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dissertation entitled

INTROGRESSION OF LATE BLIGHT RESISTANCE
FROM WILD SPECIES AND UNADAPTED GERMPLASM
TO CULTIVATED POTATO

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Plant Breeding & Genetics

Major professor

Date Aug 2, 2001

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by

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Crop and Soil Sciences

2001

ABSTRACT

Introgression of Late Blight Resistance from Wild Species and Unadapted Germplasm to Cultivated Potato

by

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Since the mid-1990's, the United States and Canada have experienced late blight (*Phytophthora infestans* (Mont.) de Bary) epidemics caused by new, more aggressive and metalaxyl resistant races that impose new disease management strategies in potato (*Solanum tuberosum* L.). Breeding offers the opportunity to identify and release advanced germplasm with late blight resistance. The general objective of this research was to introgress late blight resistance from unadapted germplasm and wild species to cultivated potato. The first effort was to combine late blight resistance from eight (B0718-3, Bertita, Bzura, Greta, Libertas, Stobrawa, Tollocan and Zarevo) unadapted cultivars with tuber quality and marketable maturity found in cultivars/advanced breeding clones adapted to North America. A total of 408 field selected clones from 95 crosses were evaluated in single- and eight-hill plots for tuber quality (tuber appearance, specific gravity and chip color). The same clones were assessed for foliar late blight reaction using a mixture of complex races of US8/A2 mating type of *P. infestans* isolates in

greenhouse and field studies in 1998 and 1999. The late blight resistant parents differ in their ability to transmit late blight resistance and tuber quality to the offspring. In addition, late blight resistance can be combined with marketable maturity and tuber quality. Moderate selection intensity for tuber quality traits can be initiated at the single-hill generation before testing for late blight resistance. The second effort was to select, within plant introduction accessions, clones with high levels of late blight resistance in greenhouse. A total of 60 selected clones representing South American species, hybrids between wild and cultivated species and cultivars/advanced breeding clones were than evaluated for their genetic diversity based on isozymes and simple sequence repeats (SSR). There is a high level of genetic diversity within and between accessions, species and ploidy levels of the late blight resistant germplasm from *S. microdontum* Bitter, *S. berthaultii* Hawkes and *S. sucrense* Hawkes that should be introgressed and combined in a breeding. The last effort was to map quantitative trait loci (QTL) conferring late blight resistance and other agronomic traits using isozymes and SSR markers in a *S. microdontum* derived population. Progeny of 110 clones and parents were field tested for foliar late blight reaction in 1999 and 2000, and for maturity, tuber number and size, yield and tuber quality in 2000. High phenotypic correlation ($r = 0.89$, $P < 0.0001$) was found for late blight reaction between years and no correlation was found between late blight with other evaluated trait. There was only one marker linked with late blight resistance and another trait (tuber size). *Solanum microdontum* has a QTL associated with foliar late blight resistance that is not associated with late maturity or any poor tuber quality traits that explains 70% of the phenotypic variance of two years of field testing. A SSR marker closely linked to the QTL that can be followed through polyploidization is suitable for using in a marker assisted selection strategy.

Dedicated to

my family

ACKNOWLEDGEMENTS

I would like to express a very special thanks to my major professor and mentor Dr. David S. Douches for believing in my potential and giving me the opportunity to pursue the Ph.D. degree in his breeding program. His incredible enthusiasm, creativity and friendship have been a great source for encouragement making the Ph.D. program much more enjoyable and productive.

I am very thankful for the committee members Dr. James Kelly, Dr. Amy Iezzoni, and Dr. Raymond Hammerschmidt for their input and contribution to my research and publication of results. Even not being part of my committee, Dr. William Kirk gave major contributions to all steps of late blight testing.

A special thanks to the "potato family", Lynn Buszka, Kim Walter-Felcher, Kaz Jastrzebski, Joe Coombs, Chris Long, Anne Lund, Beth Leary, Kelly Zarka and Jarred Driscoll. Lynn's contribution was decisive for completing the mapping studies. Kim was critical in discussing and reviewing the manuscripts. Kaz helped me to fill up my gaps of germplasm development and crossing block strategies. Joe's expertise brought easy and efficient solutions to all computer issues. Joe's and Chris' friendship were very important since my first day at MSU. After all, every one contributed in doing some serious work or being funny during those many hard days of work. I will keep those memories.

I am indebted forever with my family for being very supporting and understanding. My wife quit the job to follow my dream and now stop her career to take care of our adorable son. My parents gave their time to raise and teach me the values of love, friendship and perseverance to pursue my goals. My parents in law raised my lovely wife and wonderful mother of our son. Thanks for your support and encouragement.

Thanks also to my good friends of both the Latin American and the Brazilian Communities for being part of these important moments and supporting us here. Besides friends, I also had the extraordinary experience to work with Luis Velasquez and Dr. Irvin Widders with cucurbits, my small but important dream. After all, the Ph.D. program is gone, but good friends and good memories last forever.

I am very thankful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Ministry for Science and Technology of Brazil for all the financial aid and to the Department of Horticulture, Federal University of Santa Maria for the opportunity of taking my Ph.D. program. Also, thanks to the MSU Plant Breeding and Genetics Program for being part of this highly prestigious group.

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CHAPTER I

General Introduction

Origin and Importance of Potato

Potato (*Solanum tuberosum* L.) belongs to the family *Solanaceae* section Petota Dumortier. The origin and domestication of the potato occurred in the highlands of Perú and Bolivia 7,000 to 10,000 year ago. *Solanum stenotomum* Juz. et Bukasov ($2n = 24$) was the first domesticated species (Hawkes, 1990). There were at least four wild species involved in the evolution of domesticated potato, with *S. sparsipilum* (Bitter) Juz. et Bukasov ($2n = 24$) being the most important. A hybridization between *S. sparsipilum* and *S. stenotomum* followed by chromosome doubling formed *S. tuberosum* subsp. *andigena* Hawkes ($2n = 48$) (Hawkes, 1990; Hawkes, 1994). *Solanum tuberosum* subsp. *andigena* was brought to Chile and to Europe, where it became adapted to long day length evolving into *S. tuberosum* subsp. *tuberosum* Hawkes (Hawkes, 1994). There are seven cultivated potato species: two diploid ($2n = 24$), two triploid ($2n = 36$), two tetraploid ($2n = 48$), and one pentaploid ($2n = 60$) (Hawkes, 1990). *Solanum tuberosum* is the most important cultivated species with two subspecies. *Solanum tuberosum* subsp. *tuberosum* has long-day adaptation with widespread cultivation in temperate and

subtropical climates. *Solanum tuberosum* subsp. *andigena* has short-day adaptation and its cultivation is restricted to the Andes of South America (Correll, 1962; Hawkes, 1990). Although potato originated in South America, its worldwide distribution was from Europe at the end of the 16th century (Hawkes, 1990; Hawkes, 1994). In this research, cultivated potato refers to *S. tuberosum* subsp. *tuberosum.*

In a time frame of about 300 years, cultivated potato arose from a little known crop in the Andes area of South America to become one of the most important food crops (Hawkes, 1990; Hawkes, 1994). The importance of the potato as a food crop relies on its high yield and calorc production, excellent nutrient source, and high proportion of edible biomass (Niederhauser, 1993). The United Nation's Food and Agriculture Organization (FAO) estimated during the 1996-1998 period that an average of more than 299 million metric tons of potatoes were harvested per year, compared with wheat (595 million metric tons), maize (592 million metric tons) and paddy rice (571 million metric tons). Europe accounted for 50% of the total potato production and Russia, Poland and Germany were responsible for 48% of the European production. Asia accounted for 32% of the total production and China and India were responsible for 75% of that production. South America accounted for 4% of the total world production and Brazil, Argentina, Colombia and Perú were responsible for 89% of that production. North and Central America accounted for 9% of the total production and United States was responsible for 79% of that production (FAO, 1998). During the 1993-1997 period, the United States annually potato production averaged 20.9 million metric tons and Idaho and Washington responsible for 48% of the national production. Per capita consumption was 63.8 kg, from which 35% went to fresh market and 65% for processing as frozen (63%), chips

(18%) and dehydrated (16%). In the 1992-1996 period, potato was an average of 2.4 billion-dollar businesses in the United States (National Potato Council, 1998).

Use of Potato Germplasm in Breeding

Potato probably has the widest genetic diversity among related wild species than any other cultivated crop plant (Hawkes & Jackson, 1992). Among the 232 potato species recognized by Hawkes (1990), there were 183 with a known number of chromosomes. The vast majority of the species are diploid (136 or 74%) with the rest being triploid (7), tetraploid (27), pentaploid (3), and hexaploid (10). Despite the low level of genome differentiation in most *Solanum* species (Hawkes, 1994), the germplasm can not all be used directly for breeding due to a combination of ploidy level and endosperm balance number (EBN) incompatibility (Hawkes & Jackson, 1992). Diploid wild species can be directly crossed with dihaploids ($2n = 2x = 24$) of cultivated potato (Hermsen, 1984; Hermsen, 1994). Dihaploids occur as a result of parthenogenesis (haploid pollinator technique) (Jansky et al., 1990; Hermsen, 1994; Alfano et al., 1999) or anther culture (Tai, 1994). Dihaploid x wild species hybrids can be crossed with cultivated potato via unilateral sexual polyploidization ($4x$ - $2x$ crosses) using $2n$ gametes (Hermsen, 1994; Hutten et al., 1994). Two dihaploid x wild species hybrids that produce $2n$ pollen and $2n$ eggs can also be crossed leading to bilateral sexual polyploidization ($2x$ - $2x$ crosses) (Ortiz, 1998; Alfano et al., 1999; Hanneman, 1999).

The EBN is an arbitrary cross compatibility classification system that is based on a 2:1 maternal to parental ratio for the endosperm to be functional in a cross (Ortiz &

Ehlenfeldt, 1992; Hanneman, 1999). The diploid species with 1 EBN are distributed in North America, Central America, while only a few species are found in South America. The diploid species with 2 EBN have restricted distribution in South America and only one species in Mexico. The cultivated *S. tuberosum* subsp. *tuberosum* is an autotetraploid species with 4 EBN (Correll, 1962; Hawkes, 1990). Therefore, the knowledge and use of endosperm balance number, dihaploids and 2*n* gametes made available a wide range of the *Solanum* genetic diversity accessible to the potato breeders (Peloquin et al., 1989; Hanneman, 1999).

Part of the potato germplasm has already been characterized for a number of economically important characteristics. Sources for disease (viruses, fungi, bacteria, and nematodes) and insect resistance, temperature stress (frost and heat), glycoalkaloids and vigor for vine and flowering were reported for 111 tuber-bearing *Solanum* species (Bamberg et al., 1994). The invaluable potato germplasm also includes sources for chip-processing direct from storage, high solids content, tuber shape and color, production of unreduced gametes, endosperm viability and improvement of yield potential (Hanneman, 1989). Moreover, fine-screening evaluation has been done for more specific traits such as Colorado potato beetle resistance (Bamberg et al., 1996), tuber calcium (Bamberg et al., 1998), and late blight resistance (Douches et al., 2001) to identify resistant clones within accessions. Germplasm characterization gave the opportunity to develop cultivated primitive diploid and tetraploid potato populations using wild and cultivated species. These populations are sources of genetic diversity for characteristics present in wild species, but with a significantly increased breeding value (Mendoza, 1989).

Despite the high genetic diversity that exists in the genus *Solanum*, cultivated potato has a narrow genetic base. A few introductions of *S. tuberosum* subsp. *andigena*

with short day adaptation made the initial genetic base for long day adaptation of *S. tuberosum* subsp. *tuberosum* in Europe (Hawkes, 1994). Intense breeding efforts further narrowed the cultivated potato gene pool. High genetic similarity characterizes more than 130 potato cultivars released in North America between 1930 and 1970 (Mendoza & Haynes, 1974). The cultivars Katahdin, Early Rose and Garnet Chili (Early Rose's parent) had a very high genetic contribution to modern North American cultivars (Mendoza, 1989; Plaisted & Hoopes, 1989). Genetic diversity studies using molecular markers confirmed the relatedness among North American potato cultivars (Douches et al., 1991; Demeke et al., 1996; Provan et al., 1996) and also among European potato cultivars (Provan et al., 1999). Therefore, it is very important to increase the genetic diversity of the cultivated potato gene pool.

Late Blight and its Constraints to Potato Production and Breeding

Late blight is caused by the fungal-like oomycete *Phytophthora infestans* (Mont.) de Bary (Kamoun et al., 1999). *Phytophthora infestans* populations have very high genetic diversity and one-to-one ratio of mating types (A1 and A2) in central Mexico (Toluca Valley), supporting that area as its center of origin (Fry & Spielman, 1991). New evidences showed that central Mexico was the center of diversity, but it was not the center of origin (Ristaino et al., 2001). Late blight has assumed importance as a potato disease since the middle of the 1840s (Wastie, 1991), and more recently late blight is present in almost all potato growing areas (Ross, 1986; Henfling, 1987; Kamoun et al., 1999). Late blight is notable because it is the disease that caused the Irish potato famine

(Fry & Goodwin, 1997a) and because late blight devastating speed and destructive potential were a major stimulus for the development of the science of plant pathology (Fry & Goodwin, 1997b). *Phytophthora infestans* causes both foliar destruction and tuber decay (Ross, 1986) and ranks as the most devastating potato disease worldwide (Fry & Goodwin, 1997a; Kamoun et al., 1999). World yield losses in potato caused by *P. infestans* were estimated to exceed $2 billion annually (Kamoun et al., 1999).

Major changes occurred in the *P. infestans* population composition in the United States from 1991 to 1992 (Goodwin et al., 1995a) and in Canada in the mid-1990s (Peters et al., 2001), including the appearance of more aggressive and metalaxyl resistant genotypes. The new US8 genotype of *P. infestans* was shown to be more aggressive on potato foliage (Johnson et al., 1997) and on tuber tissue (Lambert & Currier, 1997). Metalaxyl resistant genotypes were first identified in western Washington in 1990 (Deahl et al., 1993). Since then, the most complex and virulent isolates, including either A1 or A2 mating type, were isolated from that location (Deahl et al., 1993; Goodwin et al., 1995b). The occurrence of both A1 and A2 mating types can result in sexual reproduction thus increasing the potential of pathogen evolution and is also necessary for the formation of oogonia that develops into oospores (Deahl et al., 1995; Goodwin et al., 1995a). Oospores can survive in adverse conditions for months or even years (Fry & Goodwin, 1997a) and can be source of primary inoculum in the soil. Oospore formation may increase the amount of fungicide applied to foliage and seed tubers (Dorrance et al., 1999) and may change the actual disease management strategies (Umaerus & Umaerus, 1994). The A2 mating type became more common in United States populations during 1992 and 1993 (Goodwin et al., 1995b) and Canada in 1994 and 1995 (Peters et al.,

2001). In the United States, the most aggressive and metalaxyl resistant US8 genotype was found in 23 states in 1994 and 1995 (Fry & Goodwin, 1997a).

The late blight epidemic of 1994 in the United States was caused by the US8 genotype (Inglis et al., 1996), the most complex genotype collected in western Washington during 1996 and 1997, which three out of six isolates had ten virulence factors (Dorrance et al., 1999). The high aggressiveness of the US8 genotype of *P. infestans* was attributed to metalaxyl resistance and/or increase in parasitic fitness (Inglis et al., 1996). Moreover, greater aggressiveness (faster lesion expansion rate and sporulation time) of the US8 genotype of *P. infestans* compared with US1 genotype explains why US8 genotype required more protectant fungicide for adequate epidemic suppression than US1 genotype (Kato et al., 1997). The average number of fungicide applications for late maturing potato cultivars increased from 2.5 in 1994 to 8.2-12.3 in the northern and southern Columbia Basin in 1995, respectively. The total estimated cost to control late blight in Columbia Basin in 1995 was estimated at 30 million dollars (Johnson et al., 1997).

An important tool in managing late blight resistance is host plant resistance. Vertical resistance (R-gene or specific) is conferred by R-genes and is effective against a narrow range of pathogen races (Henfling, 1987). Attempts to introgress vertical resistance from *S. demissum* Lindl. to cultivated potato were initiated in the early 1900s and pursued until at least the 1960's. These efforts resulted in the development of many late blight resistant potato cultivars (Umaerus et al., 1983). A total of 11 R-genes can be recognized that were introgressed from *S. demissum* to cultivated potato (Wastie, 1991). Vertical resistance offered no solution for the late blight problem because *P. infestans* evolved and overcame the resistance (Ross, 1986). Horizontal resistance (field, partial or

general) is effective against a broad range of pathogen races, may be expressed in different stages in the pathogen cycle from initial infection to production of sporangia (Henfling, 1987; Umaerus & Umaerus, 1994) and seems to be the only durable type of resistance to *P. infestans* (Colon et al., 1995b; Umaerus et al., 1983; Kamoun et al., 1999). Horizontal resistance was first employed in breeding and was the only type of host resistance available before the identification of R-genes from *S. demissum* (Umaerus et al., 1983). The level of horizontal resistance to late blight in potato has been increased through recurrent selection (Henfling, 1987), which recombines genes from different sources of resistance to build stronger and more durable resistance to late blight (Colon, 1999). Since *S. demissum* exhibits both vertical and horizontal resistance (Wastie, 1991), the presence of horizontal resistance to late blight in cultivated potatoes could be expected.

The development of genetic resistance to late blight in potatoes is one of the major objectives of many breeding programs (Colon et al., 1995a). Besides horizontal resistance, breeding for late blight resistance presents more challenges due to the relationships among foliar resistance, tuber resistance and late maturity. No correlation between foliar and tuber resistance was found among progeny of parents differing in both resistances (Stewart et al., 1992), but high correlation was found when using a small sample of parents (Stewart et al., 1994). A strong positive correlation was found between foliar resistance (horizontal resistance) and late maturity (Ross, 1986; Umaerus et al., 1983). Foliar resistance and late maturity were mapped to the same region of chromosome V (Collins et al., 1999; Oberhagemann et al., 1999) and QTLs for early maturity were associated with late blight susceptibility (Ewing et al., 2000). However, QTLs conferring lower levels of resistance in both foliage and tubers were mapped in

other regions of the genome and they were not associated with late maturity (Collins et al., 1999). These findings suggest that increasing resistance in the foliage is possible by accumulating genes located in regions of the genome not linked with tuber susceptibility and late maturity.

Mapping Potato for Late Blight Resistance and other Important Traits

Cultivated potato is highly heterozygous, exhibits tetrasomic inheritance and has inbreeding depression (Bradshaw, 1994). Potato dihaploids have high levels of self-incompatibility and genetic load, which eliminates the possibility of obtaining inbred lines (Ritter et al., 1990; Leonards-Schippers et al., 1994). To overcome inbreeding depression, crosses between highly heterozygous parents are used to develop segregating F1 mapping populations (Leonards-Schippers et al., 1994). The first attempts to map potato were done at the diploid level to avoid problems of interpretation associated with tetrasomic inheritance (Meyer et al., 1998). The available diploid potato maps were based on the F1 generation (e.g. Barone et al., 1990; Collins et al., 1999; Freyre & Douches, 1994; Pineda et al., 1993; Sandbrink et al., 2000; Van Eck et al., 1994) and backcrosses to a different genotype of one or both parents (e.g. Ewing et al., 2000; Van den Berg et al., 1996a; Van den Berg et al., 1996b). More recently, tetraploid potato maps were also produced based on F1 generation of crosses with tetraploid cultivated potato (Meyer et al., 1998) and backcrosses to different potato cultivars (Naess et al., 2000). High informative potato maps were produced using restriction fragment length polymorphism (RFLP) (Gebhardt et al., 1991), amplified fragment length polymorphism

(AFLP) (Van Eck et al., 1995), a combination of morphological markers, isozymes, RFLPs, and transposons (Jacobs et al., 1995), and simple sequence repeats (SSRs) or microsatellites (Milbourne et al., 1998).

Since late blight is an important constraint for potato production, late blight resistance has been the most common characteristic mapped in potato including R-genes and QTLs associated with resistance. Of the 11 known R-genes, four were already mapped. The specific resistant gene *R1* was mapped on chromosome V (El-Kharbotly et al., 1994; Leonards-Schippers et al., 1992). *R2* was mapped on chromosome IV (Li et al., 1998). *R3*, *R6* and *R7* were mapped in a cluster on chromosome XI (El-Kharbotly et al., 1996; El-Kharbotly et al., 1994). One non-identified R-gene was mapped on chromosome X (Ewing et al., 2000). Some QTLs conferring resistance to late blight were mapped on the same regions of R-gene based resistance as on chromosomes V (Collins et al., 1999; Oberhagemann et al., 1999; Sandbrink et al., 2000) and on chromosome XI (Oberhagemann et al., 1999). These results support the idea that genes for specific and general resistance are not independent (Umaerus & Umaerus, 1994) and may be controlled by alleles at the same genetic locus or by related alleles of closely linked loci (Meksem et al., 1995). Quantitative trait loci for resistance to late blight have been mapped on all potato chromosomes (Leonards-Schippers et al., 1994; Meyer et al., 1998; Collins et al., 1999; Oberhagemann et al., 1999; Naess et al., 2000; Ewing et al., 2000; Kuhl et al., 2000; Sandbrink et al., 2000; Pande et al., 2001) based on diploid and tetraploid populations having a variety of wild species as sources of resistance.

Other important characteristics received much less attention on mapping studies than late blight resistance. Specific gravity, an important tuber quality characteristic for potato processing industry, has been shown to have 10 QTLs distributed on 6

chromosomes of the potato genome (Freyre & Douches, 1994). Long dormancy in tubers is important to avoid sprouting in storage and short dormancy is important for seed tubers used in areas with two growing seasons. Six QTLs conferring long dormancy were located on six chromosomes, being the most important on chromosome VII (Freyre et al., 1994). Among nine chromosomes controlling dormancy, a major QTL conferring long dormancy was mapped on chromosome II (Van den Berg et al., 1996a). As many as 11 QTLs on 7 potato chromosomes were associated with tuberization and tuber fresh weight per plant, with a major QTL explaining 27% of the phenotypic variation on chromosome V (Van den Berg et al., 1996b).

Potato mapping studies suggest that QTLs for late blight resistance were also associated with other important traits. A major QTL affecting foliar and tuber late blight resistance, vine maturity and vigor were all mapped in the region of the RFLP markers *GP21* and *GP179* on chromosome V (Oberhagemann et al., 1999), and foliar late blight resistance in another population (Collins et al., 1999). Other resistant genes were mapped in the same position of the potato genome as the *R1* specific gene for late blight resistance (El-Kharbotly et al., 1994; Leonards-Schippers et al., 1992), the *Rx2* gene for potato virus X resistance (Ritter et al., 1991), and *Gpa5* gene to *Globodera pallida* (Stone) resistance (Rouppe van der Voort et al., 2000). Association between QTLs for foliar late blight resistance, tuberization and vine maturity was found in four out of five chromosomes (Ewing et al., 2000). In the potato chromosome IV, a QTL conferring resistance to *G. pallida* from *S. tuberosum* subsp. *andigena* was mapped (Bradshaw et al., 1998) and a SSR marker (Stm3016) was linked to both late blight and nematode resistance conferred from different parents (Pande et al., 2001). The knowledge of QTLs conferring late blight resistance in different sources and their association with undesirable

characteristics will increase the efficiency of breeding for late blight resistance. Improved levels of more durable resistance to late blight may be achieved by pyramiding QTLs from different sources of resistance.

Objectives and Dissertation Contents

The objective of this research was to study the introgression of late blight resistance from wild species and unadapted germplasm to cultivated potato. The specific objectives were:

1) to evaluate the use of late blight resistant parents in cultivar development;
2) to identify recombinant clones possessing late blight resistance, acceptable tuber quality and maturity in early stages of selection;
3) to screen wild *Solanum* species in greenhouse using the US8 genotype A2 mating type of *P. infestans* to identify resistant clones;
4) to assess the genetic diversity of the potato germplasm with reported late blight resistance using a set of isozyme loci and SSR markers; and
5) to map QTL conferring late blight resistance and other agronomic traits using isozymes, AFLP and SSR markers in a diploid *S. microdontum* Bitter derived population.

The first attempt to introgress late blight resistance was from unadapted germplasm, since resistant sources were already identified and could be directly crossed with cultivated potato. A total of eight parents with reported late blight resistance was used to cross with a set of susceptible parents possessing acceptable maturity and tuber

quality. Progeny evaluations of these crosses are discussed in chapters two and three. Phenotypic selected clones based on overall appearance and tuber number, shape, and internal defects were evaluated for late blight resistance, vine maturity and tuber quality characteristics (tuber appearance, specific gravity and chip color). The progeny evaluation was a multi-trait evaluation of selected clones that have a common late blight resistant parent (half-sib progeny). These chapters were accepted for publication in Euphytica.

The chapter four describes the evaluation of genetic diversity in the diploid and tetraploid potato late blight resistant germplasm. The germplasm used in this study included *Solanum* wild species and hybrids between wild species and cultivated potatoes identified in a previous two-year greenhouse screening. Ten other cultivars or advanced breeding clones were also included that have reported late blight resistance. This chapter was accepted for publication in HortScience.

Based on the screening and genetic diversity studies, clones from *S. microdontum* were selected to develop mapping populations. Further selection was based on the segregation of 40 seedlings for late blight resistance. The mapping study of late blight resistance and other agronomic traits in the diploid *S. microdontum* derived population is discussed in the chapter five. The chapter six is dedicated to a general discussion and conclusions.

