = very good, 4 = acceptable, 5 = unacceptable, 6–9 = poor).
[6] Values followed by the same letter are not significantly different using Fisher's LSD ($LSD_{0.05} = 0.034$).

percentage of selected clones in 1998, the parents Tollocan, Zarevo and B0718-3 transmitted resistance to a higher percentage of their offspring than Greta, Libertas and Stobrawa. Tollocan had the lowest family mean infection in both greenhouse and field testing. Of the 118 selected clones identified in 1998, Tollocan and B0718-3 also had the highest percentage of selected clones in 1999. In terms of family means, B0718-3 had the lowest and Tollocan the second lowest mean infection in greenhouse testing in 1999. In the field testing, Tollocan had the lowest and B0718-3 the second lowest RAUDPC values, for family mean and parent. The advanced clones selected in 2000 were exclusively progeny from Tollocan and B0718-3 fam-

ilies. Tollocan and B0718-3 not only had stronger late blight resistance, but also transmitted resistance to a higher percentage of their progeny compared with any other parent. This permitted the selection among their progeny for acceptable maturity and tuber quality.

Some attempts have been made to separate qualitative treatments in distinct, non-overlapping groups to give a reasonable threshold for selection. The Scott-Knott cluster analysis method uses principal component and cluster analysis to group treatment means (Scott & Knott, 1974). Gates & Bilbro (1978) showed that Scott-Knott cluster analysis was more effective at ranking genotypes into distinct groups than the Duncan multiple range test and Fisher's LSD, even in experiments with a small coefficient of variation. The no treatment overlap property of Scott-Knott cluster analysis increases the Type I error, but detects considerably smaller differences than Fisher's LSD test does (Willavize et al., 1980). The use of principal component and cluster analysis (Platt & Tai, 1984) and residual maximum likelihood (Platt & Tai, 1998) was reported as effective for ranking potato clones in groups differing in late blight resistance. In this study, we used Dunnet's T-test and Scott-Knott cluster analysis to group clones. Dunnett's T-test showed a clear threshold for selection, but this test identified only clones with high levels of resistance (4 clones in the greenhouse test and 46 clones in the field test in 1999). The strategy of selecting only clones possessing high levels of late blight resistance may severely reduce the genetic base and thereby restrict the possibility of further selection for other important traits. However, Scott-Knott cluster analysis was able to rank the clones into three discrete groups differing in level of resistance. The highly resistant clones were ranked with the resistant parents Tollocan and B0718-3, the moderately resistant clones were ranked with the resistant parents Bzura, Greta, Libertas, Stobrawa, Bertita and Zarevo, and the susceptible clones were ranked with the susceptible cultivar Atlantic. These levels of resistance in the resistant parents were also detected by Fisher's LSD which also showed significant differences between late blight resistant parents and the susceptible cultivar Atlantic. Moreover, the field testing and Scott-Knott cluster analysis results were in concordance with a previous study based on a multi-state field trial. In that study, Haynes et al. (1998) ranked B0718-3 (3^{rd}), Bzura (5^{th}), Greta (6^{th}), Libertas (7^{th}), Bertita (8^{th}), and Stobrawa (10^{th}) among 16 cultivars previously identified as resistant. Therefore, Scott-Knott cluster analysis provided a means to determine a threshold for selecting clones possessing high and moderate levels of resistance to late blight that can be further selected for other important traits.

Combining field and greenhouse resistance testing with field determinations of vine maturity and tuber characteristics provided a good measurement of the breeding potential of the late blight resistant parents and permitted a multi-trait selection for the identification of recombinant clones. Over a three-year period, beginning with unselected crosses, clones were identified that possessed late blight resistance combined with acceptable maturity and tuber quality. These selected clones can be advanced for further evaluation or used as parents in the next cycle of recurrent selection. The advanced selected clones were from the Tollocan and B0718-3 families suggesting that the late blight resistance in these parents should be highly heritable.

Despite using a mixture of *P. infestans* isolates in our tests, the type of resistance present in Tollocan and B0718-3 has not been resolved. There were a few clones that did not show infection in the greenhouse tests. There was one clone from each Tollocan and B0718-3 families in 1998 and two clones from Tollocan family in 1999 in the greenhouse tests, but these clones were infected in the field tests. The clones with no infection in greenhouse tests may have R8 or R9 genes, since the *P. infestans* isolates used were not pathogenic in detached-leaf assays and only weakly pathogenic on the R8 and R9 differentials in the field tests. All the six advanced selected clones having Tollocan and the two advanced selected clones having B0718-3 as source of resistance were infected in the greenhouse tests. Moreover, the resistance does not appear to be associated with late maturity, since selected clones demonstrated mid-season maturity in two years of evaluations. Since late blight resistance associated with late maturity was mapped on Chromosome V (Collins et al., 1999), the resistance present in the advanced selected clones should be located in other regions of the potato genome. The resistance in Tollocan and B0718-3 may not be solely vertical. B0718-3 and the most promising Tollocan-derived selections have been included in greenhouse and field tests at Michigan State University since 1997 and have showed consistently high levels of resistance to late blight. Strong horizontal resistance to late blight from different wild species associated with more simple inheritance has recently been mapped in the cultivated potato background. The cultivar Stirling has *S. demissum* Lindl. as source of resistance (Meyer et

al., 1998), in which one major quantitative trait locus (QTL) explaining 30% of the phenotypic variance was mapped to the chromosome IV (Pande et al., 2001). A major QTL explaining 62% of the phenotypic variance for the resistance of *S. bulbocastanum* Dunal was mapped to the chromosome VIII (Naess et al., 2000). Therefore, the strong and highly heritable resistance conferred by Tollocan and B0718-3 should not be designated as R-gene based resistance at this time.

The main objective of our breeding effort was to combine resistance genes from different sources to broaden the genetic base and thus increase the degree and durability of late blight resistance. Before intercrossing these resistance sources, it was necessary to combine late blight resistance with acceptable maturity, to increase adaptation and to reduce the effect of late maturity over resistance. Selected clones possessing strong resistance to late blight from Tollocan and B0718-3 are being crossed with selected clones possessing moderate resistance from Libertas, Stobrawa and Zarevo. Intercrossing these late blight resistant clones should also increase the probability of recombinants carrying foliar and tuber resistance, since Libertas (Platt & Tai, 1998) and Zarevo (Douches et al., 2001) are reported to transmit tuber resistance. Also, pyramiding genes from different sources (and in this case different levels of resistance) may build more durable resistance to late blight (Colon, 1999). Combining genes from different sources will be more effective when the QTLs for late blight resistance are mapped in the parents.

In summary, progeny evaluation was valuable to identify parents to use in breeding for late blight resistance. Moreover, the combination of greenhouse and field testing for late blight with field evaluations for maturity and tuber quality gave the possibility for multi-trait selection that resulted in the identification of recombinant clones after three years of evaluation. These selected clones can be used as parents in recurrent selection for combining sources of resistance and can continue been evaluated for germplasm release. The results indicate that this breeding approach can be used to select parents for late blight resistance breeding and to identify superior clones with high levels of late blight resistance and marketable vine maturity and tuber quality.

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