AN ABSTRACT OF THE THESIS OF

Ryon J Ottoman for the degree of Master of Science in Crop Science presented on July 17, 2006.

Title: Marker-Assisted Selection for Resistance to Potato Virus Y

Abstract approved:

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Potato Virus Y (PVY) imposes serious limitations on potato yield, quality and tuber seed production. Traditional PVY screening requires artificial inoculation under controlled conditions followed by ELISA to test for PVY. This method is very tedious and time consuming thus prohibiting the screening of large segregating populations. The genes Ry_{adg} from *S. tuberosum* ssp. *andigena* and Ry_{sto} from *S. stoloniferum* provide extreme resistance to PVY. The objective of this work is to assess the usefulness of molecular markers for determining the allelic configuration of parental material containing Ry_{adg} and Ry_{sto} genes and as an early selection tool for predicting PVY resistance. To achieve this, two segregating populations for Ry_{adg} and Ry_{sto} were screened with molecular markers, inoculated artificially and tested for PVY by ELISA. Ninety-six percent of the segregating lines for the Ry_{adg} gene showed coincidence between results for molecular markers and ELISA at 40 days after inoculations. Both ELISA and molecular marker results fit a 1:1 (resistant:susceptible) segregation ratio indicating the presence of Ry_{adg} as a simplex. In the population segregating for Ry_{sto} only 84% of the segregating lines

showed coincidence between results for molecular markers and ELISA at 40 days after inoculations. The molecular markers results fit a 1:1 segregation ratio whereas the ELISA results indicated that a second gene/allele was likely providing resistance to PVY. Markers associated with Ry_{adg} and Ry_{sto} were successfully used for Marker-Assisted Selection in the Pacific NW Potato Breeding program. In addition, advanced potato clones were evaluated for presence of Ry_{adg} and Ry_{sto} PVY resistance sources. The lines LBR2, BO718-3 and EGA97061-4 showed patterns of resistance for PVY associated with Ry_{adg} based on molecular marker evaluations. Additional sources of PVY resistance, not detected with the markers used in this study, are likely present in other advanced potato germplasm evaluated.

Marker-Assisted Selection for Resistance to Potato Virus Y

by

Ryon J. Ottoman

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented July 17, 2006
Commencement June 2007

Master of Science thesis of Ryon J. Ottoman presented on July 17, 2006.

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ACKNOWLEDGEMENTS

I would like to recognize my advisors Dr. M. Isabel Vales and Dr. Alvin Mosley for providing me with guidance in technical aspects and for providing me with this opportunity to develop myself as a scientist in the realm of plant breeding. Thanks also to my committee members Dr. Chuck Brown, Dr. Dan Hane and Dr. Mina McDaniel for their service on my graduate committee and for advice and encouragement along the way.

Personal from the Cereals and biotechnology lab and USDA small fruits lab deserve credit for supplying technical advice and equipment for the research process as does James Crosslin from the US Dept. of Agriculture/ARS, Prosser, WA for providing the verified PVY^{0} in tobacco tissue and the virus buffer. In addition, support staff including Solomon Yilma, Steve James, Kandy Marling, and Hollis Baley deserve recognition for their contributions to my success.

I wish to recognize the Department of Crop and Soil Sciences in the college of Agricultural sciences at Oregon State University for providing me a place to work and supplemental funding. Thanks to the Oregon Potato Commission and USDA-CSREES for providing funding for materials and supplies.

All of what I have accomplished in reaching this point and future accomplishments resulting from this degree would not have been possible without the steadfast support from my family and friends to whom I will be ever grateful.

CONTRIBUTION OF AUTHORS

Dr. M. Isabel Vales played a part in all aspects of this undertaking, from experimental design to manuscript preparation, Dr. Alvin Mosley for manuscript editing and discussions, Dr. Chuck Brown for very useful discussions in genetic analysis and potato breeding, Dr. Dan Hane for conducting the field screening of the advanced selections for PVY resistance and useful discussions on plant breeding and on the topic of Potato Virus Y. Oscar Gutbrod for useful conversations on the topic of potato virus Y and for collaborating on the visual observations for virus expression, and Solomon Yilma for encouraging the use of markers in the Oregon breeding program and many useful discussions.

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Marker-Assisted Selection for Resistance to Potato Virus Y

Chapter 1

General Introduction

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Introduction

The Potato (*Solanum tuberosum* L.) is a valuable source of human nutrition. It can help supply the necessary caloric intake and several important nutrients for the growing world population. A large potato (approximately 148 grams) contains 100 kilocalories (commonly expressed as calories), and no fat and is a very good source of vitamin C, potassium, and fiber (http://www.healthypotato.com/). Potatoes rank fourth in world production behind wheat, rice, and maize. Potato production has increased rapidly in developing counties more than either wheat or rice (Scott et al., 2000). Maintaining virus free seed stocks is a major challenge for potato growers in both developing and developed countries.

Virus infections in cultivated potatoes cause a range of consequences from yield reduction to internal tuber defects. Economically damaging viruses typically belong to the *Poler (luteo)*, *Poty, Potex,* and *Carla* families (Lawson and Stace-Simth, 2001). Potato Virus Y (PVY) is a Potyvirus. There are three common strains of PVY: Y^o, Y^n, and Y^c (Brunt, 2001; Thieme and Thieme, 2005). In addition, recombinant strains of PVY have been reported: PVY^{ntn}, PVY^nW, and $PVY^{n:o}$ (Chrzanowska, 1987; Kerlan et al., 1999; McDonald and Singh, 1996; Weidemann, 1988). Potato Virus Y symptoms are highly cultivar dependant and may include vein necrosis, mottling, yellowing of leaflets, leaf-dropping, stunted growth, and premature plant death (deBokx and Huttinga, 1981). Depending on the cultivar, time of infection, and growing conditions, yield losses associated with PVY range from 10-80% (deBokx and Huttinga, 1981; Rykbost et al., 1999). Under field conditions the virus is spread in a non-persistent manner by more than 30 species of aphids (deBokx and Piron,

1990; Harrington and Gibson, 1989; Harrington et al., 1986; Hoof, 1977; Hoof, 1980; Katis and Gibson, 1985; Kostiw, 1979; Piron, 1986; Ryden et al., 1979; Sigvald, 1984). Once a plant is infected, the virus moves into the phloem and spreads throughout the plant including the tubers (deBokx and Huttinga, 1981) which may later be used as seed.

Several methods are used for managing PVY in production fields. Seed tubers for commercial potato production are commonly grown in a limited-generation certification scheme where restrictions are placed on how long (years or generations) seed may be certified based on the percentage of plants infected with viruses and other diseases (Franc, 2001; Gutbrod and Mosley, 2001). Pesticides are applied to control aphid populations and thereby limit the spread of viruses in seed and commercial production. Genetically resistant cultivars containing genes introgressed from wild relatives of potato provide a desirable alternative to chemical insecticides.

The cultivated potato originated in the Andes Mountains between Peru and Bolivia, but closely related species have been found from Chile to Central America, Mexico, and Northward into Utah (Bamberg and del Rio, 2005; Hawkes, 1994). Potatoes occur in a polyploidy series with a chromosome base number of 12 ranging from diploid to pentaploid. Most cultivated potatoes are tetraploid (*Solanum tuberosum* spp. *tuberosum*, 2n= 4x= 48 chromosomes) (Cribb and Hawkes, 1986; Rabinowitch and Levy, 2001). Improving the potato, especially quantitative and recessively controlled traits, is complicated by its tetrasomic inheritance (Rabinowitch and Levy, 2001). Tetraploid potato species exhibit five possible allelic configurations for a particular locus. The configurations could be AAAA (quadruplex), AAAa

(triplex), AAaa (duplex), Aaaa (simplex), and aaaa (nulliplex). In comparison, diplod species have three classes of genotypes, e.g. AA, Aa aa (Watanabe et al., 2005). The segregation ratio in autotetraploids may also be affected by the way quadrivalents are formed and random crossing over events due to the distance between the centromere and locus in question (Allard, 1960). Breeding cultivated tetraploid potatoes is further complicated by self-incompatibility and crossing barriers that inhibit gene introgression from wild *Solanum* species (Rabinowitch and Levy, 2001). Potatoes also suffer from strong inbreeding depression leading to a decline in overall fitness, loss of vigor, and malformed tubers (Watanabe et al., 2005). Therefore, development of a cultivated disease resistant potato is not a trivial task.