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CHAPTER II

Half-Sib Progeny Evaluation and Selection of Potatoes Resistant to the US8 Genotype of *Phytophthora infestans* from Crosses between Resistant and Susceptible Parents

Abstract

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 ≤ 10% infected foliar area in the greenhouse test or ≤ 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 and 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 2.1). 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 2.2). 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 2.3). 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.

Table 2.1. Late blight resistant parents (top row) and susceptible adapted cultivars (above and

Table 2.1. 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	MSB110-3	MSC127-3	MSC127-3	MSA097-1Y	MSC127-3	MSA091-1	MSA091-1	MS716-15
MSC122-1	MSC084-A	MSE234-7	MSE234-7	MSC127-3	MSE234-7	Allegany	MSA097-1Y	NorValley
MSC127-3	MSC108-2	MSF077-8	MSF077-8	MSC135-4	MSF134-1	Chaleur	MSA199-19	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	

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 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 overcome all Black's differentials except R8 and R9, which were weakly pathogenic.

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 x 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 randomized 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 eight

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 from inoculation 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), with 1.0 being the maximum RAUDPC value. See more details in the appendix.

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).

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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 2.2). 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. See Fig. A.1 in the appendix.

Foliar infection in the field showed a large range of RAUDPC values for all families (Table 2.2). 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. See Fig. A.2 in the appendix.

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 2.3). 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. See Table A.1 in the appendix.

Table 2.2. Number of evaluated clones and foliar late blight reaction for family mean based upon a

Table 2.2. Number of evaluated clones and foliar late blight reaction for family mean based upon greenhouse and field testing in 1998.

Late Blight	Evaluated	Greenhouse Testing (%)[1]		Field Testing (RAUDPC)[2]	
Families	Clones	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 2.3. 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.

The greenhouse testing in 1999 showed a wide range of foliar infection only for the Tollocan family (Table 2.3). 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. See Fig. A.3 in the appendix.

In the 1999 field testing, the B0718-3 and Tollocan families showed a wide range of RAUDPC values (Table 2.3). 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. See Fig. A.4 in the appendix.

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 2.4). 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 2.4). 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 2.5). Only two families were represented in these new advanced selected clones; Tollocan with six and B0718-3 with two clones. See also Table A.2 in the appendix.

Table 2.4. Scott-Knott cluster groups of clones and resistant parents differing in late blight resistance in the

Table 2.4. 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] Underlined 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 2.5. Foliar late blight reaction, maturity and tuber quality performance of advanced [illegible]

Table 2.5. 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 x Tollocan
MSJ459-4	0.009 a	2	3.0	1.072	4	Lenape x Tollocan
MSJ461-1	0.016 ab	3	2.0	1.079	2	NY88 x Tollocan
MSJ459-3	0.019 ab	3	2.0	1.079	3	Lenape x Tollocan
MSJ319-1	0.031 ab	3	2.0	1.086	4	B0718-3 x W870
MSJ458-2	0.044 b	2	1.0	1.077	2	Krantz x Tollocan
MSJ456-2Y	0.045 b	3	2.0	1.082	5	Conestoga x Tollocan
MSJ317-1	0.050 b	3	1.5	1.072	4	Prestile x 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).

Discussion

The eight late blight resistant parents differed in the frequency of individuals of the offspring and in the level of late blight resistance transmitted. Based on the 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 families. Tollocan and B0718-3 not only had higher levels of 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 (four 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. See Fig. A.5 in the appendix.

Despite using a mixture of *P. infestans* isolates in these 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. High levels of 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. Selected clones possessing high levels of 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 being 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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CHAPTER III

Early Generation Selection for Potato Tuber Quality in Progeny of Late Blight Resistant Parents

Abstract

Developing disease resistant cultivars is one of the major objectives for a potato (*Solanum tuberosum* L.) breeding program, but many resistant clones have not achieved commercial acceptance because of late maturity and non-marketable tuber characteristics. Selection for tuber quality should have greater emphasis in breeding disease resistant cultivars. The objectives of this study were to evaluate the ability of late blight (*Phytophthora infestans* (Mont.) de Bary) resistant parents to transmit chip-processing (tuber appearance, specific gravity, and chip-color) or tablestock (tuber appearance) quality to the offspring and to compare selecting for tuber quality in single-hill versus eight-hill clonal generations. Crosses were made among eight unadapted potato cultivars (B0718-3, Bertita, Bzura, Greta, Libertas, Stobrawa, Tollocan, and Zarevo) with reported late blight resistance with adapted susceptible cultivars/breeding clones to generate 95 populations (4,750 seedlings). Approximately 10% of the progeny from each cross were

selected from single-hill plots based on tuber appearance, number, shape, and internal defects. These selected clones (408) were evaluated for tuber appearance, specific gravity, and chip-color. The same evaluations in the following year were made on tuber samples from eight-hill plots. Libertas and Tollocan were the best parents for transmitting chip-color; B0718-3, Zarevo, and Tollocan for transmitting tuber appearance; and Bzura, Libertas, and Zarevo for transmitting high specific gravity to the highest percentage of the offspring. Overall, 50% and 56% of the clones based on single- and eight-hill clonal generations, respectively, were considered to possess chip-processing quality; over 90% of the clones had acceptable tablestock quality. A total of 71% of the clones possessing acceptable chip-processing and 95% of the clones possessing acceptable tablestock quality selected in both clonal generations were identified in single-hill plots. The evaluation of tuber quality characteristics in single-hill plot not only permitted the identification of clones with acceptable chip-processing and tablestock, but also increased the amount of clonal information for the following generation of selection. In crosses between late blight resistant and susceptible clones, selection for tuber quality traits can be initiated in single-hill clonal generation using a moderate selection intensity and precede late blight testing.

Introduction

Breeding for disease resistance is a common objective for many crop species. Overemphasis on improving disease resistance can limit yield and other important traits because of genetic bottlenecks (Kelly et al., 1998). In potato (*Solanum tuberosum* L.),

late blight (*Phytophthora infestans* (Mont.) de Bary) is the most devastating disease worldwide (Fry & Goodwin, 1997; Kamoun et al., 1999) and genetic host plant resistance is one of the major objectives for breeding (Colon et al., 1995).

Although breeding for late blight resistance has had greater priority in the last century than for any other pathogen, the potato market in many countries is dominated by late blight susceptible cultivars (Umaerus et al., 1983). As an example, among 147 cultivars and breeding lines evaluated against US8 genotype of *P. infestans* in greenhouse experiments, two-thirds were classified as very susceptible (Douches et al., 1997). In fact, there is no North American cultivar currently in use that has an adequate level of late blight resistance (Helgeson et al., 1998). In contrast, potato breeders have made great progress over the last century for early maturity, chip-color, tuber appearance, and specific gravity (Douches et al., 1996) and cultivars with good chip-processing quality have been released (Love et al., 1998). Since late blight resistance is not the trait that confers enough advantage for a clone to become a successful cultivar (Umaerus et al., 1983), late blight resistance needs to be combined with tuber quality, acceptable maturity, and other agronomically important traits.

In order to combine characteristics, a selection/evaluation procedure capable of identifying desirable clones at the early generation stage is required. Selection in single-hill plot reduces the cost for clonal maintenance and also permits the identification of superior parents for use in other cross combinations (Thill & Peloquin, 1995). In addition, an efficient selection strategy should be able to significantly reduce the number of selected clones and to keep superior ones for later generations of selection (Tai & Young, 1984). Since superior tuber quality has been the market-limiting trait that characterizes the cultivars released in the past century (Douches et al., 1996; Love et al.,

1998), this trait should be evaluated and selected for as early as possible in a potato breeding program.

The objectives of this study were to evaluate the ability of late blight resistant parents to transmit chip-processing (chip-color, tuber appearance, and specific gravity) or tablestock (tuber appearance) quality to the progeny and to compare selecting for tuber quality in single-hill versus eight-hill clonal generations.

Material and Methods

Eight unadapted cultivars (B0718-3, Bertita, Bzura, Greta, Libertas, Stobrawa, Tollocan, and Zarevo) with reported late blight resistance were crossed with adapted susceptible cultivars/breeding clones to generate 95 populations segregating for late blight resistance, marketable tuber quality, and maturity as described in Chapter 2. For each cross, 50 seedlings (4,750 seedlings total) were transplanted at the Michigan State University Montcalm Experiment Station, MI in 1997 with 75 cm within-row spacing between plants. At harvest, approximately 10% of the best offspring from each cross were selected based on tuber appearance, number, shape, and internal defects, which resulted in 408 clones (8.9%). These clones were grouped according to their late blight resistant parent and were planted in 1998 in eight-hill plots with 30 cm within-row spacing. In this study, family refers to half-sib progeny of the respective late blight parent (i.e. B0718-3 family, Bertita family, etc).

All tubers harvested from single-hill plots and a random sample of tubers (5.1-8.3 cm diameter) from eight-hill plots was used for tuber appearance and specific gravity

evaluations. Tuber appearance was evaluated on a scale 1 to 5 of increasing defects (1 = excellent, as cultivar Atlantic; 2 = very good; 3 = acceptable; 4 = poor; and 5 = very poor). Specific gravity was measured on a minimum 2 kg sample using the formula [weight in air / (weight in air - weight in water)] with 1.080 or greater considered acceptable for chip-processing. One tuber and five-tuber samples (two slices/tuber), respectively from single- and eight-hill plots, were used for chip-color evaluation. Chip-color was evaluated on a scale 1 to 9 of increasing color darkness (1 - 2 = excellent; 3 = very good, as cultivars Atlantic and Snowden; 4 = acceptable; 5 = unacceptable; and 6 - 9 = poor). From here after, tuber quality refers to the combination of tuber appearance, specific gravity, and chip-color.

Tuber quality data were analyzed using a mixed model (SAS PROC MIXED) including families, years (single- and eight-hill clonal generations) and the interaction family x year as fixed effects. Variance components were estimated by restricted maximum likelihood. The same analysis was done considering only years as a fixed effect. The significance of fixed effects was tested by the F type III test. Family means were compared by Fisher's least significance difference (LSD) at $\alpha = 0.05$. Pearson correlation analysis was done to compare tuber quality data from single- and eight-hill clonal generations. Correlation analysis was done at the clonal level for individual late blight families and combined analysis of all families. Correlation analysis was also done at the family level (late blight family means). All these analysis were done following the procedures of SAS (SAS Institute, 1995).

Results

Progeny performance for tuber quality

Families, years (single- and eight-hill clonal generations) and the interaction family x year as fixed effects were significant ($P < 0.01$) for all tuber quality traits, except for specific gravity between years. When year was considered as a fixed effect, there was not a significant ($P > 0.05$) difference between years for chip-color and specific gravity. The performance of the late blight families for tuber quality in single-hill plot in 1997 showed that, on average, families had marginal chip-color (4.2), acceptable tuber appearance (2.6), and high specific gravity (1.087) (Table 3.1). The Libertas family had the best chip-color, but did not significantly differ from Zarevo, Tollocan, and Stobrawa families. For tuber appearance, the B0718-3 family had the best ratings, but did not significantly differ from the Tollocan family. The Libertas family was superior for both specific gravity and chip-color and the Tollocan family for chip-color and tuber appearance.

The average performance of the families was slightly better in eight-hill plots than in single-hill plot (Table 3.2). Greta and B0718-3 families had the best chip-color. The B0718-3 family had the best and the Zarevo had the second best average tuber appearance rating. For specific gravity, Libertas, Zarevo, and Bzura families had the highest values. In eight-hill plot, only the B0718-3 family performed the best for more than one trait (chip-color and tuber appearance).

The correlation analysis at the clonal level showed significant coefficients between single- and eight-hill clonal generations for the combined analysis of all late blight families for all tuber quality traits (Table 3.3). Tuber appearance had the smallest

(0.27) and specific gravity had the highest correlation coefficient (0.67). Considering individual late blight families, significant correlations between single- and eight-hill clonal generations for all individual families were found only for chip-color. Libertas family for specific gravity and B0718-3, Greta, Libertas, Stobrawa, and Tollocan families for tuber appearance had no significant correlation between single- and eight-hill clonal generations. At the late blight family level, high correlation coefficients were found for tuber appearance ($r = 0.78$, $P < 0.05$) and for specific gravity ($r = 0.92$, $P < 0.01$), but no significant correlation was found for chip-color (data not shown).

Table 3.1. Progeny performance of eight late blight resistant parents for tuber quality in single-hill plot in 1997.

Late Blight Families[1]	Evaluated Clones	Chip-Color[2] (ratings)	Tuber Appearance[3] (ratings)	Specific Gravity[4]
Libertas	52	3.7 a[5]	2.8 cdef	1.098 a
Zarevo	92	4.0 ab	2.5 bc	1.094 b
Tollocan	71	4.0 ab	2.4 ab	1.084 e
Stobrawa	34	4.1 ab	2.6 bcd	1.089 cd
Greta	28	4.3 bc	3.0 def	1.088 cd
B0718-3	59	4.3 bc	2.2 a	1.077 f
Bertita	40	4.3 bc	3.1 f	1.080 f
Bzura	32	4.7 c	2.6 bcd	1.091 bc
Average	51	4.2	2.6	1.087

[1] All evaluated clones are half-sibs in relation to the late blight resistant parent.

[2] Evaluated as a scale 1 to 9 of increasing color darkness.

[3] Evaluated as a scale 1 to 5 of increasing defects.

[4] Formula [weight in air / (weight in air - weight in water)].

[5] Means in columns followed by the same letter are not significantly different using Fisher's LSD at $\alpha = 0.05$.

Table 3.2. Progeny performance of eight late blight resistant parents for tuber quality in eight-hill plot in 1998.

Late Blight Families[1]	Evaluated Clones	Chip-Color[2] (ratings)	Tuber Appearance[3] (ratings)	Specific Gravity[4]
Greta	28	3.2 a[5]	2.5 c	1.090 b
B0718-3	59	3.7 ab	1.4 a	1.078 d
Zarevo	92	3.8 bc	1.7 b	1.095 a
Bertita	40	3.9 bcd	2.5 c	1.080 cd
Libertas	52	4.0 bcde	2.5 c	1.094 ab
Bzura	32	4.2 cdef	2.5 c	1.097 a
Tollocan	71	4.3 def	2.3 c	1.082 c
Stobrawa	34	4.4 f	2.2 c	1.090 b
Average	51	3.9	2.2	1.092

[1] All evaluated clones are half-sibs in relation to the late blight resistant parent.

[2] Evaluated as a scale 1 to 9 of increasing color darkness.

[3] Evaluated as a scale 1 to 5 of increasing defects.

[4] Formula [weight in air / (weight in air - weight in water)].

[5] Means in columns followed by the same letter are not significantly different using Fisher's LSD at $\alpha = 0.05$.

Table 3.3. Correlations at the clonal level between single- and eight-hill clonal generations for individual late blight families and combined for all families.

Late Blight Families	Traits	Specific Gravity (SG)	Tuber Appearance (TA)	Chip-Color (CC)
B0718-3	SG	0.43**		
	TA		ns[1]	
	CC			0.28*
Bertita	SG	0.56**		
	TA		0.041*	
	CC			0.60***
Bzura	SG	0.44*		
	TA		0.48**	
	CC			0.63***
Greta	SG	0.59**		
	TA		ns	
	CC			0.43*
Libertas	SG	ns		
	TA		ns	
	CC			0.38*
Stobrawa	SG	0.68***		
	TA		ns	
	CC			0.72***
Tollocan	SG	0.30*		
	TA		ns	
	CC			0.36*
Zarevo	SG	0.65***		
	TA		0.25*	
	CC			0.46***
Combined	SG	0.67**		
	TA		0.27**	
	CC			0.42**

[1] ns = not significant, * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

Selection of clones with acceptable tuber quality in single-hill plot

Libertas, Stobrawa, Zarevo, and Tollocan families had the highest percentage of clones with acceptable chip-color (Table 3.4). Greta and Bzura were the parents that transmitted chip-color to the smallest percentage of its offspring. A selection based on a light chip-color (ratings ≤ 3) in single-hill plot eliminated 18% of the clones that would be selected in eight-hill plot. Alternatively, using the chip-color rating of 4 (acceptable quality) as a threshold for selection in single-hill plot eliminated only 7% of the clones that had ratings ≤ 3 in eight-hill plot. The percentage of discarded clones with acceptable chip-color in eight-hill plots varied from 0% (Stobrawa family) to 25% (Greta family).

Stobrawa, B0718-3, Zarevo, Tollocan, and Bzura transmitted acceptable tuber appearance (ratings ≤ 3) to more than 90% of the progeny (Table 3.5). An average of 32% of the clones that would have been rejected from single-hill plot for tuber appearance had good tuber appearance (ratings ≤ 2) in eight-hill plot. However, a selection criterion of tuber appearance ratings ≤ 3 would have eliminated only 4% of the clones that would be selected in eight-hill plot. The percentage of discarded clones with acceptable tuber appearance in eight-hill plot varied from 0% (Bzura family) to 14% (Greta family).

Bzura, Libertas, Zarevo, and Stobrawa transmitted acceptable specific gravity to more than 90% of the offspring (Table 3.6). The selection of clones with specific gravity ≥ 1.080 (acceptable range) in single-hill plot resulted in 82% selection and would have eliminated only 4% of the clones that would be selected in eight-hill plot. The percentage of discarded clones that had acceptable specific gravity in eight-hill plot varied from 0% (Bzura and Stobrawa families) to 11% (Greta family).

Table 3.4. Percentage comparison of clones with acceptable chip-color quality in single- and eight-hill clonal generations.

Late Blight	Chip-Color Ratings ≤ 3[2]		Chip-Color Ratings ≤ 4[2]	
Families[1]	Selected	Not Selected[3]	Selected	Not Selected[3]
Libertas	44.2	9.6	80.8	1.9
Stobrawa	32.4	11.8	76.5	0.0
Zarevo	28.3	22.8	71.7	5.4
Tollocan	52.1	11.3	69.0	7.0
Bertita	32.5	17.5	60.0	7.5
B0718-3	28.8	20.3	59.3	11.9
Greta	21.4	53.6	57.1	25.0
Bzura	21.9	9.4	56.3	3.1
Total	34.3	18.4	67.6	7.1

[1] All evaluated clones are half-sibs in relation to the late blight resistant parent.

[2] Evaluated on a scale 1 to 9 of increasing color darkness in single-hill plots.

[3] Not selected with chip-color ratings ≤ 3 in eight-hill plot.

Table 3.5. Percentage comparison of clones with acceptable tuber appearance quality in single- and eight-hill clonal generations.

Late Blight	Tuber App. Ratings ≤ 2[2]		Tuber App. Ratings ≤ 3[2]	
Families[1]	Selected	Not Selected[3]	Selected	Not Selected[3]
Stobrawa	35.3	50.0	100.0	0.0
B0718-3	67.8	28.8	94.9	3.4
Zarevo	48.9	40.2	93.5	3.3
Tollocan	49.3	22.5	91.5	1.4
Bzura	50.0	15.6	90.6	0.0
Libertas	34.6	34.6	86.5	9.6
Greta	21.4	35.7	82.1	14.3
Bertita	22.5	27.5	72.5	7.5
Total	44.4	32.1	90.0	4.4

[1] All evaluated clones are half-sibs in relation to the late blight resistant parent.

[2] Evaluated on a scale 1 to 5 of increasing defects in single-hill plots.

[3] Not selected with tuber appearance ratings ≤ 2 in eight-hill plot.

Table 3.6. Percentage comparison of clones with acceptable specific gravity quality in single- and eight-hill clonal generations.

Late Blight	Specific Gravity ≥ 1.080[2]	
Families[1]	Selected	Not Selected[3]
Bzura	100.0	0.0
Libertas	98.1	1.9
Zarevo	96.7	1.1
Stobrawa	91.2	0.0
Greta	89.3	10.7
Tollocan	76.1	8.5
Bertita	55.0	7.5
B0718-3	52.5	6.8
Total	82.1	4.4

[1] All evaluated clones are half-sibs in relation to the late blight resistant parent.

[2] Formula [weight in air / (weight in air - weight in water)].

[3] Not selected with specific gravity ≥ 1.080 in eight-hill plot.

Identification of clones with acceptable chip-processing or tablestock quality

The identification of clones with acceptable chip-processing quality was based on the threshold for selection identified for each tuber quality characteristic (Tables 3.4, 3.5, and 3.6). The selection was first done for chip-color ratings ≤ 4 followed by tuber appearance ratings ≤ 3 and a specific gravity ≥ 1.080. Using this selection criterion, a total of 206 clones (50%) and 228 clones (56%) were identified in single- and eight-hill clonal generations, respectively, as possessing acceptable chip-processing quality. A total of 146 clones were selected in both clonal generations, which was 71% of the clones identified in single-hill plot.

The identification of clones for tablestock was done based solely on tuber appearance ratings ≤ 3. A total of 367 clones (90%) and 387 clones (95%) were selected, respectively, in single- and eight-hill plot. Five parents (Zarevo, Stobrawa, B0718-3, Tollocan, and Bzura) transmitted acceptable tablestock quality to more than 90% of the progeny in both generations of selection. A total of 350 clones were selected in both clonal generations, which was 95% of the clones identified in single-hill plot.

Discussion

In this study, unadapted late blight resistant parents were crossed with adapted susceptible clones to select for tuber quality traits. Selection within these crosses was also done for foliar late blight resistance based upon greenhouse and field tests, in which 80 clones were identified as possessing moderate to strong late blight resistance to the US8 genotype of *P. infestans* (Bisognin et al., 2001). Cultivar releases over the past

century suggest that tuber quality should be considered a market-limiting trait (Douches et al., 1996). Therefore, even in breeding disease resistant cultivars, tuber quality needs to be a high priority for selection and these results showed that selection for tuber quality could be initiated at the single-hill plot. Since Tollocan and B0718-3 transmit a higher level of late blight resistance to the highest percentage of the offspring (Bisognin et al., 2001), these two parents offer the best chance for combining resistance with tuber quality traits. Tollocan and B0718-3 are also the best candidates to apply the strategy proposed here, in which selection for tuber quality precedes selection for late blight resistance.

The selection of parents for their potential to transmit important traits to the offspring is an important step in a potato breeding program (Tai & Young, 1984; Thill & Peloquin, 1995). In this study we identified late blight resistant parents that also transmit tuber quality traits to the offspring. B0718-3 and Tollocan families had the highest tuber appearance ratings in single-hill plot. The B0718-3 family also had the highest tuber appearance rating average and the highest percentage of selected clones with a tuber appearance rating ≤ 2. Bzura, Zarevo, and Libertas families had the highest specific gravity in both generations of selection and the highest percentage of selected clones. The fact that late blight resistant parents were crossed with a different number and, in most cases, to different susceptible parents might have influenced family performance. Therefore, all family differences found in this study should not be attributed solely to the late blight resistant parent, but it was clear that those parents do differ in tuber quality traits transmitted to the offspring. Bzura and Stobrawa were crossed to the same susceptible parents and Greta was crossed to four out of five susceptible parents. Greta family had the highest chip-color rating and Bzura family had the highest specific gravity in eight-hill plot. Moreover, late blight family differences can not be attributed to the

phenotypic selection done at harvest time, since the same selection intensity was applied to all crosses.

Considering only the percentage of selected clones, Libertas, Stobrawa, Zarevo, and Tollocan were the best parents for transmitting chip-color; B0718-3, Stobrawa, Zarevo, and Tollocan for transmitting tuber appearance; and Bzura, Libertas, Zarevo, and Stobrawa for transmitting specific gravity to the highest percentage of the offspring. The Stobrawa family, for chip-color, and the Stobrawa and Bzura families, for tuber appearance and specific gravity, had all selected clones identified in single-hill plot, but the Greta family had the highest percentage of non-selected clones for all traits. Stobrawa, Bzura and Greta have similar contribution of susceptible parents. Therefore, the percentage of selected clones was effective in showing differences among late blight families when selection for tuber quality was applied at the single- or eight-hill clonal generations. Interaction between parents with years would increase the percentage of discarded clones as in the case of Greta family.

Attempting to breed for tuber quality traits in potato, a phenotypic selection based on tuber appearance, number, shape, and internal defects at harvest time in single-hill plot was able to reduce the number of evaluated clones from 4,750 to 408. Comparing with other traits considered for phenotypic selection, Tai (1975) determined that tuber appearance was the only trait to directly affect selection and Neele et al. (1991) found tuber yield to be the decisive component for selection. From the 408 clones, 68% possessed acceptable chip-color, 90% possessed acceptable tuber appearance, and 82% possessed desirable specific gravity in single-hill plot. If a moderate selection, based upon tuber appearance ratings ≤ 3 for tablestock, were employed no more than 4% of the

clones discarded in eight-hill plot would have been selected for each trait (chip-color, tuber appearance, and specific gravity) in eight-hill plot.

The high percentage of clones selected in both clonal generations is supported by the significant coefficients of correlation obtained between single- and eight-hill clonal generations. Correlation was considered the best estimate to determine relationship between early generations of selection in potato breeding (Maris, 1988). Chip-color and tuber appearance had smaller correlation coefficients than specific gravity at the clonal level, while at the family level, there was no correlation between generations for chip-color. As a consequence, with higher correlation coefficients, 96% of clones with desirable specific gravity ($\geq$ 1.080) in eight-hill could be identified in single-hill plot using the same selection criteria. High correlation was expected for specific gravity because this trait has been previously reported to have a small genotype x environment interaction (Killick & Simmonds, 1974). Haynes & Wilson (1992) also found high positive correlation for specific gravity between the two first generations in the field.

The correlation between single- and eight-hill clonal generations suggests that moderate selection intensity should be applied in single-hill plot for tuber appearance and chip-color. Tuber appearance had lower correlation coefficients at the clonal level than chip-color and had no significant correlations between single- and eight-hill clonal generations for five individual families. Tuber appearance was also the only trait significantly affected by year (single- and eight-hill clonal generations). Chip-color had significant correlations for all individual late blight families, but small correlation (0.42) for all combined families. As opposed to specific gravity, selecting clones with desirable tuber appearance (ratings $\leq$ 2) and chip-color (ratings $\leq$ 3) would eliminate a significant percentage of clones that would have desirable quality based on eight-hill plot. However,

selecting clones with acceptable tuber appearance (ratings ≤ 3) and chip-color (ratings ≤ 4) in single-hill plot would discarded a very small percentage of clones that would be selected in eight-hill plot with desirable quality. Tai (1975) also found low correlation for tuber appearance at the clonal level, but medium to high correlations at the family level. Neele et al. (1991) found high heritability estimates for tuber appearance components such as tuber shape (0.61), regularity of tuber shape (0.60), skin color (0.86), eye depth (0.69), number of tubers (0.54), and average tuber weight (0.64).

The fact that clones with desirable quality, for specific gravity, and clones with acceptable quality (moderate selection intensity), for chip-color and tuber appearance, can be applied at single-hill plot is very important, since there is a gain of one year in the selection process for tuber quality. Thill & Peloquin (1995) reported that selection decisions for cold chip-processing at the single-hill plot did not differ from those in late generations and could potentially save four years in the breeding cycle. A low to moderate selection pressure in early generations was found in other studies as the best choice to reach a balance between gain from selection and elimination of valuable clones (Tai & Young, 1984; Maris, 1988). Neele et al. (1989) determined that phenotypic selection in early generations was optimized when about 32% of the clones were selected in the first clonal generation. Anderson & Howard (1981) found a higher number of discarded than selected clones comparing the first two generations of selection. In comparison, the post-harvest selection used here reduced the initial number of clones from 4,750 to 206, through selection for chip-processing quality (4.3% of selected clones), and to 367, through selection for tablestock quality (7.7% of selected clones). However, the evaluation in single- and eight-hill clonal generations has some key differences that should be considered in the selection process. The performance of plants

grown either from small greenhouse tubers or from transplants is often very distinct from that of the same plants grown from regular sized seed tubers (Davies & Johnston, 1974). In addition, difference in the in-row spacing (75 cm vs. 30 cm) may influence plant competition. These factors can reduce the heritability in single-hill plot for many traits resulting in poor selection efficiency (Tai & Young, 1984). Sample size is another concern for evaluations in single-hill plot. The accuracy of the specific gravity estimation decreases rapidly for samples smaller than 10 tubers (Lulai & Orr, 1979).

Different traits are of primary importance when developing cultivars with chip-processing or tablestock quality. Chip-color is the most important trait for the chip-processing industry (Thill & Peloquin, 1995) followed by tuber appearance (freedom from internal and external defects) and high dry matter. For tablestock cultivars, tuber appearance is the most important trait (Dale & Mackay, 1994). If the objective is to develop cultivars for chip-processing industry and tablestock, the tuber quality information from single-hill plot could be used to assist in making better decisions in later generations of selection for other traits including disease resistance. Also, multitrait selections based on data from different environmental conditions might increase the probability of identifying clones possessing an acceptable balance of key agronomic traits. Haynes & Wilson (1992) found that the probability of selecting the same clone in the later generation was 1.7 and 1.9 times higher for clones selected based on horticultural characteristics than on specific gravity.

In summary, a moderate selection intensity for tuber quality traits (chip-color ratings ≤ 4, tuber appearance ratings ≤ 3 and a specific gravity ≥ 1.080) can be initiated at the single-hill plot in crosses to select for late blight resistance. The identification of superior clones for tuber quality in single-hill plot reduces each selection cycle in one

year and reduces the number of clones for late blight testing. Intermating selected clones, a higher percentage of clones possessing acceptable chip-processing or tablestock quality is expected in following cycles of genotypic recurrent selection. Moreover, Tollocan and B0718-3 are the best parents for improving late blight resistance and offer the best opportunity for the application of tuber quality selection in advance of disease resistance selection for combining desirable traits. The progenies of Tollocan and B0718-3 could also be combined with the offspring of other high valuable sources of late blight resistance for the development of cultivars with durable resistance.

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CHAPTER IV

Genetic Diversity in Diploid and Tetraploid Late Blight Resistant Potato Germplasm

Abstract

An understanding of the genetic relationship within potato germplasm is important to establish a broad genetic base for breeding purposes. The objective of this study was to assess the genetic diversity of potato (*Solanum tuberosum* subsp. *tuberosum* Hawkes) germplasm that can be used in the development of cultivars with resistance to late blight caused by *Phytophthora infestans* (Mont.) de Bary. Thirty-three diploid and 27 tetraploid late blight resistant potato clones were evaluated for their genetic diversity based on 11 isozyme loci and nine microsatellites. A total of 35 allozymes and 42 polymorphic microsatellite fragments was scored for presence or absence. The germplasm was clustered based on the matrix of genetic similarities and the unweighted pair group means analysis of the isozyme and microsatellite data, which were used to construct a dendrogram using NTSYS-pc version 1.7. Twenty-three allozymes and DNA fragments were unique to the wild species. The diploid *Solanum* species *S. berthaultii* Hawkes and *S. microdontum* Bitter formed two distinct phenetic groups. Within *S.*

microdontum, three sub-groups were observed. The tetraploid germplasm formed another group, with *S. sucrense* Hawkes in one sub-group and the cultivated potato and Russian hybrids in another sub-group. Based upon the genetic diversity and the level of late blight resistance, *S. microdontum* and *S. sucrense* offer the best choice for improved late blight resistance from genetically diverse sources. This potato germplasm with reported late blight resistance should be introgressed into the potato gene pool to broaden the genetic base to achieve stronger and more durable resistance.

Introduction

The cultivated potato and its wild relatives belong to the genus *Solanum* L. sect. *Petota* Dumort. There are seven cultivated and 225 wild potato species, according to the most recent taxonomic treatment of Hawkes (1990), which include diploid ($2n = 24$), tetraploid ($2n = 48$), hexaploid ($2n = 72$) and a few triploid ($2n = 36$) and pentaploid ($2n = 60$) cytotypes (Spooner & van den Berg, 1992). The cultivated potato *S. tuberosum* subsp. *tuberosum* Hawkes is an autotetraploid ($2n = 4x = 48$) that originated in South America.

Despite the wide genetic diversity that exists in the genus *Solanum*, the use of closely related germplasm in breeding programs has resulted in high genetic similarity among more than 130 potato cultivars released in North America between 1930 and 1970 (Mendoza & Haynes, 1974). The pedigrees of most cultivars can be traced back to the cultivars Early Rose and one of its parents, Garnet Chili (Plaisted & Hoopes, 1989).

Moreover, cultivars released between 1950 and 1970 have a high genetic similarity and may have reached a yield plateau (Mendoza & Haynes, 1974).

Molecular markers have been used to confirm the relatedness among North American potato cultivars. Coefficients of similarity ranged from 0.51 to 0.89 among 28 potato cultivars based on random amplified polymorphic DNA (RAPD) (Demeke et al., 1996) and from 0.44 to 0.81 among 18 potato cultivars from different origins based on simple sequence repeats (SSR) or microsatellites (Provan et al., 1996). An identical chloroplast DNA (T-type) restriction pattern was found among 10 historically important potato cultivars that traced back through Garnet Chili to Rough Purple Chili, indicating that there is only one maternal lineage (Douches et al., 1991). The predominance of the T-type cytoplasm derived from Rough Purple Chili was found in the modern European cultivated gene pool. Rough Purple Chili was introduced in Europe after the 1840's late blight epidemic and was extensively used as female parent (Provan et al., 1999).

Late blight, caused by the fungal-like oomycete *Phytophthora infestans* (Mont.) de Bary, is the most devastating potato disease worldwide (Fry & Goodwin, 1997; Kamoun et al., 1999) and causes both foliar destruction and tuber decay (Ross, 1986). The development of genetic resistance to late blight in potatoes is one of the major objectives in many breeding programs (Colon et al., 1995a) and has resulted in the release of late blight resistant germplasm (Goth & Haynes, 1997; Corsini et al., 1999). Essential studies on breeding potato for late blight resistance have been done, such as identification of resistance sources (Colon & Budding, 1988; Colon et al., 1995c; Douches et al., 2001a), components of resistance (Colon et al., 1995a,b,c), and phenotypic stability of resistance (Haynes et al., 1998). These reported late blight resistance sources are of different origin and ploidy levels and have variable levels of

resistance. Combining these sources in a breeding program will establish a broad genetic base in the cultivated potato from which the probability of selecting superior offspring is increased. Moreover, improvements in yield, adaptation, tuber quality and disease resistance can be achieved by broadening the genetic base of potato breeding populations (Mendoza & Haynes, 1974).

The objective of this study was to assess the genetic diversity of this potato germplasm with reported late blight resistance using a set of isozyme loci and microsatellite markers. These data can be used to characterize this germplasm that can be introgressed into cultivated gene pools to enhance late blight breeding efforts and concurrently broaden the genetic base of cultivated potatoes.

Material and Methods

The potato late blight resistant germplasm used in this study was identified using a greenhouse fine-screening technique (Douches et al., 2001a) and represents different origins and ploidy levels (Table 4.1). Of the total of 60 evaluated clones, 36 were from South America (2 species), 14 were tetraploid hybrids (wild x cultivated potato), and 10 were tetraploid advanced breeding clones or cultivars from North America (5), Poland (3), Sweden (1), and Russia (1). For simplification, all accessions or cultivars will only be referred to by their respective code identification (Table 4.1).

Table 4.1. Pedigree of *Solanum* accessions or cultivars used in this study and their origin, ploidy level and clone identification.

Accessions or Cultivars	Code ID[1]	Pedigree	Origin	Ploidy Level (2*n*)	Clone ID Number
PI 595507	ber1	*S. berthaultii* Hawkes	South America	2*x*	3,8,12,16,20
PI 498104	ber2	*S. berthaultii*	South America	2*x*	19
PI 458358	mcd1	*S. microdontum* Bitter	South America	2*x*	8
PI 473170	mcd2	*S. microdontum*	South America	2*x*	17,33
PI 498124	mcd3	*S. microdontum*	South America	2*x*	1,5,6,7,12,17,20,21,25
PI 595509	mcd4	*S. microdontum*	South America	2*x*	12
PI 595510	mcd5	*S. microdontum*	South America	2*x*	10,14,16,19,22
PI 595511	mcd6	*S. microdontum*	South America	2*x*	2,3,5,13,14,18,22,23,25
PI 595512	scr1	*S. sucrense* Hawkes	South America	4*x*	16,17,19
VIR 595516	K97	((*S. megistacrolobum* Bitter x Gatchinski) x Umbra) x Fausta	Russia	4*x*	10,18
VIR 595517	K98	(*S. verrucosum* Schltdl. x MPI 50.140/5) x MPI 50.140/5	Russia	4*x*	1,17
VIR 595518	K99	(*S. microdontum* x Atzimba) x Earlaine	Russia	4*x*	1
VIR 595519	K100	(*S. polytrichon* Rydb. x Anoka) x Runo	Russia	4*x*	2,6
VIR 595520	K101	((*S. microdontum* x MPI 50.140/5) x Boone) x Desiree	Russia	4*x*	9
VIR 595521	K102	*S. berthaultii* x *S. tuberosum* subsp. *andigena* Hawkes	Russia	4*x*	5
VIR 595522	K103	*S. vernei* Bitter & Wittm. x MPI 50.140/5	Russia	4*x*	19
VIR 595523	K104	(*S. gourlayi* Hawkes x Hannibal) x Hannibal	Russia	4*x*	8,20,21
VIR 595524	K105	*S. berthaultii* x Taiga	Russia	4*x*	20
AWN86514-2	AWN86514-2	KSA195-96 x Ranger Russet	USDA, Aberdeen, ID	4*x*	
B0718-3	B0718-3	B0286-3 x B9933-27	USDA, Beltsville, MA	4*x*	
Bertita	Bertita	Ac25953 x Ac25959 Ac = (*S. tuberosum* subsp. *andigena* x (*S. demissum* Lindley x *S. tuberosum* L.))	Mexico	4*x*	
Bzura	Bzura	((PG-232 x (PG-96 x Mira-1)) x ((Prosna x (Z10465 x Z951))	Poland	4*x*	
Greta	Greta	Unica x ((Magnum x (Early Rose x Patersis Victoria))	Sweden	4*x*	
Libertas	Libertas	((Record x (Trenctria x Energie)) x ((Bravo x Energie) x (Rode Star x Pepo))	Netherlands	4*x*	
MSG274-3	MSG274-3	Tollocan x Chaleur	MSU Breeding Program	4*x*	
Stobrawa	Stobrawa	Mira-1 x ((MPI55.957/54 x (MPI50.140/5 x MPI44.1016/10))	Poland	4*x*	
Tollocan	Tollocan	((58-ER-1 x (Loman x HOL-32)) x ((((Juanita x (((Loman x ((Anita x (AC25953 x USDA2131-3))))	Mexico	4*x*	
Zarevo	Zarevo	7692C/68 (*S. demissum* Lindley, *S. tuberosum* subsp. *andigena* *S. leptophyes* Bitter, *S. tuberosum*) x Berka (*S. demissum*)	Russia	4*x*	

[1] Code is for USDA Plant Introduction identification in this study, but the clone identification number is the same as in the US Potato Genebank, NRSP-6, WI.

The genetic diversity of the potato late blight resistant germplasm was assessed using isozyme and microsatellite markers. The isozyme analysis was carried out using crude protein extraction from a newly expanded leaflet (approximately 120 mg), resolved in a horizontal 10% starch gel by electrophoresis with two buffer systems. Each accession or cultivar was sampled and run twice. Tissue processing, electrophoresis, staining, and nomenclature were done as described in Douches & Quiros (1988). Eleven isozyme loci of seven enzyme systems were scored according to Douches & Quiros (1988) and Douches & Ludlam (1991). Malate dihydrogenase (*Mdh-1* and *Mdh-2*), 6-phosphogluconic dehydrogenase (*6-Pgdh-3*), phosphoglucose isomerase (*Pgi-1*), and isocitric acid dehydrogenase (*Idh-1*) were resolved with a histidine-citrate pH 5.7 buffer system (Stuber et al., 1988). Diaphorase (*Dia-1* and *Dia-2*), glutamate oxaloacetate transaminase (*Got-1* and *Got-2*) and phosphoglucomutase (*Pgm-1* and *Pgm-2*) were resolved with a lithium-borate pH 8.3 buffer system (Stuber et al., 1988). Nine of these isozyme loci have been previously mapped to six distinct potato linkage groups. *Mdh-2* and *Idh-1* were mapped to linkage group I, *Pgm-1* to III, *Pgm-2* to IV, *Mdh-1*, *6-Pgdh-3* and *Dia-1* to V, *Got-2* to VII, and *Got-1* to VIII (Bonierbale et al., 1988; Freyre & Douches, 1994). For statistical analysis, each allele was recorded as 1 for presence or 0 for absence.

DNA amplification, using nine pairs of microsatellite primers, was carried out in a total volume of 20 µl containing 1X REDTaq™ PCR reaction buffer, 1 unit of REDTaq™ DNA polymerase (Sigma-Aldrich Co., St. Louis, MO.), 20 ng of each dNTP, 25 ng of each microsatellite primer, and 50 ng of template DNA. The sequence of eight pairs of primers (*G28WXST*, *STPROINI*, *ST STP*, *STACCAS3*, *STWIN12G*, *POTM 1-2*, *ST13ST*, and *STLS1*) are published (Ashkenazi et al., 2001). The other primer combination, potato

inhibitor *IIK* locus, was also used by Provan et al. (1996) and Milbourne et al. (1997), and the sequences are identified as STIIKA. Four microsatellites have a known position on the potato linkage map. The loci G28WXST, STACCAS3 and POTM 1-2 map to linkage groups VIII, VII and VI, respectively (Veilleux, personal communication). The potato inhibitor IIK locus maps to the linkage group III (Meyer et al., 1998).

All amplifications were carried out on a Thermolyne Amplitron® (Barnstead™ Thermoline Corporation, Dubuque, IA.) thermal cycler. The protocol was as follows: 1) initial denaturation at 94 °C for 4 min; 2) 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 1.5 min; and 3) final extension at 72 °C for 5 min. The completed reaction products were held at 4 °C until electrophoretic separation using a 3% Metaphor™ Agarose (FMC Bioproducts, Rockland, ME.) gel with TBE (90 mM tris-borate, 90 mM boric acid and 2 mM EDTA) buffer. The gels were run at 100V for 4 h at 10 °C, stained with ethidium bromide (1 $\mu g \cdot ml^{-1}$) for 45 min, visualized under UV light, and photographed. Each microsatellite fragment was scored as 1 for presence and 0 for absence. Fragment sizes were estimated using a 50 bp DNA ladder (Gibco BRL, Grand Island, NY.) in each gel.

For statistical analysis, data were scored as the presence or absence of alleles (isozymes) or fragments (microsatellites). The mean number of alleles per locus, the proportion of polymorphic loci and the mean expected heterozygosity (Nei, 1972) were estimated per accession or group of clones based on allelic frequency data. For these parameters, we did not consider allele dosage for isozymes and we evaluated DNA fragments per pair of microsatellite primers. Genetic similarity was calculated using Nei and Li's (1979) computation:

$$GS_{xy} = 2N_{xy} / (N_x - N_y)$$

where N_x and N_y are the number of bands for each genotype, and N_{xy} is the number of bands in common between the two genotypes. The unweighted pair group means analysis (UPGMA) results were used to draw the dendrogram. The distance matrix and the dendrogram were constructed with NTSYS-pc version 1.7 (Rohlf, 1992). Cophenetic correlation coefficients were used to measure the distortion between the similarity matrix and the resultant dendrogram (Rohlf & Sokal, 1981).

Results And Discussion

General genetic diversity in the late blight resistant germplasm

The total mean number of alleles per locus for isozymes was 3.18 and for microsatellites was 4.67 (Table 4.2). To have a better understanding of the genetic diversity, we divided the germplasm by species or group of clones. For example, there were 27 *S. microdontum* Bitter clones that we sub-divided by PI numbers. We also separated cultivated PI (Russian hybrids) from cultivated potato. Based upon both marker systems, *S. sucrense* Hawkes (wild tetraploid) had one of the lowest mean numbers of alleles per locus. On the other hand, Russian hybrids and cultivated potatoes showed the highest number of alleles per locus. *Solanum sucrense* also had the smallest proportion of polymorphic loci, indicating low genetic diversity among evaluated clones. In other marker analyses, *S. sucrense* was previously assessed to have higher levels of genetic diversity (Hosaka & Hanneman, 1991; Bamberg et al., 2000). An underestimation of *S. sucrense* diversity was expected, since only three clones of one accession were evaluated. The highest proportion of polymorphic loci was observed in *S.*

berthaultii Hawkes and Russian hybrids for isozymes and Russian hybrids and cultivated potato for microsatellites. No trend was observed to distinguish species or group of clones using mean of expected heterozygosity (Table 4.2). In general, there were accessions or wild species with a similar level of genetic diversity compared to cultivated potato. However, direct comparisons between diploid and tetraploid species should not be made, since tetraploids have the potential to have greater heterozygosity than diploids. In summary, genetic markers showed that a high level of genetic diversity is distributed among wild diploid, and wild tetraploid and cultivated *Solanum* species with reported late blight resistance.

Sources of unique allozyme alleles

A total of 35 allozymes was detected in the late blight resistant germplasm. The presence of allozymes that were observed in the cultivated group was similar to the Douches et al. (1991) study of 112 North American cultivars and advanced breeding clones. Evaluating the genetic diversity in 2379 accessions of *S. tuberosum* subsp. *andigena* Hawkes, Huamán et al. (2000) identified 38 allozymes, however 2 allozymes had a frequency of only 0.02%.

Isozyme analysis revealed numerous allozymes in the wild germplasm that were not found in the cultivated potato group. Nine allozymes (26%), absent from the cultivated potato group, were present in other tetraploid clones and diploid *Solanum* species (Table 4.3). Ber1 and ber2 had six alleles not present in cultivated potatoes and *Pgi-1*^{5} was unique to *S. berthaultii*. *Solanum microdontum* had seven allozymes that were absent in cultivated potatoes. Allozymes unique to *S. microdontum* were present in mcd6 (*Mdh-1*^{6}) and mcd5 (*Pgm-2*^{1}). *Pgi-1*^{3} was found only in one clone (mcd3-7).

Solanum sucrense had only one allozyme (*6-Pgdh-3*^{3}) not found in the cultivated potato group. The Russian hybrids had three alleles not found in cultivated potatoes, but these alleles were found in only one clone and were also present in wild species.

Sources of unique microsatellite fragments

Forty-two of the 43 DNA fragments from nine pairs of microsatellite primers were consistently amplified and polymorphic. High levels of polymorphism were also found in other genetic studies using microsatellites in cultivated potatoes (Provan et al. 1996; Milbourne et al., 1997; Meyer et al., 1998). Six fragments were absent in the cultivated potato group (Table 4.4). The Russian hybrids did not possess any unique fragments, but did have two fragments that were not found in the cultivated potato group. *Solanum microdontum* possessed five fragments absent from the cultivated potatoes and three fragments associated with three microsatellite loci (G28WXST, POTM1-2, and STLS1) were present in all evaluated clones.

Of the 23 alleles and microsatellite fragments absent in cultivated potatoes, three alleles and two DNA fragments were present in Russian hybrids. These results were, in part, expected since Russian hybrids are hybrids of wild and cultivated potato. However, only four hybrids evaluated here (K99, K101, K102, and K105) have common wild species in their pedigrees. Therefore, the isozyme and microsatellite analyses showed that Russian hybrids carry some genetic diversity from wild species and this diversity is much more accessible in the genetic background to combine with cultivated potatoes.

Table 4.2. Number of evaluated clones per accession or group of genotypes, number of alleles/locus, proportion of polymorphic loci and expected heterozygosity in the potato late blight resistant germplasm.

Germplasm	Clones	Alleles/Locus (no.)		Polymorphic Loci (%)		Expected Heterozygosity[2]	
Code[1]	(no.)	Isozymes	Microsatellites	Isozymes	Microsatellites	Isozymes	Microsatellites
Ber1, ber2	6	2.36	2.78	60	38	0.687	0.625
Mcd1, mcd2, mcd4	4	1.91	3.00	40	36	0.688	0.580
Mcd3	9	2.18	3.67	57	57	0.704	0.532
Mcd5	5	1.64	3.22	31	43	0.678	0.593
Mcd6	9	1.91	3.78	37	60	0.579	0.554
Scr1	3	1.45	2.78	11	21	0.566	0.530
Russian hybrids	14	2.45	4.00	63	71	0.626	0.536
Cultivated potato	10	2.36	4.00	51	69	0.587	0.556
Total	60	3.18	4.67				

[1] Ber = *S. berthaultii*, mcd = *S. microdontum*, and scr = *S. sucrense* and numbers refers to different accessions. See Table 4.1 for details.

[2] Expected heterozygosity is the average of Hn within diploid or tetraploid groups. $Hn = 1 - \Sigma p^2$ (Provan et al., 1996).

Table 4.3. Unique allozymes present in unadapted late blight resistant germplasm and absent in cultivated potato.

		Percent of Individuals that Carry the Unique Allozyme							
		Diploid *Solanum* Species ($2n = 2x$)					Tetraploid Germplasm ($2n = 4x$)		
Isozyme System[1]	Unique Allozyme	Ber[2] 1 & 2	Mcd[2] 1,2 & 4	Mcd 3	Mcd 5	Mcd 6	Scr[2] 1	Russian Hybrids	Cultivated Potato[3]
MDH	*Mdh-1*^{6}	0	0	0	0	89	0	0	0
MDH	*Mdh-2*^{1}	17	0	0	20	100	0	7	0
6-PGDH	*6-Pgdh-3*^{3}	83	0	11	0	78	100	0	0
PGI	*Pgi-1*^{3}	33	0	11	0	0	0	0	0
PGI	*Pgi-1*^{5}	17	0	0	0	0	0	0	0
GOT	*Got-2*^{1}	17	0	0	0	0	0	7	0
GOT	*Got-2*^{4}	0	75	78	80	0	0	7	0
GOT	*Got-2*^{6}	33	0	0	0	67	0	0	0
PGM	*Pgm-2*^{1}	0	0	0	40	0	0	0	0
No. of Alleles	9	6	1	3	3	4	1	3	0

[1] MDH = malate dehydrogenase, 6-PGDH = 6-phosphogluconic acid dehydrogenase, PGI = Phosphoglucose isomerase, GOT = glutamate oxaloacetate transminase, and PGM = phosphoglucomutase.

[2] Ber = *S. berthaultii*, mcd = *S. microdontum*, and scr = *S. sucrense* and numbers refers to different accessions. See Table 4.1 for details.