Different sources of germplasm, both wild and cultivated, provide resistance to PVY as shown in Table 1.1. Transgenic resistance is also available through genomic modification. The first transgenic PVY resistant potato was reported by Stark and Beachy, (1989). Currently, induced gene silencing or post-transcriptional gene silencing is a developing approach to developing virus resistance (Berger and German, 2001). However, the methodology of transgenic potatoes will remain only experimental until consumer acceptance is gained.

Natural virus resistance in potato germplasm is classified into three distinct groups: hypersensitivity, tolerance and extreme resistance. Hypersensitivity is a necrotic response to PVY infection and is regulated by *N* genes that provide resistance to specific strains (Barker, 1996). Field immunity where no virus spread occurs under field conditions is associated with the hypersensitive response (Swieżyński, 1994). Plants that carry a high concentration of PVY, but show little damage as a result, are

classified as tolerant (Swieżyński, 1994). Extreme resistance prevents PVY from replicating throughout the plant and is governed by *R* genes (Cockerham, 1970).

Genes conferring extreme resistance to PVY have been identified in *S. tuberosum* spp. *andigena* (Muñoz et al., 1975), *S. hougasii* (Cockerham, 1970) and *S. stoloniferum* (Cockerham 1970). Genetic mapping efforts located the extreme resistant Ry_{adg} gene (Ross, 1986) on chromosome XI (Hämäläinen et al. 1997). Also the gene Ry_{sto} from *S. stoloniferum* was mapped near Ry_{adg} on chromosome XI (Brigneti et al., 1997). The pedigree of the mapping population used for Ry_{sto} was considered unreliable (Gebhardt and Valkonen 2001). Also, some have questioned whether the Ry_{adg} gene was indeed derived from *S. stoloniferum* or from other unknown wild sources (Brown and Corsini, 2001). Despite all the questionable pedigree information, an effort has been made to develop several molecular markers linked to the Ry_{adg} and Ry_{sto} for Marker-Assisted Selection (MAS).

Using different germplasm derived from *Solanum stoloniferum,* the Ry_{sto} gene was remapped to chromosome XII using UBC 875_{980} with a linkage distance of 13.7 cM. (Flis et al., 2005). Using the Restriction Fragment Length Polymorphism (RFLP) probe, GP122 located on chromosome XII (Gebhardt et al. 2001) converted into a Cleaved Amplified Polymorphic sequence (CAPs) marker digested with *EcoRV.* It was discovered that GP122 was 1.2 cM from $Ry\text{-}f_{sto}$ (Flis et al., 2005). In another study (Song et al., 2005) the Ry_{sto} resistance was again localized on chromosome XII but in a different location than GP122. It was located between the probes GP268 and TG28. The PCR based primer STM0003 with a band of 111 bp was shown to co-

segregate with the extreme resistance for PVY from Ry_{sto} with a LOD threshold over 3.0 (Song et al. 2005)

The resistance from *S. tuberosum* spp. *andigena* was mapped utilizing the RFLP marker TG508, identified as tightly linked to the Ry_{adg} locus with an estimated map distance of 2.0 cM on chromosome XI (Hämäläinen et al., 1997). That marker was then used to aid in the development of the Resistant Gene-Like (RGL) DNA fragment, ADG2 (Hämäläinen et al., 1998). The ADG2 fragment was found to be 77% homologous to the gene N that provides resistance to tobacco mosaic virus in *Nicotiana glutinosa* and 53% homologous to RPP5 which confers resistance to *Peronospora parasitica* (Sorri et al., 1999). It was later discovered that the fragment ADG2 corresponds to the nucleotide-binding domain (NBS) characteristic of the class of *R* genes containing a C-proximal leucine-rich repeat (LRR) region (Ellis et al., 2000; Vidal et al., 2002). A gene *Y-1* was isolated and characterized from ADG2. When *Y-1* was transformed into potato plants, systematic PVY infection was reported (Vidal et al., 2002). Therefore, the *Y-1* gene and, Ry_{adg} are different but tightly linked. A Sequenced-Characterized Amplified Region (SCARs) (RYSC3) that covers the kinase motifs within ADG2 (Kasai et al., 2000) and a CAPS marker based on the ADG2 fragment (Sorri et al., 1999) have been developed for the *Y-1* gene. These markers could hasten the development of PVY resistant cultivars.

The development of a PVY resistant cultivar traditionally begins by crossing resistant lines with susceptible lines that have complementary qualities. After successful crossing, seedling tubers are produced from true potato seeds during the first year. The seedling tubers are field-planted as single hill units the second year.

Selections are made at this stage based on visual characteristics (mainly tuber size, shape and uniformity) at the end of the growing season. In the Oregon selection scheme (Figure 1.1), single-hill selections advance to four-hill observation plots at two field locations and are reselected for acceptable appearance. Selections from the four-hill plots are evaluated for virus resistance by mechanical inoculation with PVY during the winter. Susceptible clones are discarded and resistant clones are advanced to a preliminary trial to evaluate yield, appearance, and processing qualities. After data from the preliminary trial has been reviewed, the selections may be advanced into larger yield trials or used as recurrent parents.

Chapter 2 of this study demonstrates that markers linked to the Ry_{adg} gene (Kasai et al., 2000; Sorri et al., 1999) can be used for MAS for PVY resistance in potato breeding. In Chapter 3 we follow a similar approach using markers linked to the Ry_{sto} gene developed by Song et al. (2005). Additionally, we also screened advanced entries from the Tri-State, Western Regional and National Late Blight trials for PVY resistance using markers associated with the PVY extreme resistance provided by the Ry_{adg} and Ry_{sto} genes. General conclusions and recommendations for MAS for developing PVY resistant cultivars are presented in Chapter 4.

Table 1.1. Germplasm shown to be resistant to PVY, type of resistance, inheritance, gene symbol, and chromosome location. From Brown and Corsini (2001)

Germplasm source	Type of resistance	Inheritance, gene symbols	Chromosome location	Citations
S. stoloniferum	Extreme	Ry_{sto}	XII	(Cockerham, 1970)
S. tuberosum ssp. *andigena*	Extreme	Ry_{adg}	XI	(Muñoz et al., 1975)
S. hougasii	Extreme	Ry_{hou}	Not determined	(Cockerham, 1970)
S. phureja	Relative	Polygenic	Not determined	(Vallejo, 1995)
Series Etuberosa	Variable	Not determined	Not determined	(Valkonen, 1992)
S. demissum	Hypersensitivity	Ny_{dms}	Not determined	(Cockerham, 1970)
S. chacoense	Hypersensitivity	Ny_{chc}	Not determined	(Cockerham, 1970)
S. tuberosum	Hypersensitivity	Ny_{tbr}	IV	(Celabi-Topark et al., 2002)

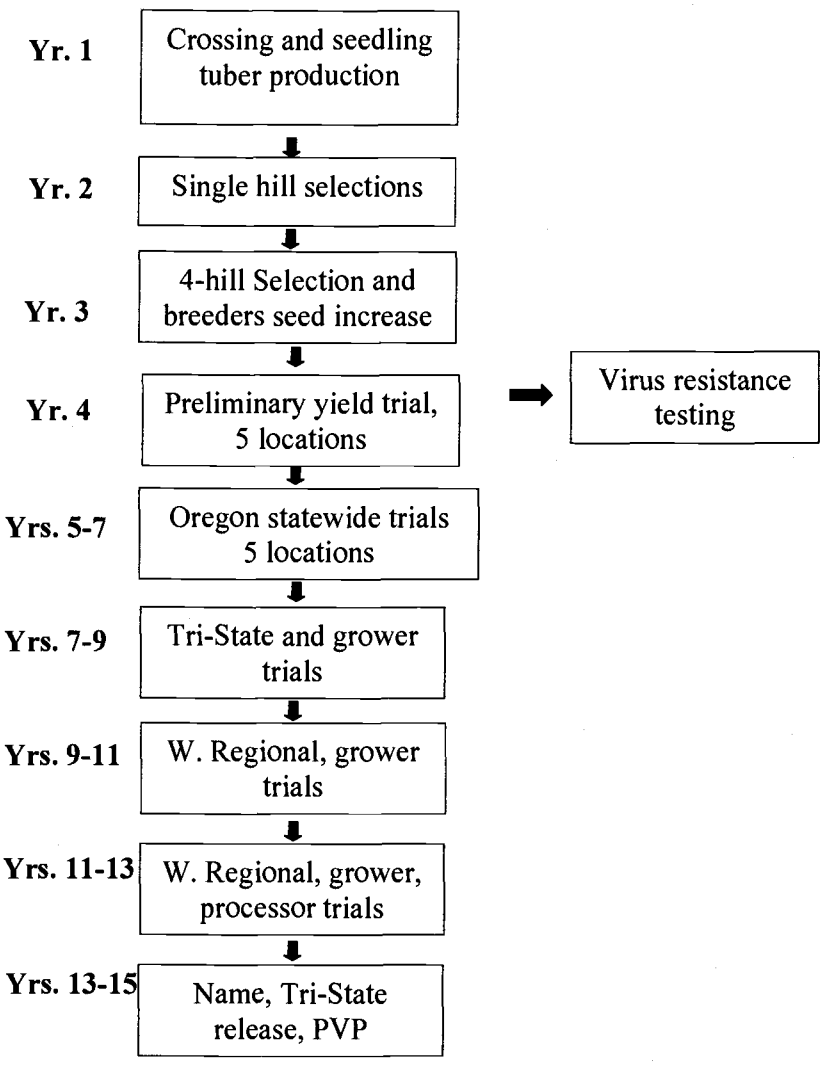

Figure 1.1. A traditional virus resistance potato breeding program from crossing to naming and release.

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Implementation of Marker-Assisted Selection (MAS) for PVY Resistance (Ry_{adg} gene) in a Potato Breeding Program

Chapter 2

Ryon J. Ottoman

Implementation of Marker-Assisted Selection (MAS) for PVY Resistance (Ry_{adg} gene) in a Potato Breeding Program.

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Abstract

Artificial inoculation under controlled conditions followed by ELISA is traditionally used to screen for PVY resistance. This method is very tedious and time consuming and prohibits screening of large segregating populations. Efforts have been made to identify and genetically map resistance to PVY. The gene Ry_{adg} from *S. tuberosum* ssp. *andigena* provides extreme resistance to PVY. This gene was mapped to chromosome XI and user-friendly PCR-based DNA markers have been developed. The objective of this work was to assess the usefulness of molecular markers linked to Ry_{adg} for determining the allelic configuration at the Ry_{adg} locus in the PVY resistant tetraploid potato clone OR00030-1 and as an early selection tool for predicting PVY resistance. To achieve this, a full-sib tetraploid population segregating for Ry_{adg} was artificially inoculated with PVY and evaluated for virus resistance using ELISA and screening with molecular markers RYSC3 and ADG *Bbv*I linked to Ry_{adg}. Ninety-six percent of the segregating lines for the Ry_{adg} gene showed coincidence between results for molecular markers and ELISA at 40 days after inoculations Ninety-six percent of the population screened with molecular markers were in agreement with the ELISA results at 40 days after inoculation. Discrepancies between marker and ELISA results could be caused by escapes from inoculation, errors in ELISA or PCR assays, recombination between the markers and the Ry_{adg} gene and/or time of evaluation. Segregation of the ELISA and molecular marker results in the full-sib population indicated the presence of Ry_{adg} as a simplex in the PVY resistant parent (OR00030-1). This information was taken into account for screening two full-sib segregating populations under field conditions. From 316 clones, nine (2.8%) were selected at the

single hill level based on visual tuber characteristics. Four (44.4%) contained markers associated with the PVY resistance gene Ry_{adg}. By using MAS for PVY resistance at the Ry_{adg} we reduced the number of PVY susceptible lines retained for succeeding field evaluations, and thereby increased the odds of generating PVY resistant potato varieties.

Keywords: Potatoes, PVY, Ry_{adg}, Extreme resistance, Marker-Assisted Selection, ELISA.

Introduction

Viruses cause serious problems in potato (*Solanum tuberosum* L.) production. Potato Virus Y (PVY) is a potyvirus and a pathogen of great concern to both commercial and seed potato growers. The vegetative propagation of potato seed enables systemic viruses to persist from one year to the next resulting in an overall decline in productivity. There are three common strains of PVY: Y^o, Y^n, and Y^c (Brunt, 2001). Recombinant strains have also been documented including PVY^{ntn}, PVY^nW, and $PVY^{n:o}$ (Chrzanowska, 1987; Kerlan et al., 1999; McDonald and Singh, 1996; Weidemann, 1988).

Potato virus Y is problematic because it is spread in a non-persistent manner by more than 30 species of aphids (deBokx and Piron, 1990; Harrington and Gibson, 1989; Harrington et al., 1986; Hoof, 1977; Hoof, 1980; Katis and Gibson, 1985; Kostiw, 1979; Piron, 1986; Ryden et al., 1979 Sigvald, 1984) and difficult to eradicate from seedlots. The expression of PVY infection includes vein necrosis, mottling, yellowing of leaflets, leaf-dropping, and premature plant death (deBokx and Huttinga, 1981) but symptom expression is highly cultivar specific. Depending on the cultivar, time of infection, and environment, yield losses associated with PVY range from 10-80% (deBokx and Huttinga, 1981; Rykbost et al., 1999). Once the plant is infected, the virus moves in the phloem and spreads throughout the plant including tubers held for seed (deBokx and Huttinga, 1981).

Methods for controlling the spread of PVY include both direct and indirect approaches. Direct PVY control is achieved by roguing infected plants and applying insecticides to control aphid populations. Indirect PVY control involves the use of

limited-generation potato seed production. Restrictions are placed on how long (generations or years) seed may be retained based on the percentage of plants infected with viruses and other pathogens (Franc, 2001; Gutbrod and Mosley, 2001). Despite the use of certified seed and chemical controls, PVY remains a serious problem. The development of PVY resistant cultivars is the most environmentally friendly and cost-effective solution to this problem.