[3] From Table 4.1: AWN86514-2, B0718-3, Bertita, Bzura, Greta, Libertas, MSG274-3, Stobrawa, Tollocan, and Zarevo.

Table 4.4. Unique microsatellite fragments (bp) present in unadapted late blight resistant germplasm and absent in cultivated potato.

		Percent of individuals that carry the unique microsatellite fragments							
		Diploid *Solanum* species ($2n = 2x$)					Tetraploid germplasm ($2n = 4x$)		
Microsatellite locus	Unique fragments	Ber[1] 1 &2	Mcd[1] 1,2 & 4	Mcd 3	Mcd 5	Mcd 6	Scr[1] 1	Russian hybrids	Cultivated Potato[2]
G28WXST	650	0	100	100	60	56	67	57	0
STSTP	240	0	0	0	0	22	0	14	0
STWIN12G	280	0	0	0	0	56	0	0	0
POTM 1-2	145	0	75	78	100	67	0	0	0
ST13ST def 4	245	0	0	0	0	0	33	0	0
STLS1	90	17	0	89	20	100	0	0	0
No. Fragments	6	1	2	3	3	5	2	2	0

[1] Ber = *S. berthaultii*, mcd = *S. microdontum*, and scr = *S. sucrense* and numbers refers to different accessions. See Table 4.1 for details.

[2] From Table 4.1: AWN86514-2, B0718-3, Bertita, Bzura, Greta, Libertas, MSG274-3, Stobrawa, Tollocan, and Zarevo.

Genetic similarity among late blight resistant clones

The assessment of either 35 allozymes or 42 polymorphic microsatellites alleles was insufficient to completely discriminate diploid from tetraploid species. The cophenetic correlation coefficient between the similarity matrix and the dendrogram was 0.79 and 0.78, respectively for isozymes and microsatellites. *Solanum microdontum*, *S. berthaultii* and *S. sucrense* belong to the series *Tuberosa* (wild) and *S. tuberosum* subsp. *tuberosum* belong to the series *Tuberosa* (cultivated) in the subsection *Petotae* (Hawkes, 1994). In both dendrograms, clones of *S. microdontum* were clustered in different groups with *S. berthaultii*, *S. sucrense* or cultivated potatoes (data not shown).

A combined data analysis from isozyme and microsatellite markers was able to distinguish diploid from tetraploid species and also separate *S. microdontum* from *S. berthaultii*. The cophenetic correlation coefficient between the similarity matrix and the dendrogram data was 0.82. The late blight resistant germplasm formed three groups, two with each diploid wild species (*S. berthaultii* and *S. microdontum*) and one with tetraploid germplasm (*S. sucrense*, Russian hybrids, and cultivated potatoes) (Fig. 4.1). The genetic similarity between *S. berthaultii* and *S. microdontum* groups, excluding mcd4, was 0.58. Within *S. microdontum*, four distinct sub-groups were formed according to accessions (mcd1, mcd2, and mcd3; mcd4; mcd5; and mcd6). Overall, the maximum genetic similarity (0.93) was between Mcd3-1 and Mcd3-5. The tetraploid germplasm group (group 2) formed two sub-groups: one with the wild tetraploid species (*S. sucrense*) and another with Russian hybrids and cultivated potatoes, with a genetic similarity of about 0.65. All cultivars were grouped with Russian hybrids with a genetic similarity of about 0.77. The cultivars B0718-3, Tollocan and AWN86514-2, reported as highly resistant to late blight, separated into three sub-groups among cultivated potatoes.

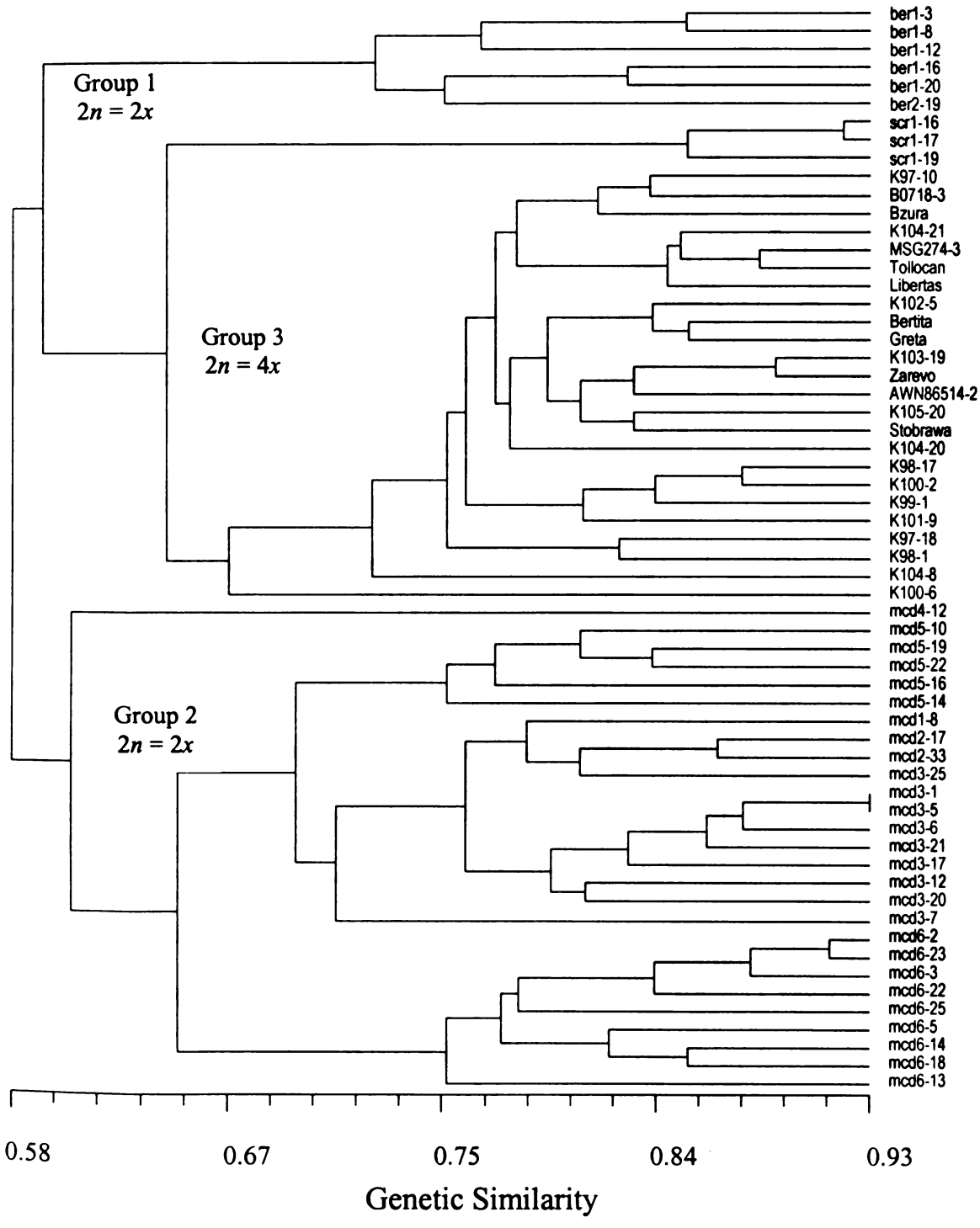

Figure 4.1. Genetic similarity among diploid and tetraploid potato germplasm with reported late blight resistance based on 35 allozymes, encoding 11 isozyme loci, and 42 polymorphic DNA fragments in nine pairs of microsatellite primers. Code for clones and pedigrees follow Table 4.1.

Besides the genetic diversity quantified using isozyme and microsatellite markers, different types of late blight resistance also could be present within wild and cultivated germplasm. Several minor genes with additive effects are involved in the late blight resistance of *S. tuberosum* subsp. *andigena* (part of the pedigree of K102, Bertita, and Zarevo), whereas major resistant genes are involved in the resistance of *S. sucrense* (Colon & Budding, 1988). If the genetic diversity present in the tetraploid germplasm might also include different genes for late blight resistance, clones from different sub-groups should be hybridized as a strategy to combine potential resistance sources.

Improvements of late blight resistant cultivars with broad genetic base

Pedigree (Mendoza & Haynes, 1974; Plaisted & Hoopes, 1989) and chloroplast diversity analysis (Douches et al., 1991; Provan et al., 1999) showed that a high genetic similarity characterizes many potato cultivars released in the last century. High genetic uniformity can result in vulnerability to diseases, pests and abiotic factors, and reduces gain from selection (Mendoza, 1989). Consequently, increasing genetic diversity in the cultivated potato gene pool is a goal in many breeding programs. Moreover, improving genetic diversity for non-T-type cytoplasm is important to reduce breeding problems associated with male sterility (Provan et al., 1999). The species studied here can be used to enhance efforts to breed late blight resistant tetraploid germplasm and to broaden the genetic base of the cultivated potato using simple crossing schemes ($4x$ - $4x$ and $4x$ - $2x$).

The late blight resistant germplasm differs in the level and source of resistance (Haynes et al., 1998; Douches et al., 2001a). *Solanum microdontum* (mcd3, mcd5, and mcd6) and *S. sucrense* had the highest level of resistance to the US8 genotype of *P. infestans*. Also, *S. berthaultii* and other *S. microdontum* accessions and the Russian

hybrids had moderate to high resistance (Douches et al., 2001a). The source of resistance for the hybrids K98 and K100 is Mexican *Solanum* species, whereas all other hybrids (K97, K99 and K101 - K105) have South American wild species in their pedigrees. The hybrid K102 is a cross between two South American *Solanum* species (*S. berthaultii* and *S. tuberosum* subsp. *andigena*) in which both parents could be contributing to the resistance. Among the cultivated germplasm, AWN86514-2 has high foliar and partial tuber resistance and has *S. acaule* Bitter, *S. demissum* Lindley, *S. phureja* Juz. & Bukasov, *S. microdontum*, *S. stoloniferum* Scheldl. & Bouche, and *S. tuberosum* subsp. *andigena* in its pedigree (Corsini et al., 1999) which can be contributing to its resistance. B0718-3 has foliar resistance to late blight derived from an Indian *S. tuberosum* introduction (PI383470B) selected in Mexico (Goth & Haynes, 1997). Bertita and Zarevo have *S. demissum* and *S. tuberosum* subsp. *andigena* in their pedigrees, both well-known sources of late blight resistance and these cultivars along with Bzura, Bertita, Greta, Libertas, and Stobrawa exhibit foliar late blight resistance in field evaluations (Haynes et al., 1998). Libertas is considered to have no R-gene and have both foliar (Colon et al., 1995b) and tuber resistance (Platt and Tai, 1998), probably sharing the same resistant genes with Pimpernel, Robijn, Populair and Surprise (Colon et al., 1995b). The advanced clone MSG274-3 is directly descended from the Mexican cultivar Tollocan. Tollocan and MSG274-3 have high foliar resistance to late blight in greenhouse and in field evaluations (Douches et al., 2001b).

The genetic diversity analysis showed that this germplasm could offer unique opportunities for late blight resistance breeding and that conventional breeding strategies may be useful to introgress and combine these different resistance sources. For short term strategy, combining sources of high levels of late blight resistance from different

sub-groups such as MSG274-3 and Tollocan with AWN86514-2 or with Russian hybrids possessing moderate resistance to late blight would be more productive than combining with *S. sucrense*, a wild 4*x* species. A long term breeding strategy would be to introgress the resistance from *S. microdontum* and *S. berthaultii*. The fact that two clones from the same accession of *S. microdontum* had different quantitative trait loci (QTL) conferring late blight resistance (Sandbrink et al. 2000) and that there are separate clusters in the dendrogram for the different *S. microdontum* accessions, suggest that multiple selections of *S. microdontum* should be used according to the clustering. This strategy should maximize the diversity of the late blight resistant sources.

Within germplasm having moderate to high resistance to late blight it is difficult to differentiate resistant individuals and almost impossible to select recombinant offspring based upon phenotypic tests. The association between markers and QTL permits the selection of individuals with desirable QTL from different parents (Meyer et al., 1998). Therefore, mapping QTL conferring late blight resistance is required to analytically pyramid genes from diverse genetic background.

In summary, there was high genetic diversity within and between accessions, species, and ploidy levels of the late blight resistant germplasm. Both wild diploid species *S. microdontum* and *S. berthaultii* had more genetic diversity between and, in some cases, within accessions than cultivated potatoes. This genetic diversity should be exploited using both short and long-term strategies to broaden the genetic base of the potato gene pool and to combine different sources of resistance in a breeding program to achieve stronger and more durable resistance in the offspring.

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CHAPTER V

Mapping Late Blight Resistance and other Agronomic Traits in a {[(*Solanum tuberosum* x *S. chacoense*) x *S. phureja*] x *S. microdontum*} Population

Abstract

A diploid potato population was developed with the objectives to map quantitative trait loci (QTL) conferring resistance to *Phytophthora infestans* (Mont.) de Bary and other agronomic traits using simple sequence repeats (SSR) and isozymes and to examine associations between late blight resistance and other agronomic traits. The mapping population was a cross between a late blight resistant selection of *Solanum microdontum* Bitter and a susceptible diploid advanced breeding clone. The progeny of 110 clones and the parents were tested at the Muck Soils Research Farm, Bath, MI in 1999 and 2000 for foliar late blight reaction using a mixture of complex races of US8/A2 mating type of *P. infestans*. Disease severity was quantified as the relative area under the disease progress curve based upon the percentage of foliar infection over time. The same population was also evaluated at Montcalm Research Farm, Entrican, MI for maturity, tuber number and size, yield, tuber appearance, specific gravity, and chip color. This

clone of *S. microdontum* transmitted high levels of resistance to late blight to a high percentage of the offspring. High phenotypic correlation ($r = 0.89$, $P < 0.0001$) was found for late blight reaction between years and no correlation was found between late blight with any other evaluated trait. A major QTL associated with foliar late blight resistance was located at the same position in linkage group 21 in both years of field testing. A QTL associated with vine maturity was mapped to linkage group 3. A QTL associated with tuber appearance was mapped to linkage group 1, one QTL associated with specific gravity was mapped to chromosome III and two QTLs associated with chip color were mapped to chromosomes VII and X. The major QTL associated with late blight resistance is suitable for marker assisted selection to introgress a new source of resistance to *P. infestans* to the cultivated tetraploid germplasm of potato.

Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important food crop with an annual world production of about 300 million metric tons (FAO, 1998). Among seven species of cultivated potato that originated in South America, the tetraploid ($2n = 48$) *S. tuberosum* is the most important species (Hawkes, 1990). Potato probably has the widest genetic diversity among related wild species than any other cultivated plant (Hawkes & Jackson, 1992) with more than 70% of the wild species being diploid ($2n = 48$) (Hawkes, 1994).

In the mid-1990's, the United States and Canada experienced a late blight (*Phytophthora infestans* (Mont.) de Bary) epidemic caused by new, more aggressive and metalaxyl resistant races (Goodwin et al., 1995; Peters et al., 2001) that imposed new

disease management strategies. The development of genetic resistance is a major strategy in managing late blight in potatoes and has become a major priority in many breeding programs (Colon et al., 1995a). Horizontal (field, partial or general) resistance seems to be the only durable type of resistance to late blight (Colon et al., 1995b; Umaerus et al., 1983; Kamoun et al., 1999). The level of horizontal resistance in potato is increased through recurrent selection (Henfling, 1987), which enables genes to be recombined from different resistant sources to build stronger and more durable resistance to late blight (Colon, 1999). However, the effectiveness of selecting recombinant individuals depends on mapping the quantitative trait loci (QTL) associated with resistance in different genetic sources (Meyer et al., 1998).

Diploid and tetraploid populations have been used for mapping QTLs associated with late blight resistance introgressed from a variety of wild species. Mapping potato at the diploid level avoids interpretation problems associated with tetrasomic inheritance (Meyer et al., 1998). A QTL associated with late blight resistance can also be associated with other traits. This is the case of chromosome V on which QTLs were mapped for foliar and tuber late blight resistance, vine maturity and vigor (Oberhagemann et al., 1999) and foliar late blight resistance in other populations (Collins et al., 1999; Sandbrink et al., 2000). Association between QTLs conferring foliar late blight resistance, tuberization and vine maturity was found in four out of five chromosomes (Ewing et al., 2000). Nematode and late blight resistances conferred by different parents were linked to the same microsatellite marker (Pande et al. 2001). Moreover, major and minor QTLs can be associated with late blight resistance. A major QTL from *S. bulbocastanum* Dunal explaining 62% of the phenotypic variance was mapped to chromosome VIII (Naess et al., 2000). A QTL from *S. demissum* Lindley explaining about 30% of the phenotypic variance was mapped to chromosome IV (Meyer et al., 1998; Pande et al., 2001).

Different QTLs associated with late blight resistance were identified in two clones of *S. microdontum* Bitter from the same accession. From one clone a QTL was mapped to chromosome IV and the other clone a QTL was mapped to chromosome X (Sandbrink et al., 2000).

The South American diploid species *S. microdontum* has shown high levels of resistance to late blight (Colon & Budding, 1988; Colon et al., 1995 a,c; Douches et al., 2001). Dominant gene action was identified in some crosses with susceptible clones (Colon et al., 1995c). Strong hypersensitive reaction or infection efficiency, lesion growth rate and sporulation time were associated with high levels of resistance in *S. microdontum* (Colon et al., 1995a). Among 20 accessions, including Russian hybrids of *Solanum* species with *S. tuberosum* and four South American species, Douches et al. (2001) selected 56 clones that were resistant to the US8 genotype of *P. infestans*, from which 27 clones represented three accessions of *S. microdontum*. One of these clones was used as a parent to develop mapping populations.

The objectives of this study were to map QTLs conferring late blight resistance and other agronomic traits using simple sequence repeats (SSR) and isozymes in a diploid *S. microdontum* derived population and to examine associations between late blight resistance and other agronomic traits.

Material and Methods

Selection of resistant parent and mapping population

A total of 175 clones representing six accessions of *S. microdontum* were tested in greenhouse using US8/A2 genotype of *P. infestans* in 1997. The most resistant clones

were re-tested in 1998 and 27 highly resistant clones were identified (Douches et al., 2001). The selected late blight resistant parent *S. microdontum* was identified as PI595511-5 by Douches et al. (2001). The accession was very distinct (genetic similarity of 0.58) from cultivated potato and other *S. microdontum* accessions with reported late blight resistance (Bisognin & Douches, 2001). PI595511-5 was crossed with susceptible parents and segregation for late blight was tested in a progeny of 40 individuals. The mapping population chosen was a cross between Michigan State University diploid breeding clone MSA133-57 [(*S. tuberosum* x *S. chacoense*) x *S. phureja*] with *S. microdontum* PI595511-5. A progeny of 110 clones that set tubers under greenhouse conditions (about 50% of total seedlings) was used for phenotypic evaluations and molecular analysis.

Late blight reaction in field tests

The *P. infestans* isolates (MS94-1, MS94-4, MS95-7 and MS97-2) were characterized as US8/A2 mating type as described in Bisognin et al. (2001). Those isolates overcame all known R-genes except R8 and R9 in detached-leaf assays. In the field, the isolates were weakly pathogenic only on the R8 and R9 of Black's differential clones.

The 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 to the plants. Parents and progeny were planted in three replications of three-hill plots on May 27th and on June 9th and inoculated on July 22nd and July 26th, respectively in 1999 and 2000. 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 from inoculation 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), with 1.0 being the maximum RAUDPC value (Kirk et al., 2001). See appendix for more details.

Vine maturity and tuber evaluations

Parents, progeny and check varieties were planted in non-replicated three-hill plots at the Michigan State University Montcalm Research Farm, Entrican, Michigan on May 22nd, 2000. Vine maturity was evaluated on September 24th when the cultivar Atlantic had a vine maturity rating of 1 on a 1 to 5 scale of increasing lateness (1 = early and 5 = late). All tubers used for evaluations were about 25 days after harvesting. The number and size of tubers and yield • hill^{-1} was recorded. 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 using the formula [dry weight / (dry weight – wet weight)].

Marker technology

Parents and progeny were genotyped using isozymes and SSR markers. The isozyme analysis was carried out using crude protein extraction from a newly expanded leaflet (approximately 120 mg), resolved in a horizontal 10% starch gel by electrophoresis with two buffer systems. Tissue processing, electrophoresis, staining, and

nomenclature were conducted as described in Douches & Quiros (1988). Nine isozyme loci of four enzyme systems were scored according to Douches & Quiros (1988) and Douches & Ludlam (1991). Malate dihydrogenase (*Mdh-1*), and phosphoglucose isomerase (*Pgi-1*) were resolved with a histidine-citrate pH 5.7, and glutamate oxaloacetate transaminase (*Got-1* and *Got-2*) and phosphoglucomutase (*Pgm-1* and *Pgm-2*) were resolved with a lithium-borate pH 8.3 buffer systems (Stuber et al., 1988).

Total genomic DNA used as a template for SSR analysis was isolated from young leaves of greenhouse plants. Tissue was harvested, freeze dried and then ground with glass beads. DNA was isolated from 20 mg of tissue using the DNeasy™ Plant Mini Kit (Qiagen Inc., Germany) following manufacturer's protocol.

A total of 161 pairs of SSR primers were used. All primer pairs have been published in Provan et al. (1996), Milbourne et al. (1998), and Sandbrink et al. (2000) as well as primers from 1 to 14 of Table 1A and all primers of Table 1D in Ashkenazi et al. (2001). All primer pairs were synthesized at Michigan State University and screened for polymorphism between the parents. DNA amplifications were carried out in a total volume of 20 μl containing 1X REDTaq™ PCR reaction buffer, 1 unit of REDTaq™ DNA polymerase (Sigma-Aldrich Co., St. Louis, MO.), 20 ng of each dNTP, 25 ng of each microsatellite primer, and 50 ng of template DNA. All amplifications were carried out on a Thermolyne Amplitron® (Barnstead™ Thermoline Corporation, Dubuque, IA.) thermal cycler. The protocol was as follows: 1) initial denaturation at 94 °C for 4 min; 2) 40 cycles of denaturation at 94 °C for 1 min, annealing at 50, 55 or 60 °C for 2 min depending on each primer, and extension at 72 °C for 1.5 min; and 3) final extension at 72 °C for 5 min. The completed reaction products were held at 4 °C until electrophoretic separation.

Electrophoretic separation for SSRs was done in a 3% Metaphor™ Agarose (FMC Bioproducts, Rockland, ME) or a 5% Polyacrylamide gels (Sigma-Aldrich Co., St. Louis, MO) depending on the size of amplified fragment. Metaphor agarose gels were run at 100V from 3.5 to 4.5 h at 10 °C, stained with ethidium bromide (1 $\mu g \cdot ml^{-1}$) for 45 min, visualized under UV light, and photographed. Polyacrylamide gels (34.5 x 50 cm) were run at 90 W for 2 h and 30 min in a Sequi-Gen® GT Sequencing Cell (Bio-Rad, Richmond, VA) and stained with Silver Sequence™ DNA (Promega, Madison, WI) following respective manufacturers' protocols. Fragment sizes were estimated using a 10 or 25 bp DNA ladder (Gibco BRL, Grand Island, NY) in each gel. Multiple loci of SSR markers were labeled by a letter after the marker designation.

Statistical analysis

For phenotypic data of late blight reaction, analysis of variance was done for the 1999 and 2000 data sets. Pearson correlation analysis was done to compare late blight data between years and late blight with other agronomic traits. Descriptive statistics was used to characterize population distribution for all evaluated traits. All those analysis were done following the procedures of SAS (SAS institute, 1995).

Data from the presence or absence of alleles present (heterozygous) in one and absent in the other parent for SSR and isozymes were used for linkage analysis and QTL mapping. The χ^2 test for goodness-of-fit was used to test for deviations of the expected Mendelian segregation ratio of 1:1 (presence versus absence). Linkage analysis was done with JoinMap V2.0 (Stam, 1993) using a minimal LOD score of 3.0 and maximum recombination fraction of 0.49. Map distances are presented in centi Morgans (cM) calculated by the Kosambi function (Kosambi, 1944). The QTL mapping was done with QTL Cartographer V1.13 (Basten et al., 1999), including analysis of genotype x

environmental interaction for late blight reaction. A QTL was declared significant based on threshold calculations of 1000 permutations (Churchill & Doerge, 1994).

Results and Discussion

Phenotypic evaluations

There were significant differences ($P \leq 0.0001$) among progeny clones for foliar late blight reaction in the field tests in 1999 and in 2000, between years and also clones x years interaction. The 1999 test had a higher mean and median RAUDPC, for parents and progeny, and progeny RAUDPC range than the 2000 test (Table 5.1). The RAUDPC of *S. microdontum* was 0.021 and 0.019 compared with 0.529 and 0.175 of MSA133-57, respectively for 1999 and 2000. There were clones in the progeny with 1.5-fold RAUDPC higher than MAS133-57 in both years of testing. Even with a higher artificial epidemic of *P. infestans* in the field in 1999 than in 2000, *S. microdontum* had almost the same RAUDPC values in both years, confirming its high resistance found in greenhouse tests (Douches et al., 2001). This *S. microdontum* clone had only a total of 10% infection in a late blight nursery in Toluca valley, Mexico, during the 2000 season (Lozoya-Saldaña, personal communication). See Fig. A.6 and A.7 and Table A.3 in the appendix.