Transgenic resistance to PVY is available for cultivar development (Berger and German, 2001; Stark and Beachy, 1989). But consumer rejection of transgenic potatoes makes this form of resistance a non-acceptable option at this time. Therefore virus resistance must be obtained by traditional breeding. There is a wide array of PVY resistant potato germplasm (Table 1.1) available for breeding purposes. Resistance can be classified into three distinct groups: Hypersensitivity, Tolerance, and Extreme resistance. Hypersensitivity is a necrotic response to PVY infection and is regulated by *N* genes that provide strain-specific resistance (Barker, 1996). Tolerance to PVY infection allows plants to carry a high concentration of virus but show little phenotypic damage (Swieżyński, 1994). Extreme resistance to all strains of PVY is provided by *R*-genes (Cockerham, 1970). Extreme resistance to PVY in potato has been identified in *S. stoloniferum, S. hougasii* and in *S. tuberosum* spp. *andigena*. The *S. tuberosum* spp. *andigena* resistant gene is referred to as Ry_{adg} (Ross, 1986) and is isolate non-specific to PVY infections (Mihovilovich et al., 1998).

Potatoes are autotetraploids (2n = 4x = 48), and the genetic inheritance is complex. There are five possible genotypes assuming a dominant phenotype such as Ry_{adg} RyRyRyRy (quadruplex), RyRyRyry (triplex), RyRyryry (duplex), Ryryryry

(simplex), and ryryryry (nulliplex). The segregation ratio is also influenced by gametic assortment during the first meiotic division. There are two possible models for random partitioning of chromatids: chromosome and chromatid assortment (Allard, 1960). The segregation for PVY resistance in progeny derived from the crossing of PVY resistant and susceptible parents depends on the allelic configuration of the resistant parent.

The traditional approach to classifying PVY resistance in potato plants involves artificial inoculation with the virus then testing with ELISA to confirm virus replication. Grafting and abrasion with carborundum dust are common methods for artificial inoculation of potatoes with PVY. Top grafting requires a PVY-infected scion to be grafted to the non-infected root stock for virus transmission. Using carborundum to infect plants with PVY involves lightly dusting plants then rubbing ground PVY leaf tissue on non-infected plant leaves. Both methods have drawbacks. Time required to successfully obtain results is excessive and there is a risk of spreading PVY to non-target susceptible elite breeding lines. To help facilitate the introgression of PVY resistant genes into breeding programs, molecular maps with markers-linked to PVY resistant traits have been developed for Marker-Assisted Selection (MAS) (Flis et al., 2005; Kasai et al., 2000; Sorri et al., 1999; Song et al., 2005; Vidal et al., 2002).

In an effort to maximize selection efficiency for genotypes resistant to PVY (Ry_{adg} source), research has been directed towards understanding the genetic resistance provided by Ry_{adg}. Utilizing Restriction Fragment Length Polymorphism (RFLP), marker TG508 was identified as tightly linked to the Ry_{adg} locus with an estimated

map distance of 2.0 cM. (Hämäläinen et al., 1997). That marker was then used to develop the Resistant Gene-Like (RGL) DNA fragment ADG2 which was located to a resistant gene family on chromosome XI (Hämäläinen et al., 1998). The ADG2 fragment was found to be 77% homologous to the *N* gene that provides resistance to tobacco mosaic virus in *Nicotiana glutinosa* and 53% homologous to RPP5 resistance to *Peronospora parasitica* (Sorri et al., 1999). It was later discovered that the fragment ADG2 corresponds to the nucleotide-binding domain (NBS) characteristic to the class of *R* genes containing a C-proximal leucine-rich repeat (LRR) region (Ellis et al., 2000; Vidal et al., 2002). The gene *Y-1* was isolated and characterized from ADG2. When *Y-1* was transformed into potato plants no significant resistance was observed, and systemic PVY infection was reported (Vidal et al., 2002). Therefore, gene *Y-1* and Ry_{adg} are different genes but tightly linked. Several PCR base and user friendly markers for the gene *Y-1* have been developed, (Kasai et al., 2000; Sorri et al., 1999). These markers are potential candidates for use in MAS for resistant varieties in potato breeding.

Artificial inoculation for screening PVY resistant clones is difficult and time-consuming, so PVY resistance is an ideal target for MAS. In this study we will examine the potential of using the markers RYSC3 and ADG2 to screen for PVY resistance.

The objectives of this research are: A) to determine the association of the markers RYSC3 and ADG2 *BbvI* with PVY resistant phenotypes based on ELISA and visual observations; B) to evaluate the application of MAS in an active breeding

program; and C) to screen advanced entries with markers linked to PVY resistance provided by Ry_{adg}.

Materials and Methods

The plant material used in this study included PVY resistant and susceptible potato clones, full-sib segregating populations (Table 2.1), and advanced breeding selections (Table 2.2).

OR00030-1 is a russet potato clone with good yield and quality potential (Table 2.3) and confirmed resistance to PVY. The initial cross was made in Oregon in 2000 (Figure 2.1) and it is available upon request (contact M.I Vales). OR00030-1 was artificially inoculated with PVY and found to be completely resistant based on ELISA and visual inspection. When tested with RYSC3 and ADG2 *Bbv*I (markers associated with the Ry_{adg} resistant gene), OR00030-1 presented alleles associated with resistance. AO95245-2 is a russet clone with good agronomic and processing quality and was confirmed to be susceptible to PVY based on artificial inoculations followed by ELISA and testing with RYSC3 and ADG2 *Bbv*I revealed alleles associated with PVY susceptibility. A93157-6LS is a russet clone with excellent yield and quality and low simple sugar content (more information can be found at http://www.ars.usda.gov/main/docs.htm?docid=3019). This line will be released during 2006 as 'Premier Russet'. No association with the Ry_{adg} gene was found in this line.

The full-sib segregating populations included one population for genetic studies (OR05030) and two populations for field selection (OR03145 and OR04155)

(Table 2.1). Crosses to generate these populations were made in 2005, 2003 and 2004, respectively in Corvallis, Oregon. The berries were harvested and seedling tubers were produced from the true potato seeds. The full-sib population OR05030 with 84 progeny was used for genetic studies and the corresponding parental lines were planted in greenhouses at Oregon State University in 4x4 inch plastic pots using SUN GRO™ professional blend media in a completely randomized block design with two replications. Greenhouse conditions were set at 18.3°C day and 15.5°C night and artificial light was provided for 16 hrs per day to extend the winter day length. Plants were watered and fertilized as needed. Plants were mechanically inoculated using verified PVY° (PVY) maintained in tobacco tissue that was kindly provided by James Crosslin (US Dept. of Agriculture/ARS, Prosser, WA). Two point five (2.5) grams of infected fresh tobacco tissue were ground in 25ml of cold 1mM potassium phosphate pH 8 virus buffer. Two young leaves of each plant which had previously been dusted with carborundum were lightly rubbed with cheesecloth dipped in virus buffer as shown in Figure 2.2. The inoculated leaves were marked with a hole punch and observed for virus symptoms. Visual PVY symptoms were divided into three severity classes: typical PVY virus expression, questionable PVY virus expression, and no virus expression. Typical PVY virus expression was classified as leaf mottling and vein burning. Plants with questionable virus expression displayed a slight mottling less distinct than classical PVY leaf mottling shown in Figure 2.3. Plants were evaluated at 20 and 40 days after inoculation for virus expression and ELISA.

The breeding populations OR03145 with 151 progeny and OR04155 with 165 progeny were planted as single hill units at the potato research center in Powell Butte,

Oregon, during the 2005-growing season. Seed pieces were spaced 0.91m between rows and 0.68m within rows. Standard production practices for Central Oregon were used throughout the growing season. Criteria for cultivar selection emphasized tuber size, shape and type. The selected material was stored at 3.3-4.4°C. A single apical eye was removed from each selection in early spring and planted in the greenhouse in Corvallis, Oregon. Greenhouse temperatures were held at 18.3°C day and 15.5°C night. Plants were watered as needed until large enough for DNA extraction.