There was a transgressive segregation for early vine maturity in the progeny (Table 5.1). For tuber quality evaluations, *S. microdontum* did not tuberize and seven progeny clones produced only very small tubers that were not enough for evaluation. The breeding clone MSA133-57 did not have a high yield • $hill^{-1}$, but set a small number of larger tubers with acceptable appearance and chip color and high specific gravity. Some progeny clones set a high number of tubers and, on average, the whole progeny had

smaller tuber size than MSA133-57. However, there were progeny clones with over 5-fold higher yield and better tuber appearance, specific gravity and chip color than MSA133-57, showing that wild species can be valuable sources even for high quality tuber traits. All traits had skewed distribution and only yield • $hill^{-1}$ and tuber size had kurtosis similar to zero. A set of data with this level of skewness and kurtosis would usually be transformed to normality, but as high contrasting parents were used to develop the population, a mixture distribution was expected in the progeny (Doerge et al., 1997). See Fig. from A.8 to A.14 in the appendix.

There was a high correlation ($r = 0.82$, $P \leq 0.0001$) for late blight reaction between the two years of testing and there was no correlation between late blight reaction and any other evaluated trait (Table 5.2). Among the evaluated traits, significant correlations between late blight resistance and late maturity is probably the most undesirable correlation (Ross, 1986; Umaerus et al., 1983). Since the high level of resistance to late blight in *S. microdontum* was not correlated with late maturity, the late blight resistance genes may be located in other chromosomal regions of the genome. A highly significant correlation ($r = 0.84$, $P \leq 0.0001$) was found between yield and tubers • $hill^{-1}$. Negative correlations ($P \leq 0.05$) were found among maturity with yield and tubers • $hill^{-1}$ and tubers • $hill^{-1}$ with tuber size. Significant positive correlations were found for tuber appearance with tuber size and yield • $hill^{-1}$. Yield and tuber size are components of tuber appearance. Tuber appearance is the only trait to directly affect phenotypic selection (Tai, 1975) and yield is a decisive component for selection (Neele et al., 1991).

Table 5.1. Phenotypic value of the parents, progeny size and descriptive statistics for foliar late blight reaction and other agronomic traits of a *S. microdontum* (PI595511-5) derived population.

Evaluated Traits	Phenotypic Values		Progeny	Descriptive Statistics					
	PI595511-5	MSA133-57	Size	Mean	Median	Skewness	Kurtosis	Range[1]	Std. Deviation
RAUDPC 1999[2]	0.021	0.529	110	0.261	0.176	0.78	-0.70	0.007-0.883	0.24
RAUDPC 2000[2]	0.019	0.175	110	0.108	0.066	0.54	-1.13	0.010-0.273	0.08
Maturity[3]	5.0	2.0	109	2.0	1.0	1.23	0.34	1-5	1.32
Yield • hill^{-1} (g)	---	241.7	103	619.0	583.3	0.63	0.07	22-1613	323.00
N. Tuber • hill^{-1}	---	2.0	103	15.5	13.3	0.75	0.33	1-41	8.10
T. Appearance[4]	---	3.0	103	4.0	4.0	-0.27	-0.83	2-5	0.80
Tuber Size (g)	---	120.8	103	41.0	40.6	0.34	-0.01	12-79	12.20
Specific Gravity[5]	---	1.107	103	1.086	1.086	-0.31	6.44	1.030-1.121	0.01
Chip Color[6]	---	4.0	103	5.3	6.0	-0.65	0.38	2-8	1.22

[1] Progeny range: highest and lowest value for each evaluated trait.
[2] Relative area under the disease progress curve (maximum RAUDPC = 1).
[3] Scale 1 to 5 of increasing lateness.
[4] Scale 1 to 5 of increasing defects (1 = excellent, 2 = very good, 3 = acceptable, 4 = poor, and 5 = very poor).
[5] Specific gravity calculate as [dry weight / (dry weight – wet weight)].
[6] Scale 1 to 9 of increasing color darkness (1 - 2 = excellent, 3 = very good, 4 = acceptable, 5 = unacceptable, 6 – 9 = poor).

Table 5.2. Coefficients of

Table 5.2. Coefficients of correlation among foliar late blight reaction in 1999 and 2000 (RAUDPC[1]) and other agronomic traits in 2000.

Evaluated Traits	RAUDPC-00	Maturity	Yield • hill^{-1}	Tuber • hill^{-1}	T. Appearance	Tuber Size	S. Gravity	Chip Color
RAUDPC-99	0.82****	ns[2]	ns	ns	ns	ns	ns	ns
RAUDPC-00		ns	ns	ns	ns	ns	ns	ns
Maturity			-0.20*	-0.21*	ns	ns	ns	ns
Yield • hill^{-1}				0.84****	0.28**	ns	ns	0.24*
Tuber • hill^{-1}					ns	-0.24*	ns	ns
Tuber Appearance						0.19*	ns	0.36***
Tuber Size							ns	ns
Specific Gravity								ns

[1] Relative area under the disease progress curve.

[2] ns = not significant, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and **** $P \leq 0.0001$

Marker and linkage analyses

From four isozyme systems, nine isozyme loci were polymorphic in the population. A total of 161 pairs of SSR primers was screened with DNA template from the parents and 125 were successful amplified producing readable bands. A total of 45 pairs of primers was polymorphic in metaphor agarose gels and resulted in 63 marker loci. An additional 25 pairs of primers were polymorphic in polyacrylamide gels and resulted in 46 marker loci, which totals 109 SSR marker loci (Table 5.3). A general trend could be observed between metaphor and polyacrylamide gels. Pairs of primers that amplified fragments with about 250 bp or smaller with a polymorphism between parents of about 15 bp or more could efficiently be separated in metaphor agarose gels. Pairs of primers that amplify either bigger fragments or with smaller difference between parents required separation in polyacrylamide gels. The isozyme and SSR analyses resulted in a total of 118 marker loci in the population, of which 54 were heterozygous (band present) in *S. microdontum* and 64 in MSA133-57. In addition to polymorphic primers, eight pairs of SSR primers were also homozygous in both parents and these SSRs were used to confirm the F_1 status of the mapping populations. These eight SSR markers that were fixed in the parents were examined in the progeny for the presence of co-dominant bands.

A total of 93 markers were grouped in 27 linkage groups, leaving 25 markers unlinked. The map covered 844.4 cM of the potato genome, with an average distance of 9.1 cM between markers and an average of 3.4 markers per linkage group. The length of the potato linkage maps varies from 690 cM (Gebhardt et al., 1989) to 1120 cM (Jacobs et al., 1995). The only *S. microdontum* map available was constructed using six sub-populations with total genome coverage length varying from 341 cM to 683 cM (Sandbrink et al., 2000).

Table 5.3. Simple sequence repeat markers used to map a *S. microdontum* derived population.

Original Name[1]	Genome Position[2]	Fragment Size (bp)	Separation System[3]	Original Name[1]	Genome Position[2]	Fragment Size (bp)	Separation System[3]
ST34		215	Metaphor	Stm1021a	IX	220	PAGE
ST56a		225	Metaphor	Stm1021b	IX	215	
ST56b		175		Stm1024	VIII	172	PAGE
ST56c		150		Stm1025a	III	310	PAGE
ST78		258	PAGE	Stm1025b	III	293	
ST910a		230	PAGE	Stm1025c	III	283	
ST910b		180		Stm1029	I	150	Metaphor
ST910c		135		Stm1030	II	190	Metaphor
ST1112a		306	PAGE	Stm1040		208	PAGE
ST1112b		290		Stm1041	V	95	Metaphor
ST1112c		257		Stm1051	IX	225	Metaphor
ST1112d		245		Stm1052		250	Metaphor
ST1516a		450	Metaphor	Stm1053	III	239	PAGE
ST1516b		400		Stm1056a	VIII	275	PAGE
ST1516c		240		Stm1056b	VIII	265	
ST1920a		225	Metaphor	Stm1056c	VIII	260	
ST1920b		170		Stm1064	II	224	PAGE
ST2122a		242	PAGE	Stm1072a		300	PAGE
ST2122b		235		Stm1072b		198	
ST3334		214	Metaphor	Stm1072c		190	
ST3334b		207		Stm1097		95	Metaphor
ST3940		148	PAGE	Stm1102a	I,IX	180	Metaphor
ST4142		190	Metaphor	Stm1102b	I,IX	145	
ST6162a		500	Metaphor	Stm1104	VIII	193	PAGE
ST6162b		145		Stm1105	VIII	100	Metaphor
STIIKA		250	PAGE	Stm1106	X	140	Metaphor
Stm0004a	VII	190	PAGE	Stm2003		183	PAGE
Stm0004b	VII	188		Stm2005a	XI	245	PAGE
Stm0004c	VII	162		Stm2005b	XI	200	
Stm0007	XII	280	PAGE	Stm2013		160	Metaphor
Stm0013a	V	173	PAGE	Stm2020	I	145	Metaphor
Stm0013b	V	155		Stm2022a	II	210	PAGE
Stm0015		180	Metaphor	Stm2022b	II	172	
Stm0019a	VI	200	Metaphor	Stm2028a	XII	450	Metaphor
Stm0019b	VI	175		Stm2028b	XII	290	
Stm0019c	VI	145		Stm2028c	XII	230	
Stm0020a		125	Metaphor	Stm3000		115	Metaphor
Stm0020b		100		Stm3003		350	PAGE
Stm0024	VIII	135	Metaphor	Stm3009	VII	125	Metaphor
Stm0028	VII	150	Metaphor	Stm3011	II	140	Metaphor
Stm0030a	XII	150	Metaphor	Stm3012a	IX	300	PAGE
Stm0030b	XII	110		Stm3012b	IX	293	
Stm0032	XII	140	Metaphor	Stm3012c	IX	270	

Continued

Stm0038	II	95	Metaphor	Stm3015	VIII	105	Metaphor
Stm0046		100	Metaphor	Stm3016a	IV	130	PAGE
Stm0051	X	125	Metaphor	Stm3016b	IV	115	
Stm0052	VII	105	Metaphor	Stm3023		190	Metaphor
Stm1002		200	Metaphor	STP0AC58		95	Metaphor
Stm1003	VII	230	PAGE	LECAB9		80	Metaphor
Stm1004	VII	150	Metaphor	LEGAST1		120	Metaphor
Stm1009a	VII,XI	290	Metaphor	ST13STa		90	Metaphor
Stm1009b	VII,XI	160		ST13STb		80	
Stm1009c	VII,XI	135		STRBCS1b		250	Metaphor
Stm1009d	VII,XI	105		STACCAS3		145	Metaphor
Stm1016		250	Metaphor				

[1] Primer sequences of ST markers were published by Ashkenazi et al. (2001) , STIIKA by Provan et al. (1996), Stm by Milbourne et al. (1997) and other primer sequences by Sandbrink et al. (2000).

[2] Position in the potato genome as published in Milbourne et al. (1997).

[3] More details about DNA separation (polyacrylamide and metaphor agarose gels) in the material and methods.

Simple linear regression showed that there were four SSR marker loci on the linkage group 21 linked with late blight resistance in both years of field testing and combined analysis. There were two other markers linked with late blight resistance in 1999, one on linkage group 6 and another one on linkage group 16 and one locus linked with late blight resistance on linkage group 4 in 2000. Three loci linked with vine maturity mapped on each of these linkage groups 3, 8 and 25. Among tuber traits, five loci were linked with specific gravity on chromosome III. Three loci were associated with tuber size on each of linkage groups 6 and 17 and two loci on each of linkage groups 9 and 18. There were two loci linked with chip color that mapped on chromosomes III and X. All other loci linked with traits were single loci that mapped to different linkage groups (data not shown).

Simple linear regression also showed that there were markers linked with multiple traits (Table 5.4). This analysis showed that there was no association between late blight resistance and late maturity in this *S. microdontum* population. One SSR marker (ST6162b) was linked with late blight resistance in 1999 and tuber size, in which an association in repulsion increases susceptibility to late blight and reduces tuber size. The SSR marker ST56c was also linked in repulsion and was linked with earlier maturity and lower specific gravity. For tuber traits, there were three markers (Stm0032, Stm1025c, and STRBCS1b) linked with three traits and six markers (STIIKAa, Stm0030b, Stm0046, Stm2003, Stm2028c, and Stm2028d) linked with two traits (Table 5.4).

Table 5.4. Markers associated with multiple traits in a S.

Table 5.4. Markers associated with multiple traits in a *S. microdontum* derived population.

Markers[1]	Late Blight	Maturity	Yield • hill^{-1}	Tuber • hill^{-1}	T. Appearance	Tuber Size	S. Gravity	Chip Color
ST6162b	0.046*[2]					0.008**		
ST56c		0.001*					0.034*	
STIIKAa							0.014*	0.022*
Stm0030b			0.020*	0.013*				
Stm0032			0.033*	0.023*		0.005**		
Stm0046			0.048*			0.005**		
Stm1025c					0.013*		0.000****	0.022*
Stm2003					0.048*			0.000***
Stm2028c				0.010*		0.003**		
Stm2028d				0.010*		0.003*		
STRBCS1b					0.027*	0.008*	0.006**	

[1] Primer sequences of markers ST were published by Ashkenazi et al. (2001), STIIKA by Provan et al. (1996), Stm by Milbourne et al. (1997) and STRBCS1b by Sandbrink et al. (2000).

[2] * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, and **** $P \leq 0.0001$.

Quantitative trait locus analysis

A QTL in *S. microdontum* associated with foliar late blight resistance on the linkage group 21 was detected at the same position in both years of field evaluations (Fig. 5.1). This QTL explained 42% of the phenotypic variance in 1999 within the interval between 41 and 47 cM and explained 70% of the phenotypic variance in 2000 and combined analysis within the interval between 36 and 50 cM.

The SSR marker Stm0020 was linked to late blight resistance in both years located within the region of highest LOD score of the QTL. This marker has two bands in *S. microdontum*, one segregating in the progeny with the resistant individuals and the other with the susceptible individuals. The average RAUDPC of resistant clones having the band linked with resistance was 0.156 and 0.070 and with susceptibility was 0.407 and 0.160, respectively for 1999 and 2000.

Quantitative trait loci associated with late blight resistance have been mapped in wild *Solanum* species and in hybrids among wild species resistant to late blight. In the cultivar Stirling that has *S. demissum* as source of late blight resistance, a QTL was mapped on chromosome IV (Meyer et al., 1998; Pande et al., 2001), in *S. berthaultii* on chromosomes I, III, VII, VIII and XI (Ewing et al., 2000), in *S. bulbocastanum* on chromosome VIII (Naess et al., 2000), and in *S. microdontum* on chromosomes IV, V and X (Sandbrink et al., 2000). In a wild species hybrid, QTLs were mapped on chromosomes III, V, VI and IX (Collins et al., 1999) and in another hybrid on chromosomes III, IV, V, VI, IX and XI (Oberhagemann et al., 1999). The mapping population used in this study was also a hybrid involving three wild species, but only *S. microdontum* contributed with the resistant alleles. One major QTL was found on linkage group 21 that had four SSR loci. Since none of these markers have been mapped

in potato, we were unable to assign this linkage group to a specific chromosome. However, this QTL should be different from the QTL mapped in the cultivar Stirling (Meyer et al., 1998) and the previous QTLs mapped in *S. microdontum* (Sandbrink et al., 2000). The SSR markers linked with late blight resistance in these previous studies (Stm3016 in the cultivar Stirling on chromosome IV and STPOAC58 in *S. microdontum* on chromosome V) were polymorphic in the *S. microdontum* derived population, but were not linked to late blight resistance. Moreover, the SSR marker ST13ST on the top of the linkage group 21 was not linked with late blight resistance in the *S. microdontum* population studied by Sandbrink et al. (2000).

One QTL was associated with vine maturity on linkage group 3 and had three SSR markers that were not mapped on potato (Fig. 5.2). Quantitative trait loci associated with vine maturity have been mapped to different chromosomes and regions of the potato genome. In this *S. microdontum* population, late blight resistance and vine maturity were mapped on different linkage groups. The fact that late blight resistance is not associated with late maturity suggests that the introgression of resistance can be achieved without incorporating late maturity. Previous molecular analysis shows that there is a major region conferring late blight resistance and late maturity located on chromosome V (Collins et al., 1999; Oberhagemann et al., 1999) and there are QTLs associated with late blight susceptibility and early maturity that map to other regions of the genome (Ewing et al., 2000).

1999

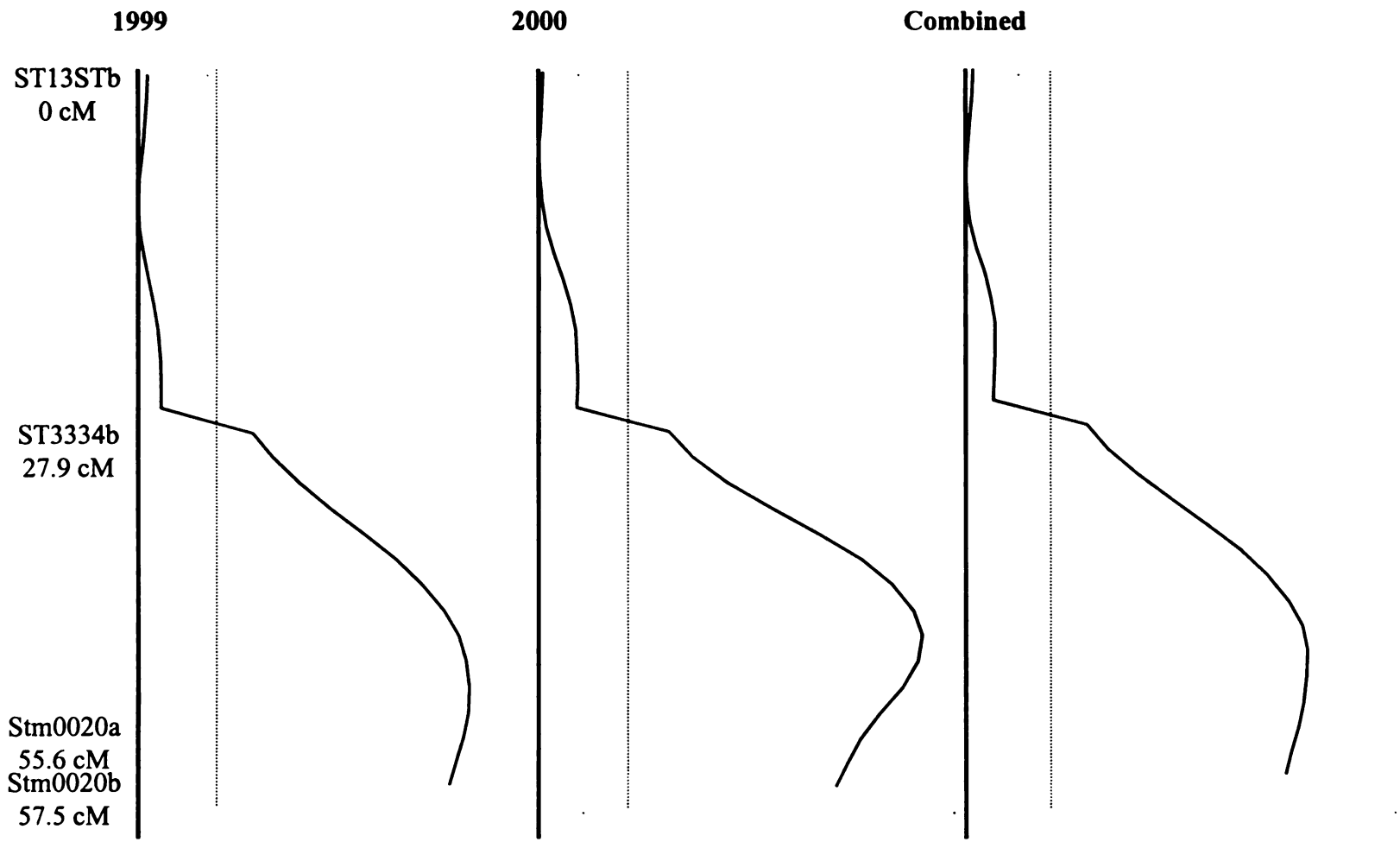

Figure 5.1. Quantitative trait loci associated with foliar late blight resistance in the field of a *S. microdontum* derived population on linkage group 21 in 1999, 2000 and combined data analysis. Primer sequences of markers ST were published by Ashkenazi et al. (2001), Stm by Milbourne et al. (1997) and ST13ST by Sandbrink et al. (2000). Vertical dashed line represents the threshold of LOD score 3.0. cM = centi Morgan.

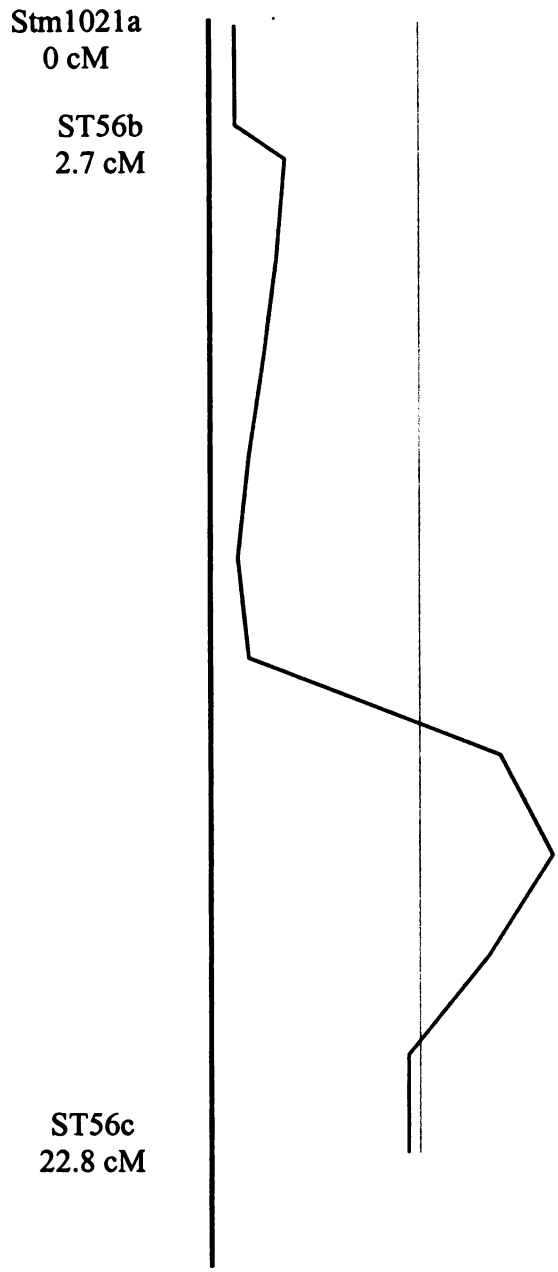

Figure 5.2. Quantitative trait loci associated with vine maturity of a *S. microdontum* derived population on linkage group 3 in 2000. Primer sequences of markers ST were published by Ashkenazi et al. (2001) and Stm by Milbourne et al. (1997). Vertical dashed line represents the threshold of LOD score 3.0. cM = centi Morgan.

Among tuber characteristics, all identified QTLs were associated with tuber quality traits (tuber appearance, specific gravity and chip color). A QTL for tuber appearance was mapped on linkage group 1 (Fig. 5.3), a QTL associated with specific gravity on chromosome III (Fig. 5.4) and two QTLs associated with chip color on chromosomes VII and X (Fig. 5.5). For tuber appearance, the three SSR loci of the linkage group have not been mapped yet. The QTLs associated with tuber quality traits were located on different chromosomes or linkage groups, indicating that each QTL can be independently selected in a marker assisted selection (MAS) program. Although there were marker loci linked to tuber quality traits in different linkage groups, only one QTL was found for tuber appearance and specific gravity and two QTLs for chip color.

For specific gravity, Freyre & Douches (1994) found 10 QTLs distributed on six chromosomes from which one QTL was located within the region of the *Pgm*-1 allozyme on chromosome III, the same region in which a QTL was mapped in this *S. microdontum* population. Douches & Freyre (1994) found six QTLs associated with chip color, but none were located on chromosome VII in the region of the allozyme *Got*-2. A QTL was found on chromosome X, but no connections can be done with the QTL mapped in *S. microdontum*, since chip color was linked to different marker loci. The identification and selection of QTLs associated with tuber quality is important because all cultivars released over the past century have superior tuber quality (Douches et al., 1996; Love et al., 1998). Therefore, it is not only important to combine different sources of late blight resistance, but also to combine resistance with early maturity and tuber quality traits. The identification of QTLs associated with late blight resistance in *S. microdontum* together with QTLs associated with vine maturity and tuber quality traits gives the possibility of pyramiding desirable QTLs in the progeny through MAS strategy.