Advanced breeding materials evaluated included clones from the 2005 Tri-State (Oregon, Washington, and Idaho) and Western Regional trials (Oregon, Washington, Idaho, Colorado, Texas and California) and clones included in the National Late Blight trials in Corvallis, Oregon, in 2005 (Table 2.2). Selections from the Tri-State and Western Regional trials were screened for field resistance to PVY at the Hermiston Agricultural Research and Extension Center. The entries were planted in two ten-hill plots randomized and replicated four times. Each plot was bordered by a spreader row of seedborne PVY-infected plants and cultural management was conducive for aphid buildup. At harvest the plots were lifted and 12 tubers were randomly selected from each plot. Two tubers were sprouted and tested for the presence of PVY using ELISA.

Potato Virus Y resistance was determined using Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) with PVY^{o-n} polyclonal antibodies (AGDIA Company, Elkhart, IN). The third petiole from artificially inoculated plants was collected and ground in a buffer ratio of 1:10 (50mg tissue: 500µl grinding buffer) using a Qiagen/Retsch MM 300 mixer mill (Qiagen Inc, Valencia, CA.) The assay was conducted using a modified method prescribed by AGDIA. Two negatives and two positive controls were used in each 96-well ELISA plate. Absorbance values were measured at 405nm (A_{405}nm) using a VERSAmax microplate reader (Molecular Devices Sunnyvale, CA.). The resistance threshold cutoff was set at absorbance levels two times greater than the mean for negative controls (Sutula et al., 1986).

Genomic DNA was extracted from 30-50mg of young leaf tissue. The leaf samples were cut into four smaller pieces and placed in Qiagen collection tubes (Qiagen Inc, Valencia, CA) and stored at -80°C until DNA was extracted. The tissue was ground using a Qiagen/Retsch MM 300 mixer mill (Qiagen Inc, Valencia, CA.) DNA was isolated as described by (Riera-Lizarazu et al., 2000). DNA concentration and quality were determined on a 1% agarose gel by comparison with lambda DNA of known concentration.

Polymerase Chain Reaction (PCR) amplification of markers linked to Ry_{adg} was carried out using a Techne thermalcycler (Techne Inc, Burlington NJ.) with primers developed by Kasai et al., (2000) and Sorri et al., (1999) (Table 2.4). Each reaction contained 0.03 U/µl of Taq polymerase, 1 X Taq buffer (Qiagen Inc, Valencia, CA), 2% sucrose in 0.04% cresol red, 0.1 mM of each deoxynuleotide, 0.5 µM of each primer and 10 ng template DNA. Reaction controls included water and

DNA from a clone (AO88266-2) known to contain alleles linked to Ry_{adg}. The PCR reaction volume was 10 µl for the marker RYSC3. The PCR program consisted of an initial denaturation step at 93°C for 9 min., followed by 35 cycles of denaturation at 94°C for 45s, primer annealing at 60°C for 45s, and extension at 72°C for 60s, followed by a final extension at 72°C for 5 min. PCR products were checked in a 2% agarose gel in 0.5 X TBE and scored as a dominant marker. Presence of a 321 base pair (bp) band was associated with PVY resistance from Ry_{adg} and absence of the band indicated association with susceptibility to PVY as in Kasai et al. (2000). The reaction volume for the ADG2 marker was 20µl. The PCR consisted of an initial denaturation step at 93° C for 2 min., followed by 35 cycles of denaturation at 93°C for 45s, primer annealing at 45°C for 45s, and primer extension at 72°C for 60s, followed by a final extension at 72°C for 5min. PCR product was checked in a 2% agarose gel in 0.5 X TBE. The PCR products of ADG2 were digested in a reaction volume of 12 µl with 126 ng of PCR product, 0.1 U/µl *BbV*I enzyme, 10 X enzyme buffer (Fermentas). Samples were digested at 65°C for 3 hours and products were then visualized on a 2% agarose gel in 0.5 X TBE and scored as a co-dominant marker. Presence of an undigested product of 355 bp was associated with the PVY resistance gene Ry_{adg}. Presence of two digested products of 270 bp and 85 bp were associated with PVY susceptibility as in Sorri et al. (1999)

The ELISA results obtained 20 and at 40 days after artificial inoculation with PVY were tested for normality using the PROC UNIVARIATE statement of SAS (SAS Institute, 2001). Chi-square tests for homogeneity were conducted to compare the ELISA results and visual PVY symptom scores (phenotype) between 20 and 40

days after inoculation. Chi-square tests for a fixed ratio were used to fit the marker and ELISA scores to the segregation of a single dominant resistance allele (simplex) in a tetraploid with tetrasomic inheritance under chromosome (1:1) or chromatid (0.87:1) assortment models (Allard, 1960). Regressions were performed using the PROC REG statements of SAS (SAS Institute, 2001) to predict PVY visual symptoms scores or ELISA results at 20 and 40 days based on the marker scores.

Results and Discussion:

The parental lines from the full-sib population of OR05030, OR00030-1 and AO95245-2 were evaluated at the genotypic level with markers associated with Ry_{adg}, RYSC3 and ADG2 *Bbv*I. OR00030-1 had patterns associated with PVY resistance (RYSC3: 321 bp band; ADG2 *Bbv*I: 355 bp band) and AO95245-2 had patterns associated with PVY susceptibility (RYSC3: no band; ADG2 *Bbv*I: 270 bp and 85 bp bands) as shown in Figures 2.4 and 2.5. These were expected patterns based on Kasai et al. (2000) and Sorri et al. (1999). At 20 days after inoculation with PVY^{O} both parental lines, OR00030-1 and AO95245-2, displayed ELISA A_{405}nm values within the PVY resistant class as shown in Figure 2.6. At the phenotypic level, OR00030-1 and AO95245-2 showed no symptoms of PVY infection at 20 days after inoculation. At 40 days after inoculation (Figure 2.7) the A_{405}nm value showed that OR00030-1 was resistant and AO95245-2 was susceptible to PVY. The phenotypic evaluations at 40 days after inoculation for AO95245-2 showed some light mottling but the symptoms were less severe than classical PVY symptom expression. The resistant clone OR00030-1 appeared to be completely healthy at 40 days after inoculation. Based on these results we recommend that ELISA tests be preformed at 40+ days after inoculation

Of the 84 plants in full-sib family OR05030 (Table 2.5) screened with the marker RYSC3, 36 contained a 321 bp band associated with resistance to PVY. No PCR amplification associated with PVY susceptibility was noted in the remaining 48 plants. Screening of the full-sib population with ADG2 *Bbv*I provided the same results: 36 plants contained a pattern associated with PVY resistance (an undigested

product of 355 bp in the case of ADG2 *Bbv*I) and 48 plants contained a pattern associated with PVY susceptibility (270 bp and 85 bp bands resulting from the digestion of the PCR product ADG2 with the enzyme *Bbv*I). The observed population segregation for the markers RYSC3 and ADG2 *Bbv*I fit the expected ratio for a single dominant gene. No significant deviations from a 1:1 (chromosome assortment) or 0.87:1 (chromatid assortment) were observed (Table 2.6). This provides convincing evidence that OR00030-1, the PVY resistant parent, has simplex allelic configuration for the gene Ry_{adg} as shown by markers linked to the trait. The use of the co-dominant marker ADG2 *Bbv*I was a good complement to confirm results from the dominant marker RYSC3 in which absence of a band indicates association with PVY susceptibility. To differentiate between PCR failure and recombination between the marker and trait, it is advisable to use two markers flanking the region where the gene/QTL for the trait of interest is located.

ELISA results for the population OR05030 at 20 and 40 days after inoculations (Figures 2.6 and 2.7) showed a bi-modal distribution. A Chi-square test of homogeneity between the numbers of resistant and susceptible plants was conducted to compare ELISA and visual symptom (phenotypic) scores obtained at 20 and 40 days after inoculation. ELISA scores obtained at 20 and 40 days after inoculation were significantly different (χ^2=5.357, P=0.02). The same result was observed when visual symptom scores obtained at 20 and 40 days after inoculation were compared (χ^2=9.636, P=0.002).

Regression analysis between the markers (RYSC3 or ADG2 *Bdv*I) linked to the Ry_{adg} gene, and the ELISA, and visual virus expression scores at 20 and 40 days

after inoculation with PVY are shown in Table 2.7. At 20 days after inoculation, the resistant allele for marker RYSC3 (same for ADG2 *Bbv*I) explained 45% of the phenotypic variation that was observed by ELISA and 29% of the variation observed by visual observation of the symptoms. Twenty eight percent (14/49) of the lines shown to be resistant by ELISA (class X_1 at 20 DAI) were found to be susceptible using molecular markers. Further, 2.9% (1/35) of the plants shown to be susceptible based on ELISA (class X_0 at 20 DAI) were scored as resistant based on molecular marker results. Of the lines declared resistant to PVY based on visual observations (class E_1 at 20 DAI), 33% (9/27) were scored as susceptible by the marker. Conversely 4.5% (1/22) expressed typical PVY mottling (class E_0 at 20 DAI) but were scored resistant by the marker. At 40 days after inoculations, the resistant allele of the marker RYSC3 (same for ADG2 *Bbv*I) explained a larger R^2 (89%). Only 2.9% (1/35) of the plants scored as resistant by ELISA (class X_1 at 40 DAI) were scored susceptible by the markers. Four percent (2/49) of the plants classified susceptible by ELISA (class X_0 at 40 DAI) were scored as resistant based on molecular marker results. Overall, at 40 days after inoculation with PVY, a 96.4% (81 of 84 plants) match (X_1, N_1 and X_0, N_0) was observed between the marker RYSC3 (same for ADG2 *Bbv*I) and ELISA scores (Table 2.5). The discrepancies between marker and ELISA results may be due to escapes from inoculation, errors in ELISA or PCR assays, recombination between the markers and the Ry_{adg} gene and/or effects of time of evaluation. At 40 days after inoculation the marker results for RYSC3 and ADG2 cut with *Bbv*I explained 85% of the phenotypic variation observed based on visual evaluation of PVY symptoms, similar to the R^2 for ELISA. The discrepancy rate (E_1

N_0 or E_0 N_1) between visual observation of symptoms and molecular markers at 40 DAI is relatively low (2.2%, 1/45) and the correlation between results for ELISA and visual observations was very high (0.99), much higher than at 20 DAI. ELISA is a more reliable method for evaluating resistance to PVY than visual observation because a relatively large number of individuals (24%) presented questionable symptoms and were not assigned to either the resistant or susceptible classes based on visual observations of PVY symptoms.

In MAS procedures, results for classifying a selection as resistant or susceptible may be obtained when plant tissue is available for DNA extraction. Conventional screening methods using artificial inoculations methods followed by ELISA testing consume valuable resources and time. Furthermore, the methods are more variable than MAS. Our results show that MAS for PVY resistant material has the potential to expedite the development of PVY resistant cultivars.

To demonstrate the use of MAS in a tetraploid potato breeding program for PVY resistant material, two full-sib families derived from crosses OR03145 and OR04155 were selected for testing (Table 2.8). The male parent OR00030-1 provided PVY resistance in both families. It was determined that OR00030-1 has the Ry_{adg} allelic configuration of a simplex, (Table 2.6) and markers (Kasai et al., 2000; Sorri et al., 1999) identify resistant and susceptible lines with 96% accuracy in the experimental full-sib population OR05030 evaluated at 40 DAI. The female parental lines were AO95245-2 and A93157-6LS (Premier Russet), respectively. A total of 316 seedling tubers were planted as single hill units at Powell Butte for field selection based on tuber shape and type. The criteria for field selection were very stringent,

taking into account the simplex configuration of the resistant parent. The probability of selecting a resistant progeny is 50%. Therefore, a minimum of four clones were selected in OR03145 and five were selected in family OR04155, representing a 2.8% selection rate on average. Screening of the selected lines with markers RYSC3 and ADG2 *Bbv*I indicated that four out of nine selections (44.4%) contained alleles associated with PVY resistance (RYSC3: 321 bp band; ADG2 *Bbv*I: 355 bp resistant band) (Table 2.8). These results are in agreement with expected segregation of PVY resistance from a simplex parent. The clones selected based on visual tuber observations and confirmed to contain alleles associated with PVY resistance are pictured (Figures 2.8, 2.9, 2.10, 2.11). These selections will be evaluated in the field in unreplicated four-hill plots in 2006. Depending on tuber appearance and preliminary quality evaluations of the selections, they will be retained for further yield and quality evaluations in the Oregon breeding program as potential cultivars. Those not meeting the quality expectations of the Tri-State breeding program (Oregon, Idaho and Washington) could be re-used as recurrent parents.

In our proposed MAS program for PVY resistance (Figure 2.12), full-sib lines derived from crossing susceptible and resistant PVY parents will be screened first as single hills and selected based on tuber type and shape. The selected lines will be screened with molecular markers associated with PVY resistance and only the lines containing alleles associated with PVY resistance will continue in the program. Using MAS for PVY resistance at the Ry_{adg}, the number of PVY susceptible lines that move to the next years of field evaluations will be reduced thereby increasing the odds of generating PVY resistant potato varieties. Evaluations of the selected lines under

artificial inoculation and/or under field conditions with high PVY pressure should be performed to confirm molecular marker results. If the initial PVY resistant parent had a single (simplex) dominant gene for extreme resistance, at least four lines should be picked from each cross to maximize the chances of getting at least one resistant clone. The use of PVY resistant parental lines with multiple copies (duplex, triplex or quadruplex) of the PVY resistant gene to improve the chances of obtaining PVY resistant lines within the full-sib progeny is desirable. In addition, the pyramiding of several genes for resistance to PVY (for example Ry_{adg} and Ry_{sto}) in a parental line would also greatly enhance the development of PVY resistance. Marker-assisted selection would provide the only means for tracking the presence or both major genes conferring extreme resistance to PVY. Further pyramiding with other disease/pest/quality genes or QTL would be highly advantageous.. This has not yet been explored in great depth in tetraploid potato breeding programs, but initial attempts using major gene-mediated pathogen resistance have demonstrated feasibility (Gebhardt et al., 2006)

In an effort to determine if the Ry_{adg} source of PVY resistance was already present in advanced tetraploid breeding germplasm, 72 advanced lines (Table 2.2) from potato breeding programs across the USA were evaluated for the presence of molecular markers RYSC3 and ADG2 *Bbv*I. Fifty eight of the 72 lines (Table 2.9) were also tested for field resistance under high PVY pressure at Hermiston Oregon. A93157-6LS and A92294-6 showed no PVY infection under field conditions. Six advanced selections showed moderate resistance to PVY (25% infection) while all other entries showed much higher infection levels. When these entries were screened

with markers associated with the Ry_{adg}, only one entry, LBR2, showed alleles associated with PVY resistance at both markers. BO718-3 and EGA97061-4 showed a pattern for ADG2 *Bbv*I associated with PVY resistance, but did not show the allele associated with PVY resistance at the RYSC3. These entries must be tested by artificial inoculation to confirm PVY resistance. Since the markers linked to Ry_{adg} could not be used to explain field resistance to PVY observed under natural infection, it will be necessary to screen the same materials for other sources of resistance. Extreme resistance to PVY could also be derived from *S. stoloniferum*. Since the Ry_{adg} source of resistance evaluated in this study is not present in Northwest advanced breeding materials, the inclusion of this type of resistance from parental lines such as OR00030-1 will contribute significantly to our breeding programs. This clone also brings good yield and quality characteristics in addition to PVY resistance.