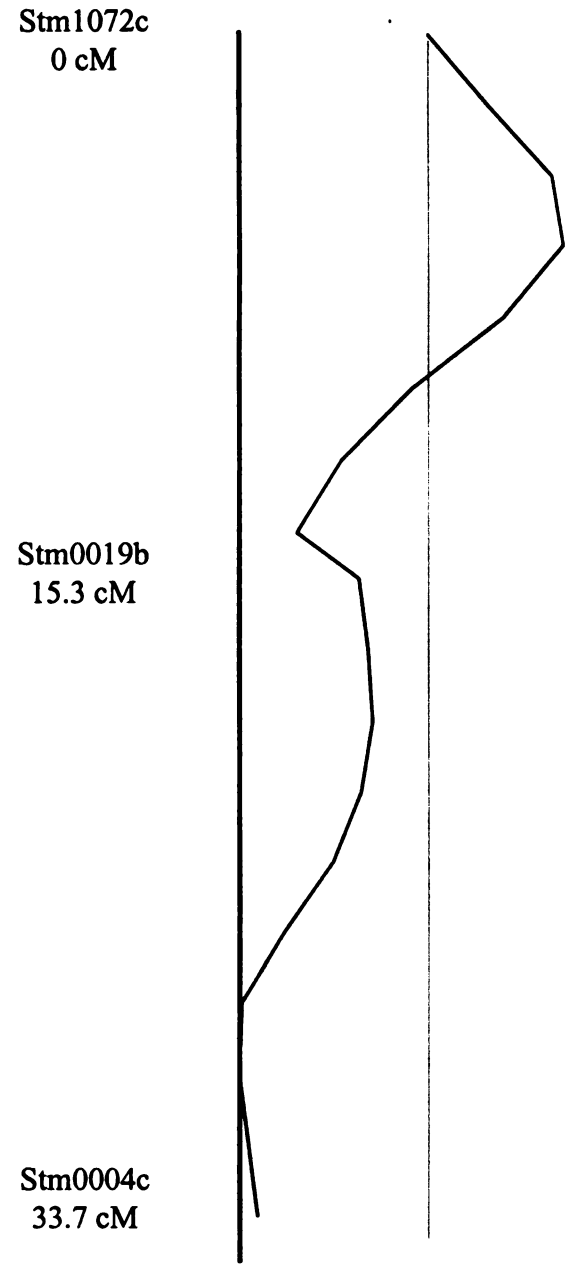

Figure 5.3. Quantitative trait loci associated with tuber appearance of a *S. microdontum* derived population on linkage group 1 in 2000. Primer sequences of markers Stm were published by Milbourne et al. (1997). Vertical dashed line represents the threshold of LOD score 3.0. cM = centi Morgan.

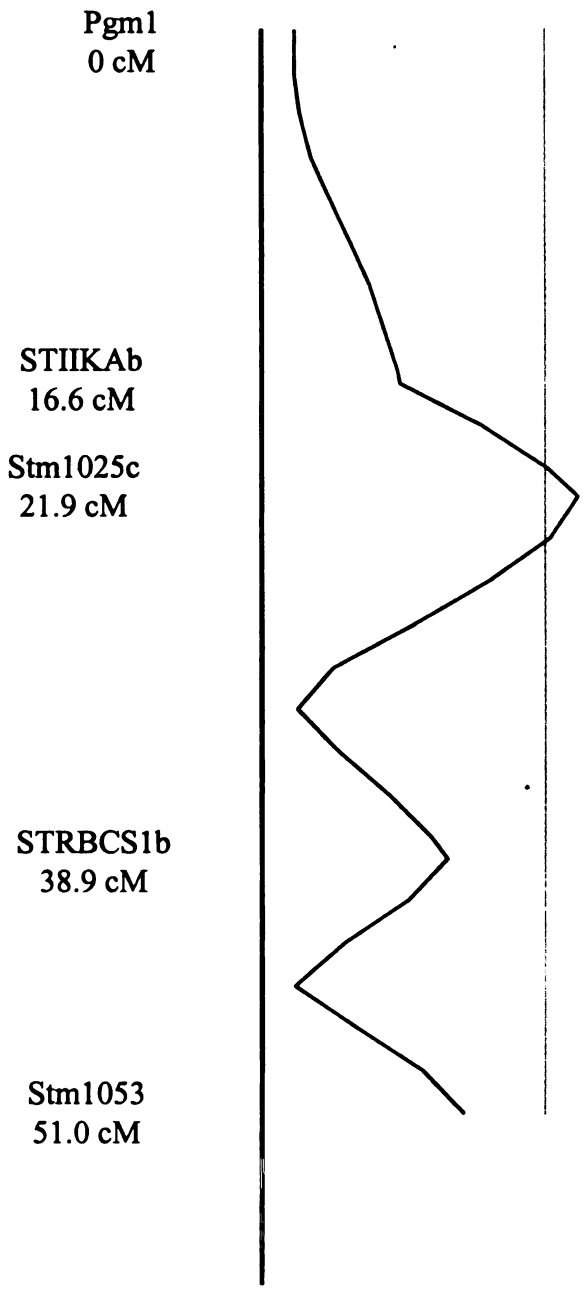

Figure 5.4. Quantitative trait loci associated with specific gravity of a *S. microdontum* derived population on chromosome III in 2000. Primer sequences of markers Stm were published by Milbourne et al. (1997), STIIKA by Provan et al. (1996) and STRBCS1b by Sandbrink et al. (2000). Vertical dashed line represents the threshold of LOD score 3.0. cM = centi Morgan.

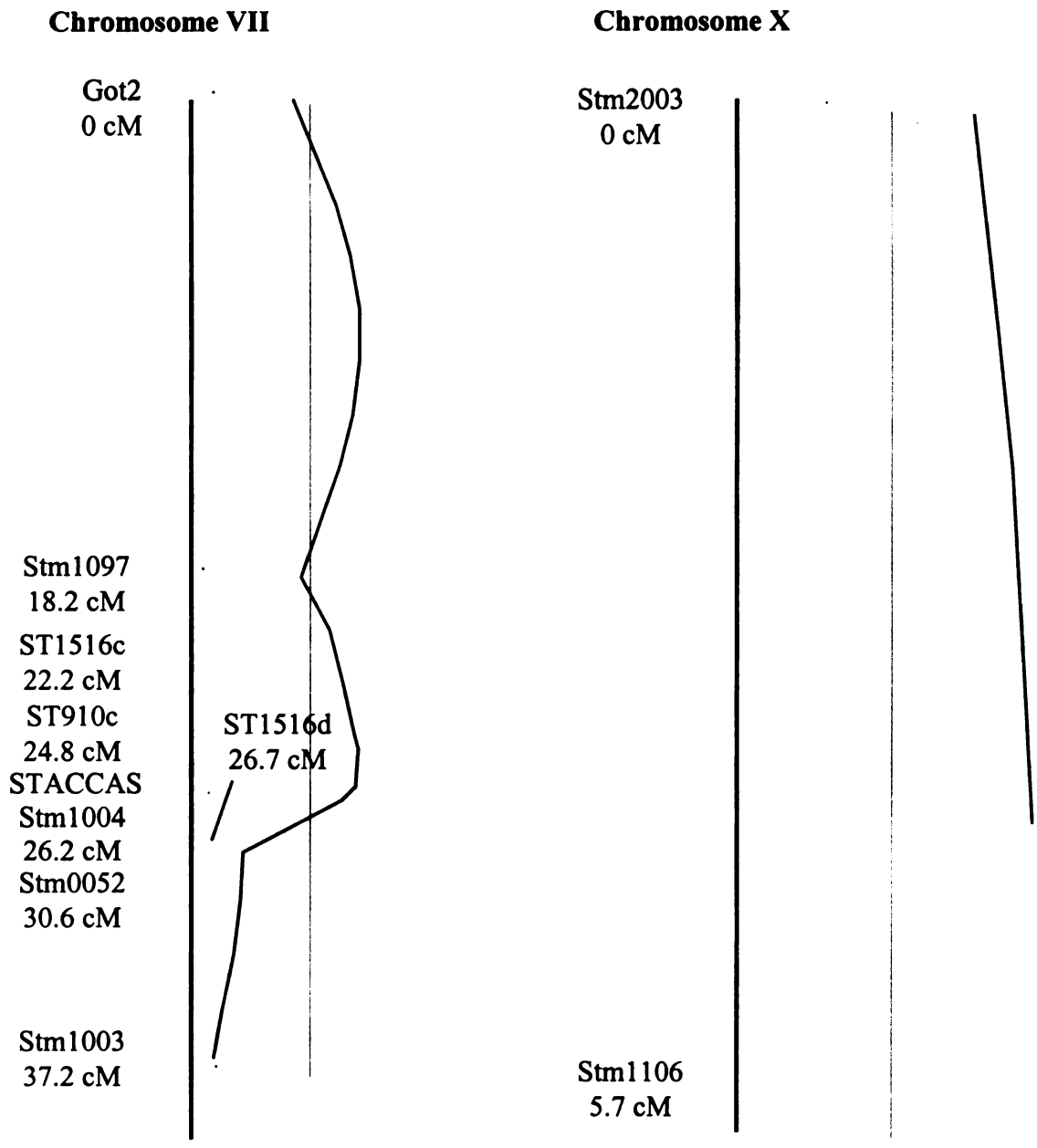

Figure 5.5. Quantitative trait loci associated with chip color of a *S. microdontum* derived population on chromosomes VIII and X in 2000. Primer sequences of ST markers were published by Ashkenazi et al. (2001), Stm by Milbourne et al. (1997) and STACCAS3 by Sandbrink et al. (2000). Vertical dashed line represents the threshold of LOD score 3.0. cM = centi Morgan.

The mapping population used in this study combined late blight resistance in *S. microdontum* with yield and tuber quality traits in the diploid breeding clone MSA133-57. All marker locus associated with late blight resistance were linked in coupling with *S. microdontum*, but the ST3334b allele from MSA133-57 contributed to a reduction in the average RAUDPC value of the combined years from 0.246 to 0.145. With the exception of chip color on chromosome X, all other marker loci linked with tuber quality traits were linked in coupling with MSA133-57, but, even on chromosome X, MSA133-57 contributed alleles that improved chip color from an average rating of 5.9 to 4.9.

The clone PI595511-5 of *S. microdontum* has a QTL associated with foliar late blight resistance located at the same position on linkage group 21 in both years of field testing. This QTL explained 42% of the phenotypic variance in 1999 within the interval between 41 and 47 cM and explained 70% of the phenotypic variance in 2000 and combined analysis within the interval between 36 and 50 cM of the linkage group. This QTL associated with foliar late blight resistance is not associated with any other undesirable trait such as late maturity or poor tuber quality. There is a SSR marker (Stm0020) that maps at 57.5 cM from the top of linkage group 21 and can be used in a MAS program. In *S. microdontum*, Stm0020 showed two bands, one linked with resistance and one with susceptibility in the progeny (Fig. 5.6A). The presence of bands in both resistant and susceptible clones of the progeny makes selection easier based on the band fragment itself instead of band presence or absence. The band linked with resistance could also be followed through polyploidization using DLB1-150 as diploid parent (Fig. 5.6 B and C). From eight progeny individuals of the cross with MSAF313-3, two had the resistant band and the only individual of the progeny of the cross with Norvalley also had the resistant band. This is a very important finding as being the first

report that a genetic marker from a diploid clone can be followed through polyploidization and used in a marker assisted selection program. The addition of more linkage data is necessary to locate the QTL associated with foliar late blight resistance in *S. microdontum* on the potato genome to a specific chromosome and determine the relationship with QTLs mapped in *S. bulbocastanum* (Naess et al., 2000) and *S. berthaultii* (Ewing et al., 2000). The next step should be to pyramid these QTLs into specific genotypes to develop potato cultivars to improve levels and durability of resistance to late blight.

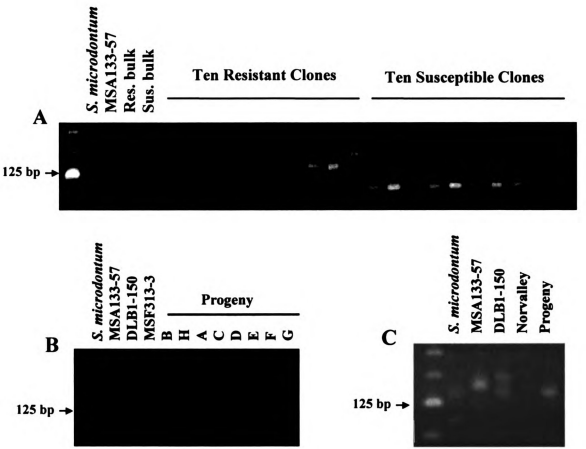

Figure 5.6. Simple sequence repeat marker Stm0020 fragments separated in 3% metaphor agarose gel run at 100 V for 4.5 hours. A) Parents, bulks and ten resistant and susceptible progeny clones, respectively. Stm0020a linked with resistance and Stm0020b linked with susceptibility, both from resistant parent *S. microdontum*. B and C) SSR marker Stm0020a followed through polyploidization. Parents of the diploid population, diploid resistant clone of the progeny and tetraploid parent with tetraploid progeny clones.

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CHAPTER VI

General Discussion and Conclusions

The experiments reported here were carried out with the objective of introgressing late blight resistance from wild *Solanum* species and unadapted germplasm into cultivated potato. The re-emergence of late blight in the United States and Canada in the mid- 1990's (Fry & Goodwin, 1997; Peters et al., 2001) as the most important potato disease re-initiated research areas such as the identification of resistance sources and the development of resistant cultivars. Unfortunately, no cultivar adapted to North American conditions had adequate level of late blight resistance (Douches et al. 1997; Helgeson et al. 1998). Therefore, the first challenge was to combine early maturity, good tuber appearance, specific gravity (Douches et al., 1996) and good chip processing quality (Love et al., 1998) with late blight resistance that was found only in unadapted cultivated germplasm.

Multi-trait evaluation of half-sib progeny of late blight resistant parents was used to combine resistance with tuber quality and marketable maturity. The results supported the use of the single-hill clonal generation as an efficient breeding strategy for identifying superior parents. Therefore, superior parents can be used more quickly in other cross combinations. Also, the earlier in the selection process that superior clones are identified

in the progeny, the more efficient the breeding process becomes. The results also showed that late blight resistance could be combined with marketable tuber quality and maturity when parents such as Tollocan and B0718-3 were crossed with susceptible parents possessing tuber quality. These two parents transmit high levels of late blight resistance to a high percentage of the offspring. A total of eight clones (six clones have Tollocan as parents and two have B0718-3 as parent) were selected possessing resistance to late blight combined with marketable maturity and tuber quality. These results are very significant, since previous reports showed positive correlation between late blight resistance and late maturity (Ross, 1986; Umaerus et al., 1983; Collins et al., 1999; Oberhageman et al., 1999) and, from eight evaluated parents, only two transmitted high levels of resistance, which justifies time and resources to select superior parents for breeding.

Potato has the widest genetic diversity among related wild species than other major cultivated plants (Hawkes & Jackson, 1992). This diversity is an invaluable source for the most important traits including late blight resistance (Bamberg et al., 1994). Some wild *Solanum* species have been recognized as resistant to late blight such as the South American diploid species *S. microdontum* Bitter and *S. berthaultii* Hawkes along with the tetraploid species *S. sucrense* Hawkes (Colon et al., 1995a,b). A fine-screening evaluation was used to identify late blight resistant clones within accessions of these three wild species and hybrids between wild species and cultivated potato. A total of 56 clones were selected as possessing high levels of late blight resistance based on two years of greenhouse tests (Douches et al., 2001). These selected clones together with 10 cultivars or advanced breeding clones were assessed for genetic diversity. The genetic diversity that exists in this potato germplasm with reported late blight resistance should

be combined and introgressed into the cultivated gene pool. These should broaden the potato genetic base of cultivated potato and concurrently achieve higher level and more durable resistance to late blight.

Short- and long-term breeding strategies should be considered for combining resistance sources. As a short-term strategy, cultivated potato and its hybrids with wild species from different sub-groups should be crossed, because hybrids already have genetic diversity incorporated from wild species combined with some adaptation. As hybrids and some cultivars have different wild species as sources of resistance, different sub-groups may be considered as different sources of late blight resistance. As a long-term strategy, resistance from the diploid species *S. microdontum* and *S. berthaultii* should be incorporated into cultivated potato. Besides being in distinct genetic groups, accessions of these two species were clustered in sub-groups with similar or even higher genetic diversity than tetraploid germplasm, suggesting that several different accessions should be used to introgress resistance. In fact, even clones of the same accession can have different genes for resistance as the case of the two clones of *S. microdontum* that have major quantitative trait loci (QTL) for late blight resistance located on different chromosomes (Sandbrink et al., 2000).

Mapping QTL conferring late blight resistance is required to select individuals with desirable QTLs from different sources (Meyer et al., 1998). *Solanum microdontum* was chosen for QTL analysis based on its high levels of resistance to late blight conferred by different mechanisms (Colon et al., 1995a,b), high percentage of previously selected clones (Douches et al., 2001), and segregation for resistance in crosses with susceptible parents. The QTL analysis showed that the *S. microdontum* PI595511-5 has one QTL conferring high levels of resistance to late blight that explains more than 50% of the

phenotypic variance in two years of field testing. This QTL was not associated with maturity or with any other evaluated trait. A simple sequence repeat (SSR) marker Stm0020 (Milbourne et al., 1998) showed two bands in *S. microdontum*, offering the advantage of showing different amplified fragments on both resistant and susceptible progeny clones. This QTL is likely to be different from those mapped by Sandbrink et al. (2000), since different SSR markers are linked with late blight resistance. Moreover, *S. microdontum* provides additional evidence that improved field resistance against complex races of *P. infestans* can be conferred by a single major QTL as found in *S. bulbocatanum* (Naess et al., 2000) and in the cultivar Stirling, a known source of horizontal and durable resistance (Meyer et al, 1998).

The resistance of *S. microdontum* was mapped at the diploid level. Sexual polyploidization can be achieved using 2*n* gametes (Hermsen, 1994; Hutten et al., 1994) in crosses with tetraploid cultivars or breeding clones (unilateral polyploidization or 4*x* - 2*x* crosses) or in crosses with other diploids that also produce unreduced gametes (bilateral polyploidization or 2*x* - 2*x* crosses) (Ortiz, 1998; Alfano et al., 1999; Hanneman, 1999). A 4*x* - 2*x* cross between MSF313-3 and the diploid clone DLB1-150 was conducted using 2*n* pollen. This cross resulted in eight plants from which two showed the marker Stm0020 linked to late blight resistance from *S. microdontum*. This is a very important finding as being the first report that a genetic marker from the diploid clone can be followed through polyploidization and used in a marker assisted selection (MAS) program at the tetraploid level. Moreover, the resistance from *S. microdontum* (via DLB1-150) can also be combined with the resistance from Tollocan and B0718-3, selected as superior parents, to broaden the genetic base and achieve higher level and more durable resistance to late blight in the offspring.

Molecular studies showed that major and minor QTLs associated with late blight resistance can also be associated with undesirable traits. Previous studies (Collins et al., 1999; Oberhagemann et al., 2000; Ewing et al., 2000) indicated that higher levels and more durable resistance in the progeny might be obtained by pyramiding minor QTLs associated with late blight resistance that are not associated with other undesirable traits such as late maturity. I am reporting here the identification of a major QTL in *S. microdontum* that is not associated with late maturity or any poor tuber quality traits. These results along with major QTLs associated with late blight resistance in Stirling and *S. bulbocastanum* suggest that high levels of resistance to late blight transmitted to a high percentage of the offspring can be conferred by a single major QTL.

A better understanding of the late blight resistance in potato can be explained by the results reported herein. The evaluation of the market limiting traits such as chip quality, specific gravity, tuber appearance and maturity together with late blight testing are possible and required for identifying recombinant clones with potential use as new varieties. There are many late blight resistant sources in which resistance is either associated with late maturity or transmitted to a low percentage of the offspring that restricts the possibility of identifying recombinant individuals. On the other hand, a few sources transmit high levels resistance to a high percentage of the offspring making possible to select for market limiting traits in advance. At the tetraploid level, Tollocan and B0718-3 were identified and recombinant clones selected in the progeny. At the diploid level, *S. microdontum* was selected, the resistance was mapped with a SSR marker closely linked to the peak of the QTL. This marker is suitable for MAS and can be followed at the tetraploid level after polyploidization.

The results reported here support the following conclusions:

1) A multi-trait progeny evaluation is valuable to identify parents and superior progeny clones to use in breeding for late blight resistance.
2) Late blight resistant parents do differ in their ability and rate to transmit late blight resistance and tuber quality to the progeny.
3) Moderate selection intensity for tuber quality traits (chip-color ratings ≤ 4, tuber appearance ratings ≤ 3 and a specific gravity ≥ 1.080) can be initiated at the single-hill generation.
4) Tuber quality evaluations at single-hill generation increase the amount of clonal data for the following generations of selections, thus improving genetic gain.
5) Tuber quality should be selected in advance of disease resistance in crosses for combining desirable traits.
6) Late blight resistance can be combined with marketable tuber quality and maturity in one cycle of selection.
7) There is a high level of genetic diversity within and among accessions, species and between ploidy levels of late blight resistant germplasm that should be introgressed in a breeding program to achieve higher level and more durable resistance in the offspring.
8) The *S. microdontum* has a major QTL associated with late blight resistance explaining more than 50% of the phenotypic variance in two years of field testing.
9) This QTL is suitable for marker assisted selection since is not associated with any other undesirable such as late maturity and poor tuber quality and explains a high percentage of the phenotypic variance.
10) High levels of late blight resistance transmitted to a high percentage of the offspring can be conferred by a single major QTL.

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APPENDICES

Table A.1. Late blight families, number of crosses and evaluated clones per family, number and percentage of selected clones with late blight resistance.

Late Blight Families[1]	Number of Crosses	Evaluated Clones[1]	Selected Clones[2]		Selected Clones[3]	
			Number	Percent	Number	Percent
Tollocan	17	71	45	64.4	37	52.1
B0718-3	15	59	17	28.8	14	23.7
Greta	4	28	7	25.0	4	14.3
Zarevo	31	92	33	35.9	13	14.1
Stobrawa	5	34	6	17.6	4	11.8
Libertas	9	52	6	11.5	4	7.7
Bertita	9	40	4	10.0	3	7.5
Bzura	5	32	1	3.2	1	3.2
Total	95	408	119	29.2	80	19.6

[1] Represent all crosses involving the same late blight resistant parent that were evaluated for tuber quality traits in 1997 and 1998 and late blight reaction in greenhouse and field in 1998.

[2] Selected clones based on infected plant area $\leq$ 10% in the greenhouse or $\leq$ 30% in the field in 1998 that were evaluated in greenhouse and field in 1999.

[3] Selected clones based on Scott-Knott cluster analysis possessing high and intermediated foliar resistance to late blight in the field in 1999.

Field Conditions for Late Blight Testing

Agronomic Practices

All experiments were conducted at the Michigan State University Muck Soils Research Station, Bath, MI (90% organic muck soil). Soils were plowed to 20 cm depth during October following harvest of preceding crops. Soils were prepared for planting with a mechanical cultivator in early May and fertilizer applied during final bed preparation on the day of planting. Fertilizers were applied in accordance with results from soil testing carried out in the spring of each year and about 250 kg N/ha (total N) was applied in two equal doses at planting and hilling. Additional micronutrients were applied according to petiole sampling recommendations and in all years. Approximately 0.2, 0.3 and 0.2 kg/ha boron, manganese and magnesium, respectively were applied as chelated formulations.

When relative humidity (RH) dipped below 80% (measured with RH sensors mounted within the canopy), a mist irrigation system was turned on to maintain RH at >95% within the plant canopy. Plots were irrigated as necessary to maintain canopy and soil moisture conditions conducive for development of foliar late blight with turbine rotary garden sprinklers (Gilmour Group, Somerset, PA, U.S.A.) at 1055 l H_2O ha/hr and managed under standard potato agronomic practices. Weeds were controlled by hilling and with metolachlor at 2.3 l/ha 10 days after planting (DAP), bentazon salt at 2.3 l/ha, 20 and 40 DAP and sethoxydim at 1.8 l/ha, 58 - 60 DAP. Insects were controlled with imidacloprid at 1.4 kg/ha at planting, carbaryl at 1.4 kg/ha, 31 and 55 DAP, endosulfan at 2.7 l/ha, 65 and 87 DAP and permethrin at 0.56 kg/ha, 48 DAP. The dates of application were similar for all years.

Disease Evaluation and Data Analysis

As soon as late blight symptoms were detected (about 7 days after inoculation, DAI), each plant within each plot was visually rated at 3 to 5 day intervals for percent leaf and stem (foliar) area with late blight lesions. The mean percent blighted foliar area per treatment was calculated. Evaluations continued until untreated plots of susceptible cultivars reached 100% foliar area diseased. Days after inoculation were used as key reference points for calculation of Relative Area Under the Disease Progress Curve [RAUDPC (1)]. For each plot and assessment date, the area under the disease progress curve (AUDPC) was estimated using the formula:

$$AUDPC = (T_{i+1} - T_i) * \left(\frac{D_{i+1} + D_i}{2} \right)$$

where T was the time in days since inoculation and D was the estimated percentage of area with blighted foliage. As foliar late blight was assessed at various time intervals, the AUDPC was estimated with the area of a right triangle whose side lengths were based on the time interval and amount of late blight in the canopy. To accumulate AUDPC for the entire season and convert it to a rate over time, the formula was:

$$RAUDPC = \frac{\sum (T_{i+1} - T_i) * \left(\frac{D_{i+1} + D_i}{2} \right)}{T_{Total} * 100}$$

Estimated AUDPC for each interval were summed, divided by the total number of days to the 100% diseased foliar area reference point in the non-treated susceptible controls, and multiplied by 100, resulting in an accumulated assessment of seasonal disease estimated as a fraction of one (RAUDPC).