Table 2.1. Full-sib families derived from crosses involving the PVY resistant clone OR00030-1.

	Parental lines[a]		
Family	Female	Male	Number of individuals
OR05030	*OR00030-1*	AO95245-2	84
OR03145	AO95245-2	*OR00030-1*	151
OR04155	A93157-6LS	*OR00030-1*	165

[a] Italic font indicates the PVY resistant potato clone containing the Ry_{adg} gene

Table 2.2. Elite potato breeding material planted in 2005 from the Tri-State, Western Regional and National Late Blight trials.

Potato clone[a]	Potato clone[a]	Potato clone[a]	Potato clone[b]
Russet Burbank	MWTX2609-2Ru	CO94165-3P/P	A9520-45
Ranger Russet	PA97B3-2	Yukon Gold	A96517-2
Russet Norkotah	TXA549-1Ru	A95074-6	AND9552-7
A96023-6	Atlantic	BTX1544-2W/Y	AWN86514-2
A96108-12	Chipeta	CO94157-2W/Y	BO692-4
A97142-3	Ivory Crisp	NDA5507-3Y/F	BO718-3
A97229-1	A91814-5	VC1002-3W/Y	BO767-2
A97287-6	BO766-3T	VC1009-1W/Y	EGA97061-4
A98104-4	CO95051-7W	VC1123-2W/Y	LBR1R2R3R4
AO96141-3	COA96141-2C	NY126W/Y	LBR2
AO98133-2	COA96142-3C	CO94157-2W/Y	LBR3 tbr
A92030-5	CO96141-4W	AOTX98137-1Ru	LBR5
A92294-6	Willamette	CO97043-14W	LBR8
A93157-6LS	DK Red Norland	AC97097-14W	MSI152-4
A95109-1	Red LaSoda	CO97233-3R/Y	OR00030-1
A95409-1	A96741-1R	CO97137-1W	AO95245-2
A96095-3	A96741-2R	CO97226-2R/R	
A96104-2	VC1075-1R	AC96052-1Ru	
AO96160-3	CO97232-1R/Y	CO97232-2R/Y	
AO96164-1	Modoc	TXDH-99-1Ru	
AOA95154-1	VCO967-2R/Y	CO97065-7W	
AOA95155-7	VC1015-7R/Y	AOTX95265-2Ru	
ATX91137-1Ru	CO94183-1R/R	AC97521-1R/Y	
CO94035-15Ru	PA99P20-2	CO97078-5R	
CO95086-8Ru	POR01PG20-12	AOTX95265-4Ru	
CO95172-3Ru	All Blue	MWTX2609-4Ru	

[a] Advanced potato clones from the 2005 Tri-State (Oregon, Washington and Idaho, USA) and Western Regional (Oregon, Washington, Idaho, Colorado and Texas, USA) trials

[b] Potato clones included in the 2005 US National Late Blight trials

Table 2.3. Average agronomic, morphological and tuber quality traits for the PVY resistant clone OR00030-1 and Russet Burbank in two trials in Hermiston and Madras, Oregon, in 2004.

Trait	OR00030-1	Russet Burbank
Yield total (Mg ha^{-1})	113.7	85.8
U.S. No. 1 yield (Mg ha^{-1})	95.8	49.2
Skin type, Russetting[a]	2.7	3.4
Average tuber size (g)	281.2	227.2
Tuber length to width ratio	1.7	1.65
Specific gravity[b]	1.076	1.078

[a] 1: no russeting, 3: medium, 5: heavy russeting
[b] Air/water method

Table 2.4. Forward and reverse primer sequences for the RYSC3 and ADG2 DNA-based markers, annealing temperatures, PCR product sizes and chromosome location. ADG2 was digested with *Bbv*I.

Marker[a]	Primer	Forward and reverse primer sequences (5'-3')	Ta	Digestion enzyme	Product sizes (bp)[b]	Chromosome	Reference
RYSC3	3.3.3s	ATACACTCATCTAAATTTGATGG	60^0C	None	(R) 321	XI	(Kasai et al., 2000)
	ADG23R	AGGATATACGGCATCATTTTTCCGA			(S) absent		
ADG2 *Bbv*I	ADG2-F	ATACTCTCATCTAAATTTGATGG	45^0C	*Bbv*I	(R) 355	XI	(Sorri et al., 1999)
	ADG2-R	ACTGAACAGCATCATGTTCAAG			(S) 270		

[a] Names used in this study
[b] R: band associated with PVY resistance, S: band associated with PVY susceptibility

Table 2.5. Segregation of the OR05030 family for resistance to PVY based on DNA-based marker evaluation and ELISA readings at 20 and 40 days after inoculation with PVY[o].

	No. of plants per genotypic class[a]		ELISA[b] 20 day		ELISA[b] 40 days	
	N_1	N_0	X_1[c]	X_0[c]	X_1[c]	X_0[c]
RYSC3	36	48	49	35	35	49
ADG2 *Bbv*I	36	48	49	35	35	49

[a] Genotypic classes: N_1: allele associated with resistance, N_0: allele associated with susceptibility

[b] ELISA: Enzyme-Linked Immunosorbent Assay

[c] Two phenotypes are distinguished by absence (X_1) or presence (X_0) of virus detected by ELISA

Table 2.6. Chi-square test to fit fixed segregation ratios in the full-sib population OR05030 based on ELISA results at 20 and 40 days after inoculations visual observations and molecular markers associated with Ry_{adg}.

Trait	Time (DAI)	Segregation ratio (Resistant: Susceptible)	Type of segregation assortment	χ^2	*P*-value
ELISA	20	1:1	Chromosome	2.330	0.126
		0.87:1	Chromatid	9.211	0.002
Phenotype	20	1:1	Chromosome	0.510	.475
		0.87:1	Chromatid	2.698	0.1
ELISA	40	1:1	Chromosome	2.333	0.126
		0.87:1	Chromatid	0.193	0.660
Phenotype	40	1:1	Chromosome	11.57	0.001
		0.87:1	Chromatid	5.7	0.016
RYSC3		1:1	Chromosome	1.714	0.190
		0.87:1	Chromatid	0.048	0.826
ADG2 *Bbv*I		1:1	Chromosome	1.714	0.190
		0.87:1	Chromatid	0.048	0.826

Table 2.7. Comparison of molecular marker, ELISA and visual determination of PVY presence at 20 and 40 days after inoculation (DAI). Bold font indicates matches between the molecular marker and testing procedure

Test	Time (DAI)	Class[a]	Genotypic class[b]		Regression equation	R^2
			N_1	N_0		
ELISA	20	X_1	**35**	14	Y= 0.27 + 0.69X	0.45
		X_0	1	**34**		
Phenotype	20	E_1	**18**	9	Y= 0.43 + 0.49X	0.29
		E_0	1	**21**		
		$E_?$	17	18		
ELISA	40	X_1	**34**	1	Y= 0.23 + 0.94X	0.89
		X_0	2	**47**		
Phenotype	40	E_1	**17**	1	Y= 0.03 + 0.91X	0.85
		E_0	1	**44**		
		$E_?$	18	3		

[a] Classes are distinguished by absence (X_1) or presence (X_0) of virus detected by ELISA or by visual observation of virus symptom expression (E_1: no PVY symptoms, E_0: PVY symptoms, $E_?$: questionable symptoms)

[b] Genotypic classes: N_1: allele associated with resistance, N_0: allele associated with susceptibility. Based on molecular marker screening with RYSC3 and ADG2 *Bbv*I.