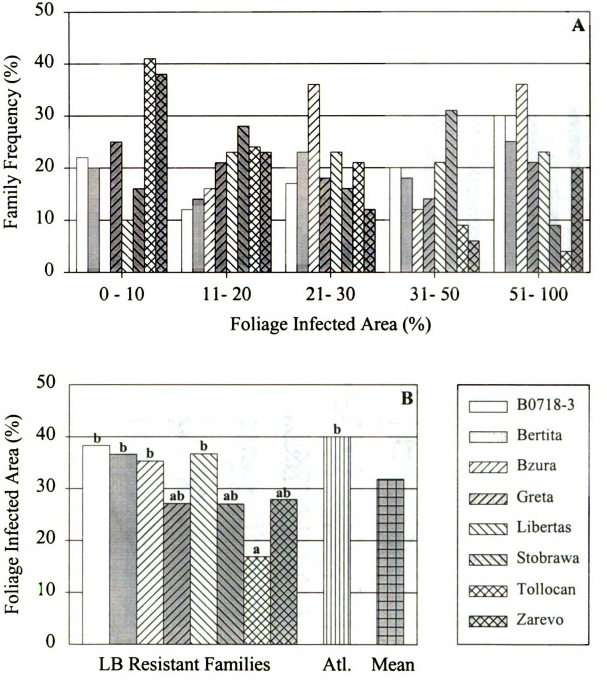

Figure A.1. A) 1998 greenhouse late blight (LB) reaction evaluated as the percentage of foliage infected area for 408 clones from crosses between late blight resistant parents (shown) and susceptible ones (top). B) Comparisons among LB resistant families and the check cv. Atlantic (bottom). Means (bars) followed by the same letter do not differ significantly using Tukey's multiple range test at P = 0.05.

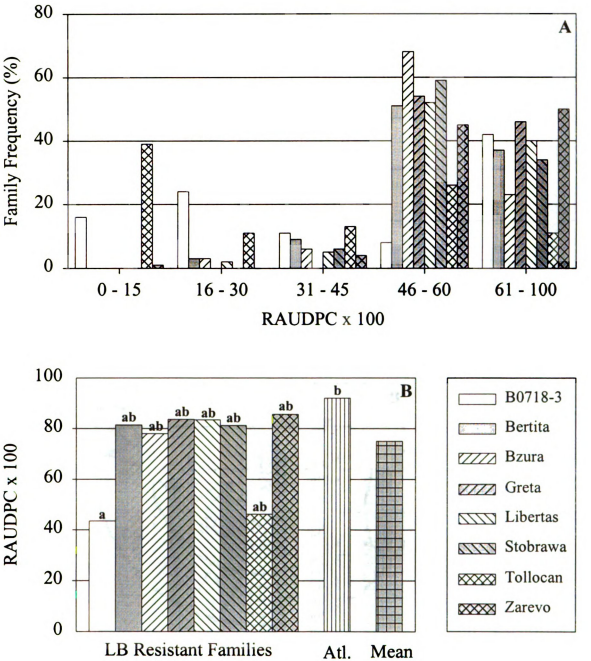

Figure A.2. A) 1998 field foliage late blight (LB) reaction expressed as the relative area under the disease progress curve (RAUDPC) for 408 clones from crosses between late blight resistant parents (shown) and susceptible ones (top). B) Comparisons among LB resistant families and the check cv. Atlantic (bottom). Means (bars) followed by the same letter do not differ significantly using Tukey's multiple range test at P = 0.05.

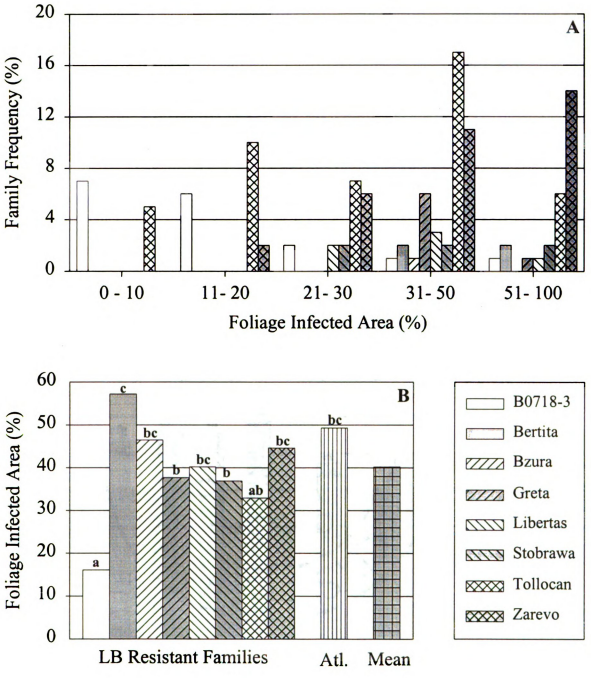

Figure A.3. A) 1999 greenhouse late blight (LB) reaction evaluated as the percentage of foliage infected area for 119 clones from crosses between late blight resistant parents (shown) and susceptible ones (top). B) Comparisons among LB resistant families and the check cv. Atlantic (bottom). Means (bars) followed by the same letter do not differ significantly using Tukey's multiple range test at P = 0.05.

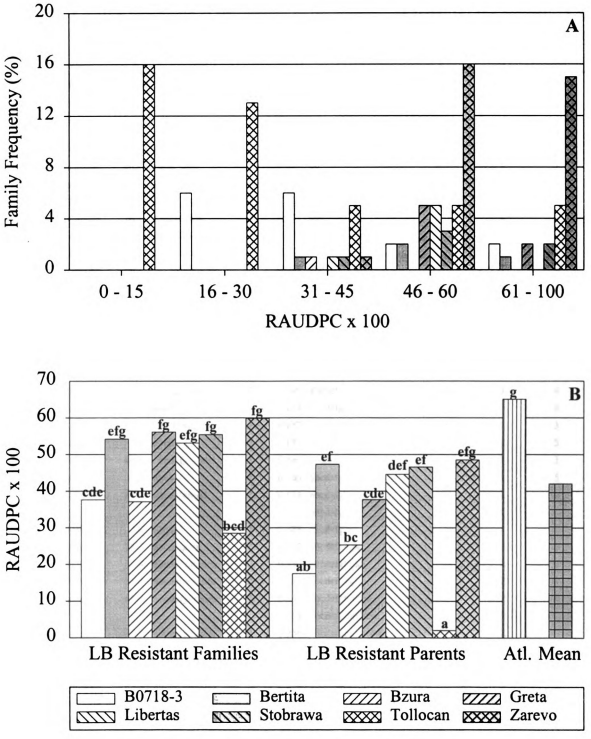

Figure A.4. A) 1999 field foliage late blight (LB) reaction expressed as the relative area under the disease progress curve (RAUDPC) for 119 clones from crosses between late blight resistant parents (shown) and susceptible ones (top). B) Comparisons among LB resistant families and the check cv. Atlantic (bottom). Means (bars) followed by the same letter do not differ significantly using Tukey's multiple range test at P = 0.05.

Table A.2. Foliar late blight (LB) reaction in the greenhouse (GH) for the percentage of infected area and in the field for the relative area under the disease progress curve (RAUDPC) and maturity in 1999.

Clone or Cultivar	LB Source	LB Evaluation in the GH			LB Evaluation in the Field			Maturity
		Foliage Infection (%)		Cluster[1]	RAUDPC		Cluster[1]	Ratings[2]
Tollocan[3]		...			0.020	a[5]	1	...
J468-2[4]	Tollocan	2.2	abc[5]	1	0.048	ab	1	4
J460-3[3]	Tollocan	14.6	abcdefghij	1	0.051	ab	1	5
J461-2[3]	Tollocan	0.1	a	1	0.053	ab	1	4
J464-5[4]	Tollocan	0.4	ab	1	0.064	ab	1	4
J461-1[4]	Tollocan	16.4	abcdefghijk	2	0.071	abc	1	3
J458-1[3]	Tollocan	14.2	abcdefghij	1	0.074	abc	1	5
J457-4[3]	Tollocan	44.4	bcdefghijklm	2	0.081	abc	1	4
J458-2[3]	Tollocan	56.7	defghijklm	1	0.095	abc	1	3
J462-2[3]	Tollocan	13.1	abcdefghi	1	0.106	abcd	1	3
J468-5[3]	Tollocan	24.4	abcdefghijkl	1	0.119	abcde	1	5
J456-4[3]	Tollocan	9.0	abcdefgh	1	0.132	abcdef	1	4
J466-4[3]	Tollocan	16.5	abcdefghijkl	3	0.133	abcdef	1	5
J459-2[3]	Tollocan	20.2	abcdefghijkl	1	0.140	abcdef	1	4
J457-2[3]	Tollocan	9.1	abcdefgh	1	0.144	abcdef	1	4
J459-5[3]	Tollocan	23.1	abcdefghijkl	2	0.149	abcdef	1	4
J453-4[3]	Tollocan	9.9	abcdefgh	1	0.154	abcdefg	1	4
J459-3[3]	Tollocan	14.7	abcdefghij	1	0.157	abcdefg	1	3
J453-2[4]	Tollocan	3.0	abcd	1	0.157	abcdefg	1	5
J459-4[3]	Tollocan	46.1	bcdefghijklm	1	0.160	abcdefg	1	3
J306-5[3]	B0718-3	9.7	abcdefgh	1	0.160	abcdefg	1	5
J459-1[3]	Tollocan	19.9	abcdefghijkl	1	0.172	abcdefgh	1	4
B0718-3[3]		...			0.175	abcdefghi	1	...
J307-1[3]	B0718-3	7.2	abcdef	1	0.183	abcdefghij	1	5
J464-4[3]	Tollocan	15.5	abcdefghij	3	0.222	abcdefghijk	2	3
J306-3[3]	B0718-3	35.4	abcdefghijklm	3	0.236	abcdefghijkl	2	5
J464-1[3]	Tollocan	38.6	abcdefghijklm	3	0.237	abcdefghijkl	2	3
J467-2[3]	Tollocan	46.9	cdefghijklm	1	0.238	abcdefghijklm	2	4
Bzura[3]		...			0.252	abcdefghijklmn	2	...
J466-3[3]	Tollocan	37.8	abcdefghijklm	1	0.256	abcdefghijklmno	2	3
J317-5[3]	B0718-3	7.9	abcdefg	1	0.263	abcdefghijklmnop	2	5
J458-3[3]	Tollocan	18.0	abcdefghijkl	1	0.265	abcdefghijklmnopq	2	4
J455-4[3]	Tollocan	43.7	abcdefghijklm	3	0.268	abcdefghijklmnopqr	2	3
J307-2[3]	B0718-3	9.4	abcdefgh	2	0.272	abcdefghijklmnopqr	2	4
J320-2[4]	B0718-3	4.6	abcde	1	0.275	abcdefghijklmnopqrs	2	5
J465-1[3]	Tollocan	35.4	abcdefghijklm	1	0.284	abcdefghijklmnopqrst	2	4
J467-3[3]	Tollocan	27.2	abcdefghijklm	2	0.294	bcdefghijklmnopqrstu	2	4
J462-3[3]	Tollocan	16.9	abcdefghijkl	1	0.303	bcdefghijklmnopqrstuv	2	4
J462-1[3]	Tollocan	43.7	abcdefghijklm	1	0.312	bcdefghijklmnopqrstuvw	2	5
J319-9[3]	B0718-3	22.6	abcdefghijkl	1	0.317	bcdefghijklmnopqrstuvw	2	4
J456-2[3]	Tollocan	17.9	abcdefghijkl	3	0.342	cdefghijklmnopqrstuvwx	2	3
J348-7[3]	Bzura	46.5	bcdefghijklm	1	0.371	defghijklmnopqrstuvwxy	2	5
J453-3[3]	Tollocan	43.2	abcdefghijklm	2	0.373	defghijklmnopqrstuvwxy	2	4
Greta[3]		...			0.377	defghijklmnopqrstuvwxyz	2	...

Continued

J319-1[3]	B0718-3	16.0	abcdefghij	1	0.378	efghijklmnopqrstuvwxyz	2	3
J317-1[3]	B0718-3	10.7	abcdefgh	1	0.382	efghijklmnopqrstuvwxyz	2	4
J448-1[3]	Stobrawa	20.8	abcdefghijkl	1	0.388	efghijklmnopqrstuvwxyz	2	5
J309-6[3]	B0718-3	10.1	abcdefgh	1	0.391	efghijklmnopqrstuvwxyz	2	5
J468-1[3]	Tollocan	39.6	abcdefghijklm	1	0.395	fghijklmnopqrstuvwxyz	2	2
J319-7[4]	B0718-3	6.2	abcdef	2	0.423	ghijklmnopqrstuvwxyza	2	2
J469-2[3]	Tollocan	50.2	cdefghijklm	3	0.439	hijklmnopqrstuvwxyzab	2	5
J332-1	Bertita	42.4	abcdefghijklm	1	0.444	ijklmnopqrstuvwxyzab	2	5
Libertas		...			0.446	ijklmnopqrstuvwxyzab	2	...
J314-3	B0718-3	7.5	abcdefg	1	0.449	jklmnopqrstuvwxyzab	2	4
J395-10	Libertas	27.7	abcdefghijklm	1	0.450	jklmnopqrstuvwxyzab	2	5
J471-5	Zarevo	14.0	abcdefghij	2	0.452	jklmnopqrstuvwxyzab	2	5
J310-3	B0718-3	7.7	abcdefg	1	0.455	klmnopqrstuvwxyzab	2	3
Stobrawa		...			0.466	klmnopqrstuvwxyzabc	2	...
J464-6	Tollocan	39.5	abcdefghijklm	1	0.468	klmnopqrstuvwxyzabcd	2	5
J476-1	Zarevo	41.7	abcdefghijklm	1	0.472	klmnopqrstuvwxyzabcd	2	4
Bertita		...			0.473	klmnopqrstuvwxyzabcd	2	...
J404-5	Libertas	45.8	bcdefghijklm	1	0.484	klmnopqrstuvwxyzabcd	2	5
Zarevo		...			0.485	klmnopqrstuvwxyzabcd	2	...
J503-1	Zarevo	38.1	abcdefghijklm	1	0.486	klmnopqrstuvwxyzabcd	2	....
J488-2	Zarevo	12.9	abcdefghi	1	0.488	klmnopqrstuvwxyzabcd	2	4
J449-5	Stobrawa	22.8	abcdefghijkl	2	0.498	lmnopqrstuvwxyzabcd	2	4
J365-2	Greta	29.7	abcdefghijklm	3	0.500	lmnopqrstuvwxyzabcd	2	4
J481-1	Zarevo	67.6	ghijklm	1	0.501	lmnopqrstuvwxyzabcd	2	5
J452-3	Stobrawa	35.0	abcdefghijklm	2	0.502	lmnopqrstuvwxyzabcd	2	3
J497-1	Zarevo	68.8	hijklm	3	0.504	lmnopqrstuvwxyzabcd	2	4
J496-2	Zarevo	42.4	abcdefghijklm	2	0.505	lmnopqrstuvwxyzabcd	2	4
J488-4	Zarevo	23.5	abcdefghijkl	2	0.509	mnopqrstuvwxyzabcde	2	3
J501-6	Zarevo	57.7	defghijklm	2	0.516	nopqrstuvwxyzabcde	2	....
J320-1	B0718-3	11.4	abcdefgh	3	0.519	nopqrstuvwxyzabcde	2	....
J499-2	Zarevo	32.2	abcdefghijklm	2	0.524	nopqrstuvwxyzabcde	2	4
J365-8	Greta	31.3	abcdefghijklm	1	0.526	opqrstuvwxyzabcde	2	5
J502-1	Zarevo	59.6	efghijklm	1	0.528	pqrstuvwxyzabcde	2	....
J492-2	Zarevo	58.9	efghijklm	1	0.531	pqrstuvwxyzabcde	2	4
J366-4	Greta	30.9	abcdefghijklm	1	0.536	qrstuvwxyzabcde	2	5
J491-3	Zarevo	56.4	defghijklm	2	0.539	rstuvwxyzabcde	2	4
J399-1	Libertas	33.2	abcdefghijklm	3	0.539	rstuvwxyzabcde	2	3
J324-2	Bertita	62.7	fghijklm	1	0.545	stuvwxyzabcde	2	4
J466-2	Tollocan	88.1	m	3	0.547	tuvwxyzabcde	2	4
J452-4	Stobrawa	51.9	cdefghijklm	1	0.549	tuvwxyzabcde	2	3
J332-6	Bertita	79.7	klm	1	0.551	tuvwxyzabcde	2	5
J467-6	Tollocan	80.2	lm	1	0.553	tuvwxyzabcde	2	2
J395-1	Libertas	19.0	abcdefghijkl	2	0.553	tuvwxyzabcde	2	5
J364-1	Greta	40.5	abcdefghijklm	3	0.560	uvwxyzabcde	2	4
J496-1	Zarevo	29.9	abcdefghijklm	3	0.565	uvwxyzabcde	3	4
J462-5	Tollocan	28.3	abcdefghijklm	1	0.568	vwxyzabcde	3	4
J494-1	Zarevo	20.6	abcdefghijkl	1	0.574	vwxyzabcde	3	3
J482-1	Zarevo	64.5	fghijklm	1	0.576	wxyzabcde	3	4
J365-10	Greta	34.5	abcdefghijklm	2	0.579	wxyzabcde	3	5
J400-3	Libertas	63.9	fghijklm	1	0.581	wxyzabcde	3	2

Continued

J405-1	Libertas	46.1	bcdefghijklm	3	0.581	wxyzabcde	3	3
J484-2	Zarevo	37.1	abcdefghijklm		0.591	xyzabcde	3	....
J465-3	Tollocan	77.0	jklm	1	0.598	xyzabcde	3	4
J364-5	Greta	56.4	defghijklm	1	0.610	xyzabcde	3	3
J463-1	Tollocan	37.9	abcdefghijklm	1	0.610	xyzabcde	3	3
J365-6	Greta	34.2	abcdefghijklm	3	0.615	yzabcde	3	3
J501-1	Zarevo	34.8	abcdefghijklm	1	0.618	yzabcde	3	4
J476-5	Zarevo	62.5	fghijklm	1	0.619	yzabcde	3	4
J468-4	Tollocan	65.0	fghijklm	1	0.622	yzabcde	3	3
J326-5	Bertita	51.5	cdefghijklm	3	0.629	yzabcde	3	3
J497-4	Zarevo	23.7	abcdefghijkl	1	0.636	yzabcde	3	4
J492-4	Zarevo	42.2	abcdefghijklm	2	0.640	yzabcde	3	3
J493-2	Zarevo	36.6	abcdefghijklm	1	0.641	yzabcde	3	4
J315-1	B0718-3	23.2	abcdefghijkl	1	0.642	yzabcde	3	4
Atlantic		49.3	cdefghijklm	1	0.648	zabcde	3	...
J492-1	Zarevo	33.1	abcdefghijklm	2	0.668	abcde	3	3
J314-1	B0718-3	54.6	cdefghijklm	1	0.670	abcde	3	4
J464-3	Tollocan	60.5	efghijklm	1	0.677	abcde	3	5
J487-1	Zarevo	26.4	abcdefghijklm	2	0.681	abcde	3	4
J482-2	Zarevo	56.2	defghijklm	1	0.690	abcde	3	4
J451-3	Stobrawa	56.9	defghijklm	1	0.694	abcde	3	3
J456-3	Tollocan	53.7	cdefghijklm	1	0.694	abcde	3	3
J450-5	Stobrawa	37.5	abcdefghijklm	1	0.695	bcde	3	3
J494-4	Zarevo	51.3	cdefghijklm	3	0.696	bcde	3	3
J501-5	Zarevo	63.9	fghijklm	1	0.697	bcde	3	3
J456-1	Tollocan	33.8	abcdefghijklm	2	0.698	bcde	3	3
J495-2	Zarevo	29.4	abcdefghijklm	2	0.701	bcde	3	4
J492-6	Zarevo	54.2	cdefghijklm	3	0.734	cde	3	3
J489-1	Zarevo	75.2	ijklm	1	0.738	de	3	2
J483-1	Zarevo	27.1	abcdefghijklm	1	0.738	de	3	3
J487-5	Zarevo	65.0	fghijklm	1	0.777	e	3	3
J315-5	B0718-3	7.2	abcdef	2	...		...	...
J455-1	Tollocan	41.2	abcdefghijklm	2	...		...	5
J487-3	Zarevo	61.6	efghijklm	2	...		...	3
Average		35.1			0.432			3.8
C.V.%		35.7			17.2			

[1] Scott-Knott cluster groups differing in late blight resistance: cluster group 1 = resistant, 2 = intermediate, and 3 = susceptible.

[2] Evaluated in 20-hill plots at MSU - Lake City Research Farm, in a scale 1-5 of increasing lateness.

[3, 4] Dunnett's T tests for alpha = 0.05 against Atlantic control in the field[3] and in the greenhouse[4].

[5] Numbers in columns followed by the same letter do not differ significantly using Tukey's multiple range test at P = 0.05.

... Not evaluated.

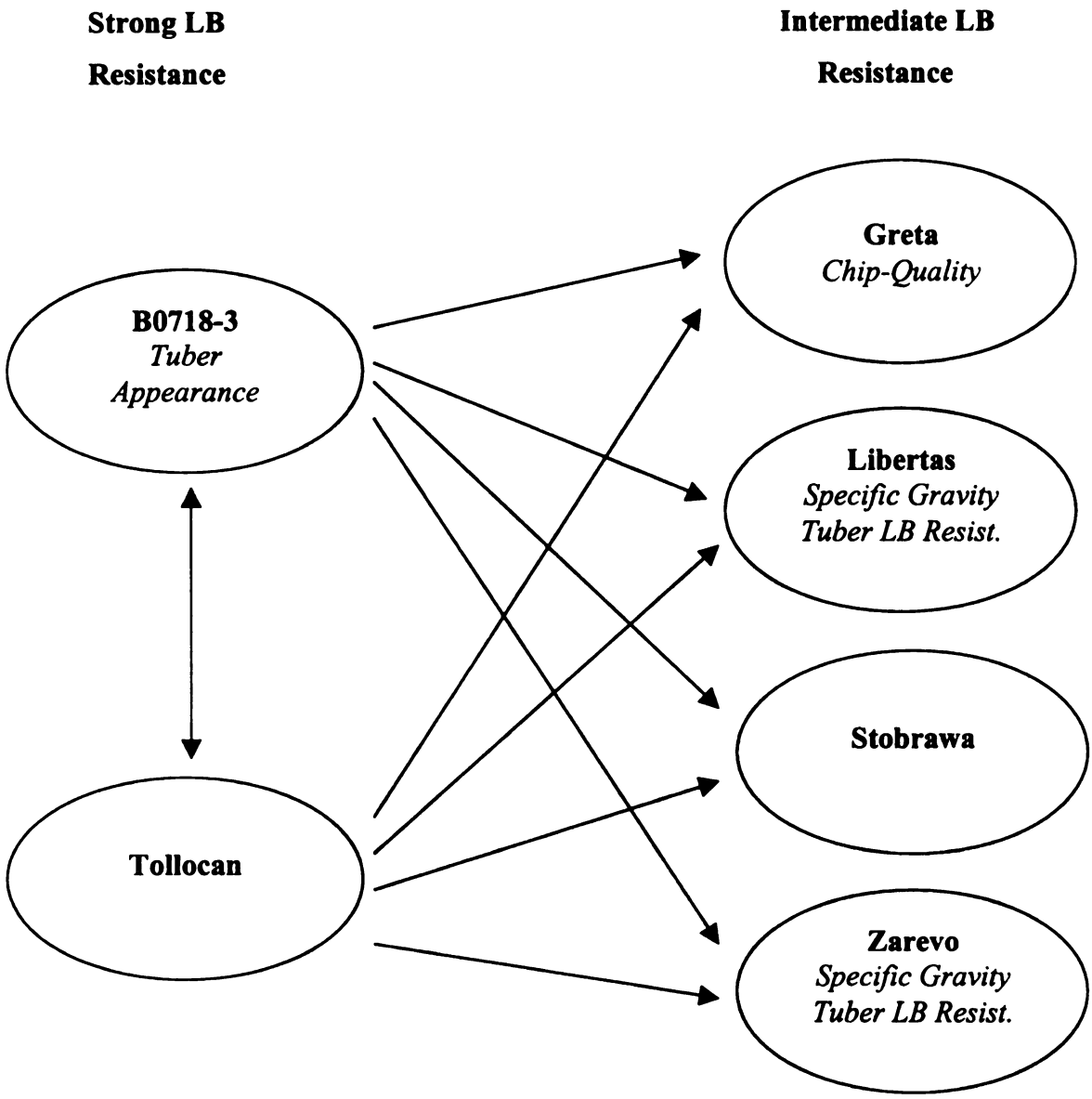

Figure A.5. Intercrossing scheme to combine late blight (LB) resistance from different sources with other important characteristics.

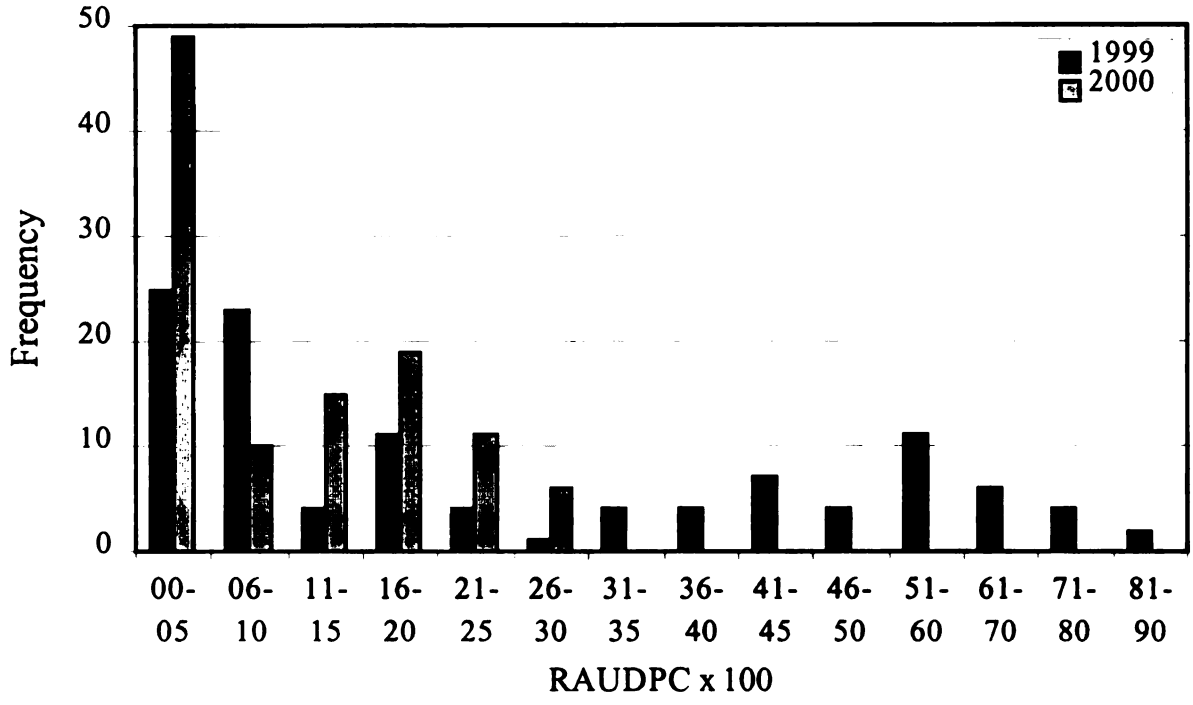

Figure A.6. Frequency distribution of a *S. microdontum* population for foliar late blight reaction in the field in 1999 and 2000. RAUDPC = relative area under the disease progress curve. Note changes in interval frequency. (RAUDPC for PI595511-5 = 0.021 and 0.019, MSA133-57 = 0.529 and 0.175, respectively for 1999 and 2000 field tests).

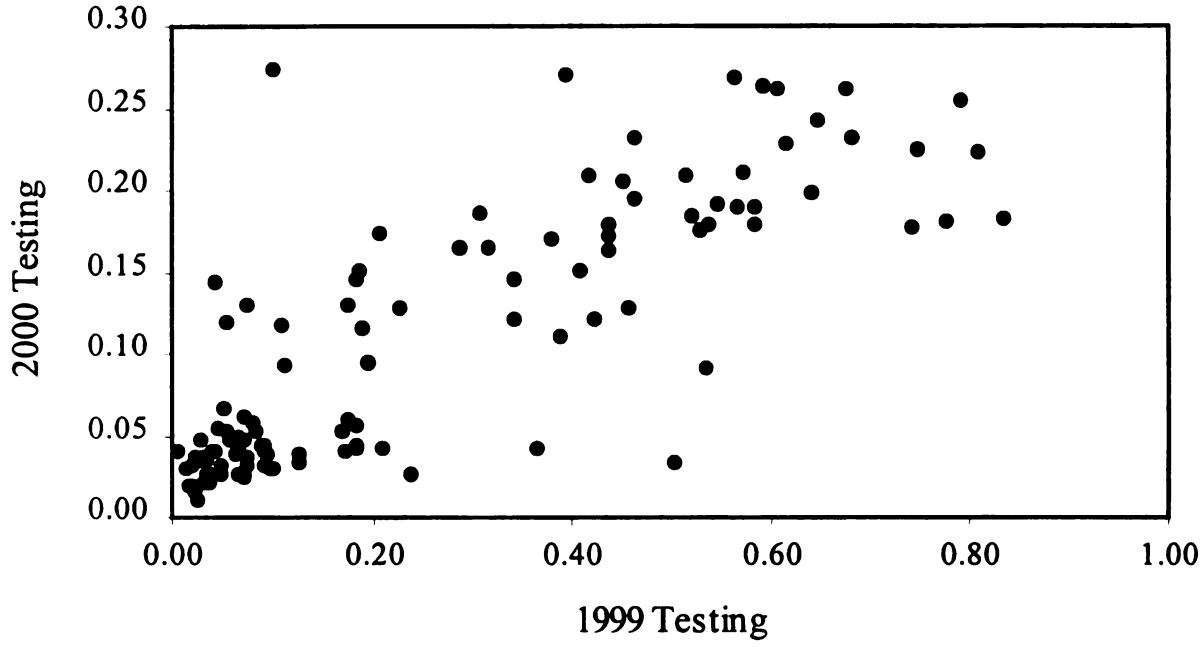

Figure A.7. Correlation of *S. microdontum* mapping population for foliar late blight reaction in the field between 1999 and 2000 (r = 0.82, P = 0.0001).

Table A.3. Foliar late blight reaction in the field based on the relative area under the disease progress curve and Scott-Knott cluster groups differing in resistance level in 1999 and 2000.

Parents and Progeny	RAUDPC[1] 1999	Scott-Knott Groups[2]	RAUDPC[1] 2000	Scott-Knott Groups[2]
176	0.025	1	0.010	1
002	0.024	1	0.016	1
PI595511-5	0.021	1	0.019	1
004	0.018	1	0.019	1
185	0.038	1	0.021	1
195	0.033	1	0.021	1
225	0.071	1	0.025	1
095	0.034	1	0.026	1
068	0.038	1	0.026	1
209	0.238	5	0.026	1
117	0.048	1	0.026	1
122	0.066	1	0.027	1
073	0.072	1	0.027	1
169	0.097	1	0.030	1
124	0.014	1	0.030	1
005	0.101	1	0.030	1
213	0.019	1	0.031	2
153	0.050	1	0.031	2
125	0.074	1	0.031	2
123	0.092	1	0.032	2
134	0.503	5	0.034	2
220	0.125	2	0.034	2
170	0.033	1	0.035	3
065	0.030	1	0.035	3
097	0.024	1	0.036	3
217	0.031	1	0.037	4
182	0.075	1	0.037	4
206	0.096	1	0.038	5
152	0.125	2	0.039	6
173	0.064	1	0.039	6
140	0.007	1	0.040	6
179	0.171	2	0.040	6
086	0.093	1	0.041	6
109	0.040	1	0.041	6
216	0.041	1	0.041	6
040	0.044	1	0.041	6
053	0.211	5	0.042	6
062	0.065	1	0.042	6
084	0.185	4	0.042	6
104	0.365	5	0.043	6

Continued

103	0.091	1	0.043	6
193	0.184	3	0.043	6
177	0.088	1	0.044	7
156	0.071	1	0.047	7
155	0.058	1	0.047	7
205	0.030	1	0.047	7
099	0.066	1	0.048	7
157	0.082	1	0.052	7
096	0.055	1	0.053	7
121	0.170	2	0.053	7
214	0.045	1	0.055	7
145	0.183	3	0.057	8
199	0.082	1	0.058	8
092	0.176	2	0.060	9
050	0.071	1	0.061	9
150	0.050	1	0.066	9
003	0.051	1	0.066	9
166	0.535	5	0.092	9
044	0.112	1	0.094	9
223	0.196	5	0.094	9
019	0.387	5	0.111	9
218	0.189	5	0.116	9
108	0.110	1	0.117	9
224	0.054	1	0.119	9
020	0.422	5	0.120	10
057	0.341	5	0.122	10
048	0.228	5	0.127	11
188	0.456	5	0.128	12
107	0.074	1	0.130	13
131	0.176	2	0.130	13
120	0.045	1	0.144	14
192	0.342	5	0.145	15
154	0.184	3	0.146	15
159	0.186	5	0.150	16
210	0.409	5	0.150	16
128	0.436	5	0.164	17
222	0.287	5	0.165	18
172	0.315	5	0.165	19
167	0.380	5	0.170	19
091	0.438	5	0.172	19
203	0.207	5	0.173	19
MSA133-57	0.529	5	0.175	19
161	0.741	7	0.177	19
076	0.582	6	0.179	20
101	0.537	5	0.179	20
129	0.438	5	0.179	20
070	0.775	7	0.180	21

Continued

111	0.833	10	0.182	22
158	0.519	5	0.184	22
219	0.309	5	0.186	22
080	0.582	6	0.189	23
051	0.565	6	0.190	23
113	0.547	5	0.192	23
102	0.463	5	0.195	24
207	0.640	6	0.198	25
012	0.451	5	0.206	26
198	0.417	5	0.208	26
164	0.514	5	0.210	26
094	0.571	6	0.211	26
115	0.807	9	0.223	27
200	0.747	7	0.225	28
078	0.615	6	0.228	29
178	0.680	7	0.231	30
221	0.462	5	0.232	30
171	0.647	6	0.241	31
058	0.789	8	0.254	32
061	0.675	6	0.261	33
008	0.606	6	0.262	33
105	0.593	6	0.263	34
183	0.563	5	0.268	35
045	0.393	5	0.271	35
100	0.102	1	0.273	36
Mean	0.260		0.106	
L.S.D.	0.433		0.067	
C.V.%	42.6		33.2	

[1] Relative area under the disease progress curve (maximum RAUDPC = 1).

[2] Scott-Knott cluster groups differing in late blight resistance.

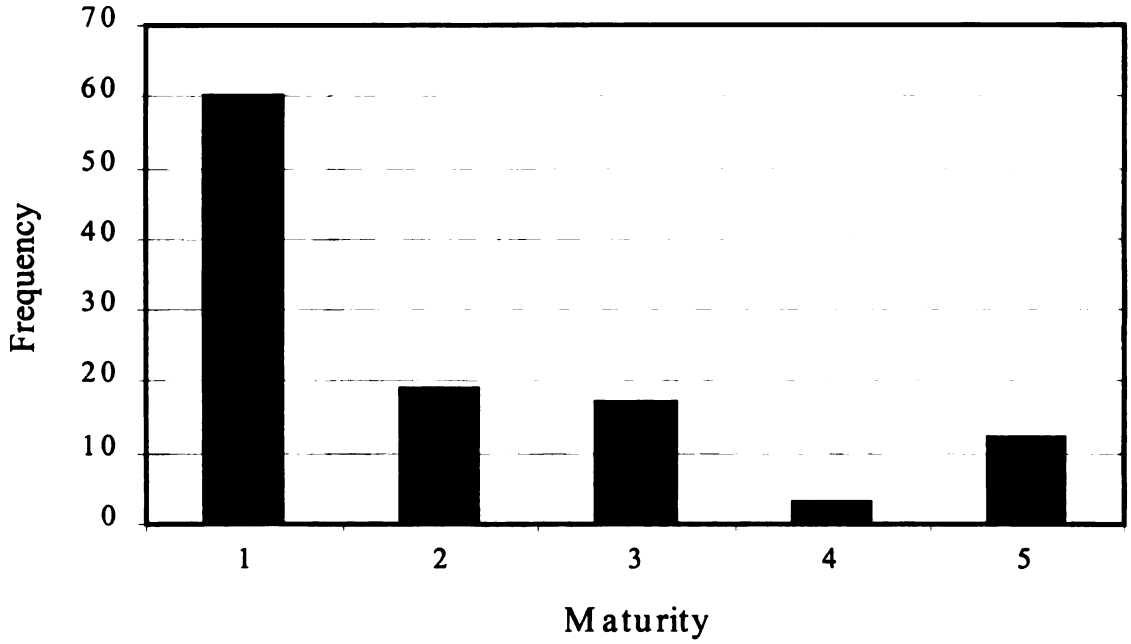

Figure A.8. Frequency distribution for vine maturity of a *S. microdontum* mapping population on a scale 1 to 5 of increasing lateness in 2000. (MSA133-57 = 2 and PI 595511-5 = 5).

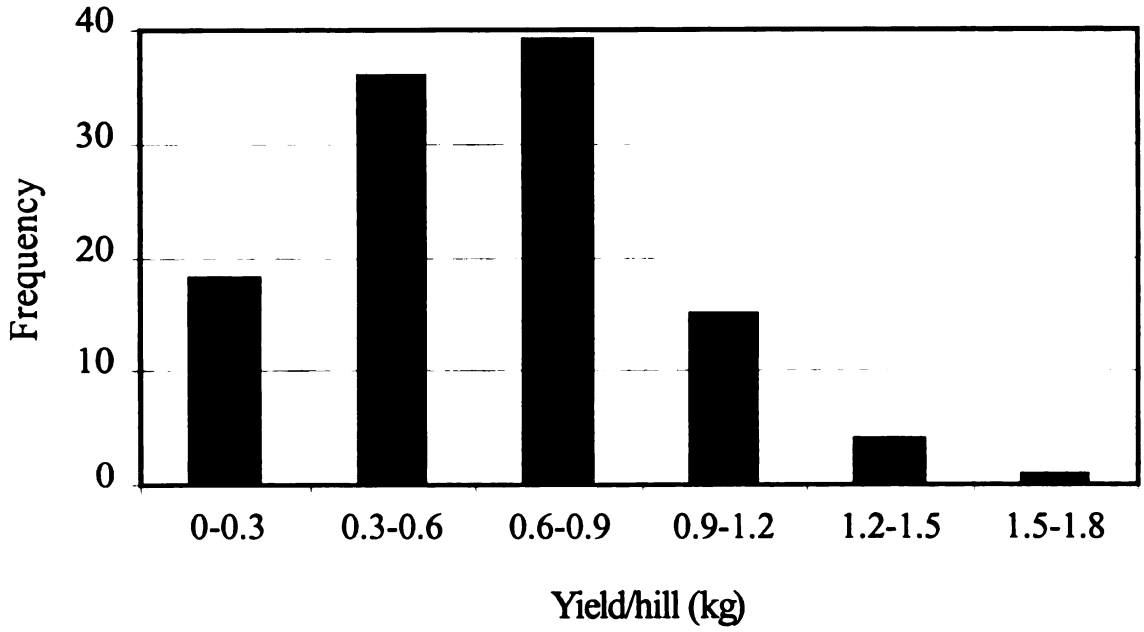

Figure A.9. Frequency distribution for yield • hill^{-1} (kg) of a *S. microdontum* mapping population in the field in 2000. (MSA133-57 = 0.242 kg).

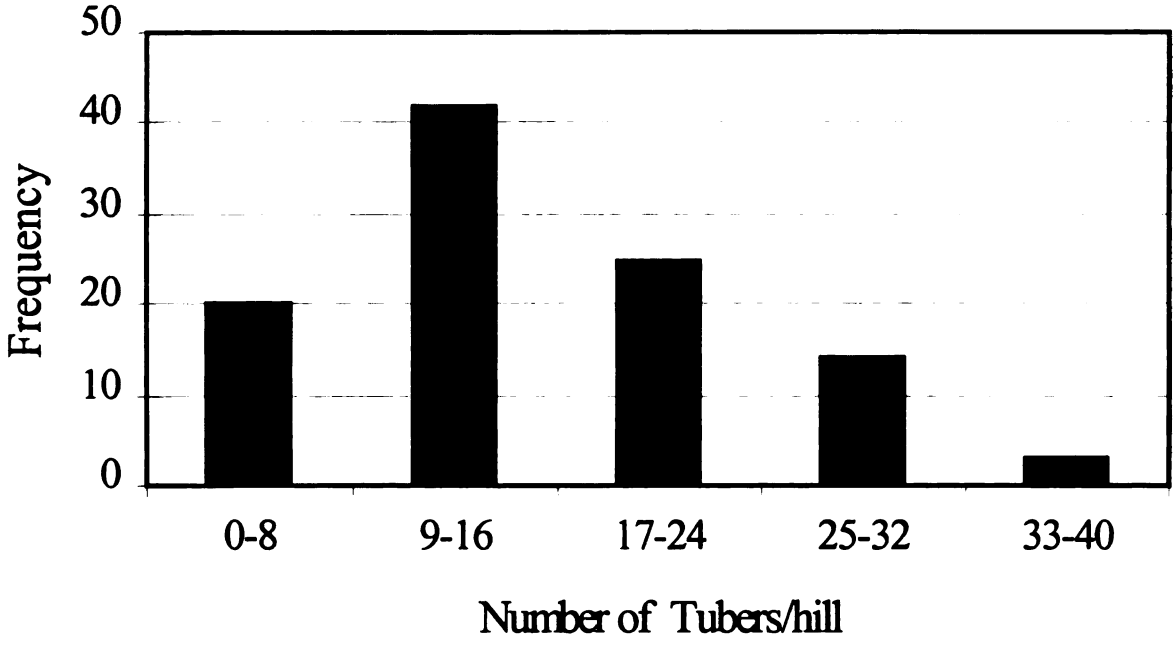

Figure A.10. Frequency distribution for number of tubers • hill^{-1} of a *S. microdontum* mapping population in the field in 2000. (MSA133-57 = 2).

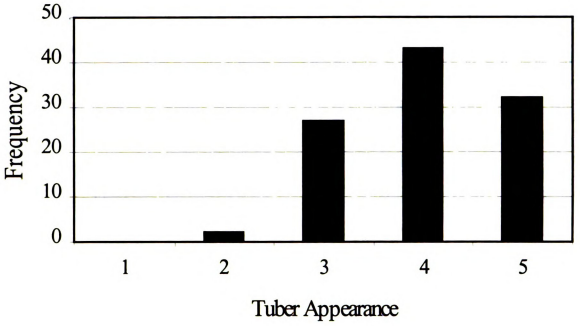

Figure A.11. Frequency distribution for tuber appearance of a *S. microdontum* mapping population on a scale 1 to 5 of increasing defects in 2000. (MSA133-57=3).

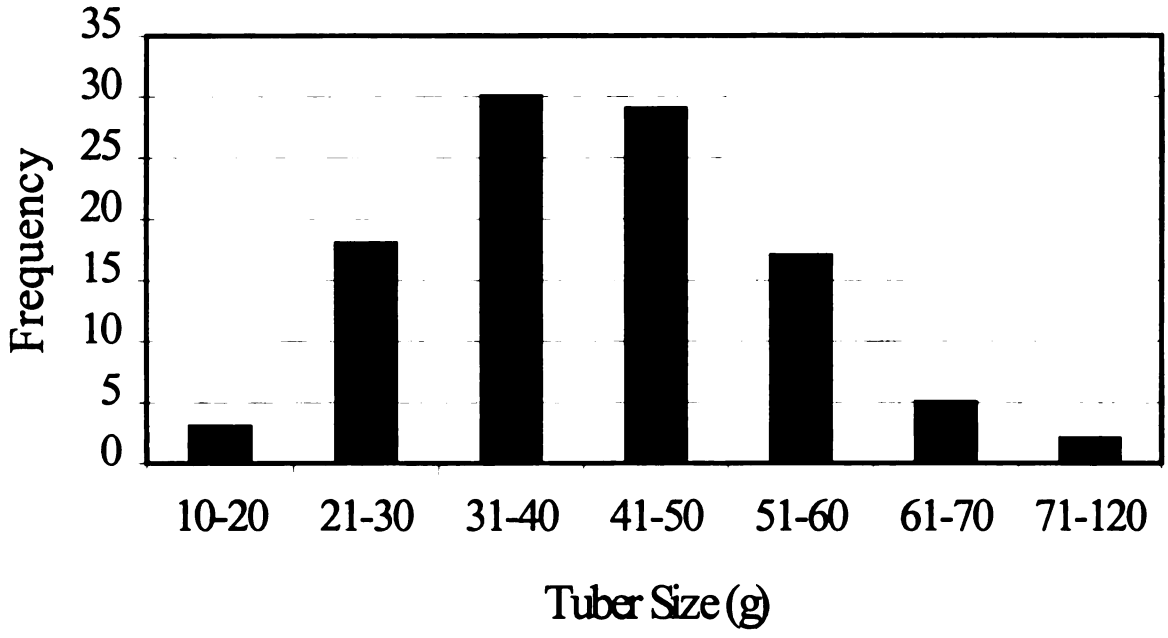

Figure A.12. Frequency distribution for tuber size (g) of a *S. microdontum* mapping population in the field in 2000. (MSA133-57 = 120.8 g).

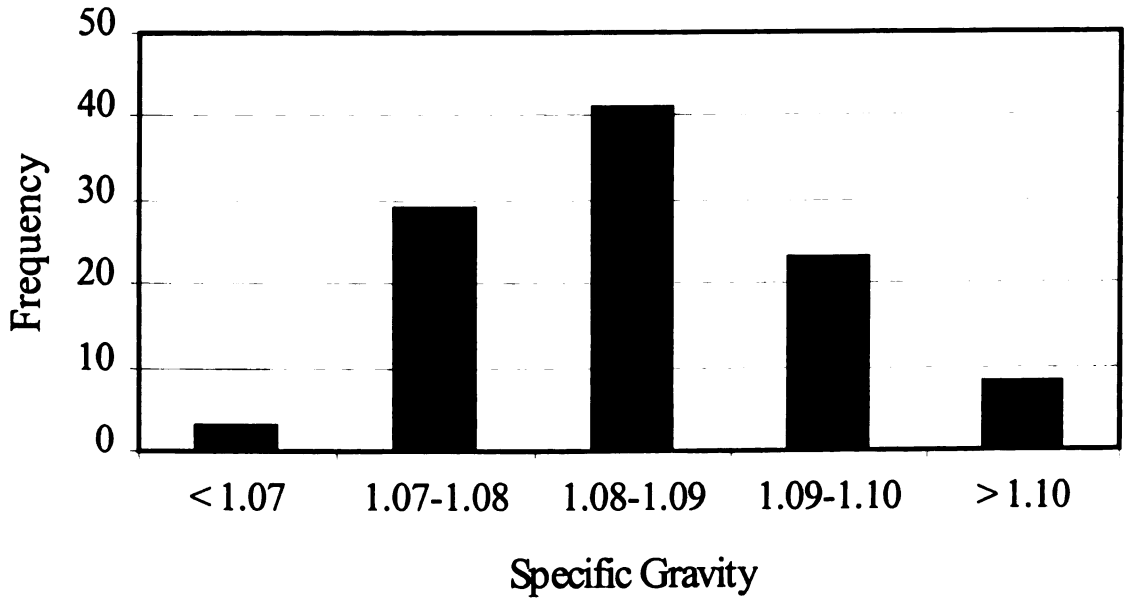

Figure A.13. Frequency distribution for specific gravity of a *S. microdontum* mapping population in the field in 2000. (MSA133-57 = 1.107).

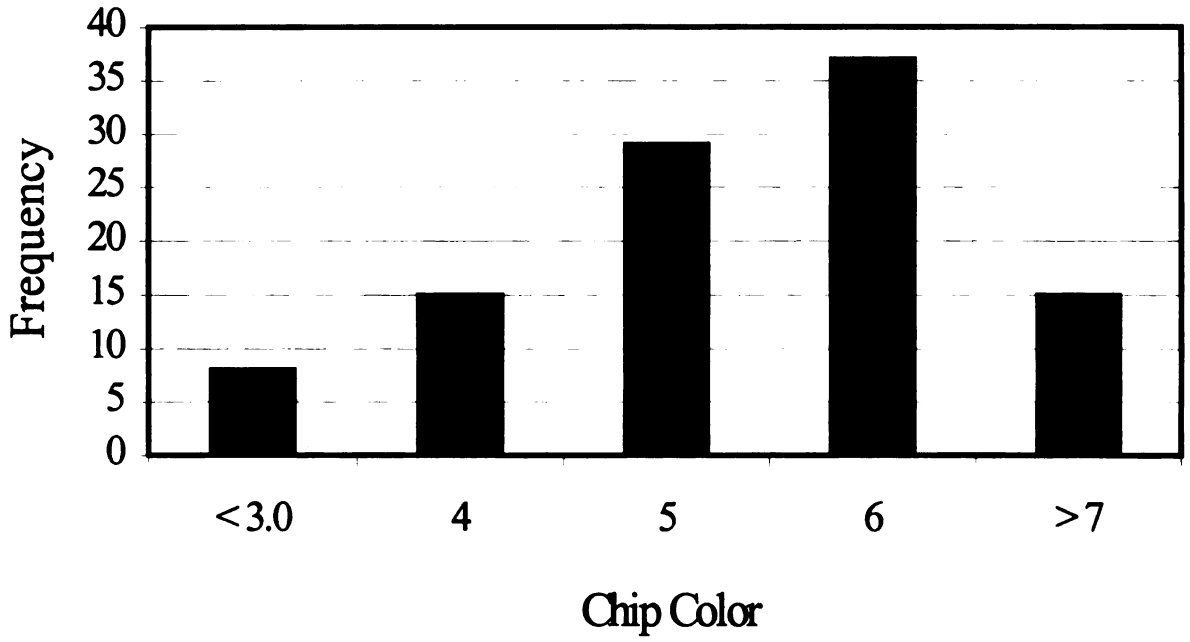

Figure A.14. Frequency distribution for chip color of a *S. microdontum* mapping population on a scale 1 to 9 of increasing darkness in the field in 2000. (MSA133-57 = 2).