Table 2.8. Full-sib individuals evaluated under field conditions as single hills in Powell Butte, OR, in 2005 and numbers of selections containing markers associated with resistance to PVY (Ry_{adg}).

Family	Number of individuals	Number of selections[a]	Number of selections containing RYSC3 and ADG2 *Bbv*I resistant alleles[b]
OR03145	151	4	2
OR04155	165	5	2
Total	*316*	*9 (2.8%)*	*4 (44.4%)*

[a] Single-hill selections based on tuber appearance
[b] Alleles associated with PVY resistance

Table 2.9. Screening of potato breeding material for alleles linked to the Ry_{adg} gene and PVY resistance under field conditions and high PVY pressure in Hermiston, Oregon in 2005.

	Marker[a]		PVY evaluations based on ELISA	
Potato clone	RYSC3	ADG2 *Bbv*I	No. of plants susc/total[b]	% infected[c]
Russet Burbank	-	-	7/7	100%
Ranger Russet	-	-	7/7	100%
Russet Norkotah	-	-	7/8	87%
A96023-6	-	-	6/8	75%
A96108-12	-	-	8/8	100%
A97142-3	-	-	8/8	100%
A97229-1	-	-	8/8	100%
A97287-6	-	-	8/8	100%
A98104-4	-	-	3/8	37%
AO96141-3	-	-	5/8	63%
AO98133-2	-	-	7/8	88%
A92030-5	-	-	6/8	75%
A92294-6	-	-	0/6	0
A93157-6LS	-	-	0/7	0
A95109-1	-	-	4/6	67%
A95409-1	-	-	7/8	88%
A96095-3	-	-	6/8	75%
A96104-2	-	-	7/8	88%
AO96160-3	-	-	4/8	50%
AO96164-1	-	-	4/8	50%
AOA95154-1	-	-	2/8	25%
AOA95155-7	-	-	2/7	29%
ATX91137-1Ru	-	-	4/6	67%
CO94035-15Ru	-	-	5/8	63%
CO95086-8Ru	-	-	8/8	100%
CO95172-3Ru	-	-	5/8	63%
MWTX2609-2Ru	-	-	8/8	100%
PA97B3-2	-	-	8/8	100%
TXA549-1Ru	-	-	8/8	100%
Atlantic	-	-	8/8	100%
Chipeta	-	-	6/8	75%
Ivory Crisp	-	-	5/8	63%
A91814-5	-	-	4/8	50%
BO766-3T	-	-	5/8	63%
CO95051-7W	-	-	3/8	37%

[a] '-' allele associated with resistance, '+': allele associated with susceptibility
[b] Number of susceptible plants/total number of individuals tested per plot.
NT: not tested

Table 2.9. Continued

	Marker[a]		PVY evaluations based on ELISA	
Potato clone	RYSC3	ADG2 *Bbv*I	No. of plants susc/total[b]	% infected[c]
COA96141-2C	-	-	6/7	86%
COA96142-3C	-	-	3/8	38%
CO96141-4W	-	-	5/7	71%
Willamette	-	-	NT	NT
DK Red Norland	-	-	3/8	38%
Red LaSoda	-	-	7/8	88%
A96741-1R	-	-	8/8	100%
A96741-2R	-	-	8/8	100%
VC1075-1R	-	-	4/8	50%
CO97232-1R/Y	-	-	NT	NT
Modoc	-	-	NT	NT
VC0967-2R/Y	-	-	5/8	63%
VC1015-7R/Y	-	-	8/8	100%
CO94183-1R/R	-	-	2/8	25%
PA99P20-2	-	-	5/8	63%
POR01PG20-12	-	-	8/8	100%
All Blue	-	-	2/8	25%
CO94165-3P/P	-	-	2/8	25%
Yukon Gold	-	-	6/8	75%
A95074-6	-	-	4/8	50%
BTX1544-2W/Y	-	-	7/8	88%
CO94157-2W/Y	-	-	7/7	100%
NDA5507-3Y/F	-	-	1/8	13%
VC1002-3W/Y	-	-	2/8	25%
VC1009-1W/Y	-	-	8/8	100%
VC1123-2W/Y	-	-	4/8	50%
NY126W/Y	-	-	6/8	75%
CO94157-2W/Y	-	-	NT	NT
AOTX98137-1Ru	-	-	NT	NT
CO97043-14W	-	-	NT	NT
AC97097-14W	-	-	NT	NT
CO97233-3R/Y	-	-	NT	NT
CO97137-1W	-	-	NT	NT

[a] '-'allele associated with resistance, '+': allele associated with susceptibility

[b] Number of susceptible plants/total number of individuals tested per plot.

NT: not tested

Table 2.9. Continued

	Marker[a]		PVY evaluations based on ELISA	
Potato clone	RYSC3	ADG2 *Bbv*I	No. of plants susc/total[b]	% infected [c]
CO97226-2R/R	-	-	NT	NT
AC96052-1Ru	-	-	NT	NT
CO97232-2R/Y	-	-	NT	NT
TXDH99-1Ru	-	-	NT	NT
CO97065-7W	-	-	NT	NT
AOTX95265-2Ru	-	-	NT	NT
AO97521-1R/Y	-	-	NT	NT
CO97078-5R	-	-	NT	NT
AOTX95265-4Ru	-	-	NT	NT
MWTX2609-4Ru	-	-	NT	NT
A9520-45	-	-	NT	NT
A96517-2	-	-	NT	NT
AND9552-7	-	-	NT	NT
AWN86514-2	-	-	NT	NT
BO692-4	-	-	NT	NT
BO718-3	-	+	NT	NT
BO767-2	-	-	NT	NT
EGA970614	-	+	NT	NT
LBR1R2R3R4	-	-	NT	NT
LBR2	+	+	NT	NT
LBR3 tbr	-	-	NT	NT
LBR5	-	-	NT	NT
LBR8	-	-	NT	NT
MSI152-4	-	-	NT	NT
AO95245-2	-	-	NT	NT
OR00030-1	+	+		

[a] '-' allele associated with resistance, '+': allele associated with susceptibility
[b] Number of susceptible plants/total number of individuals tested per plot.
NT: not tested

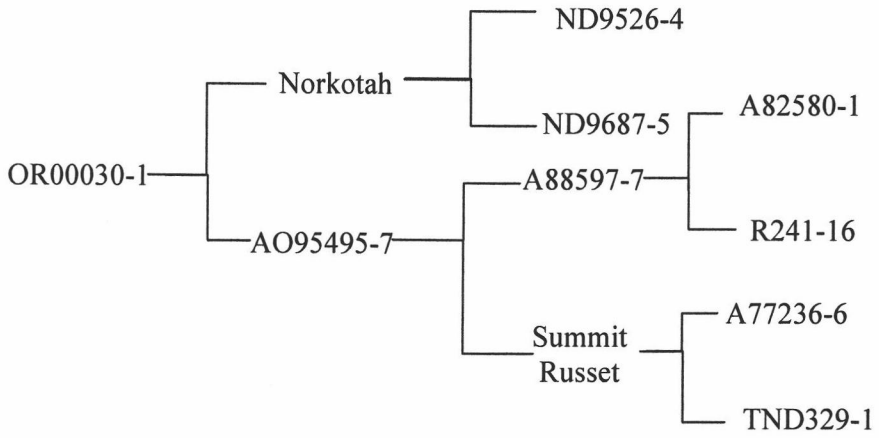

Figure 2.1. Pedigree of the PVY resistant clone OR00030-1

Figure 2.2. Mechanical inoculation of the full-sib population OR05030 with PVY^{o} using carborundum.

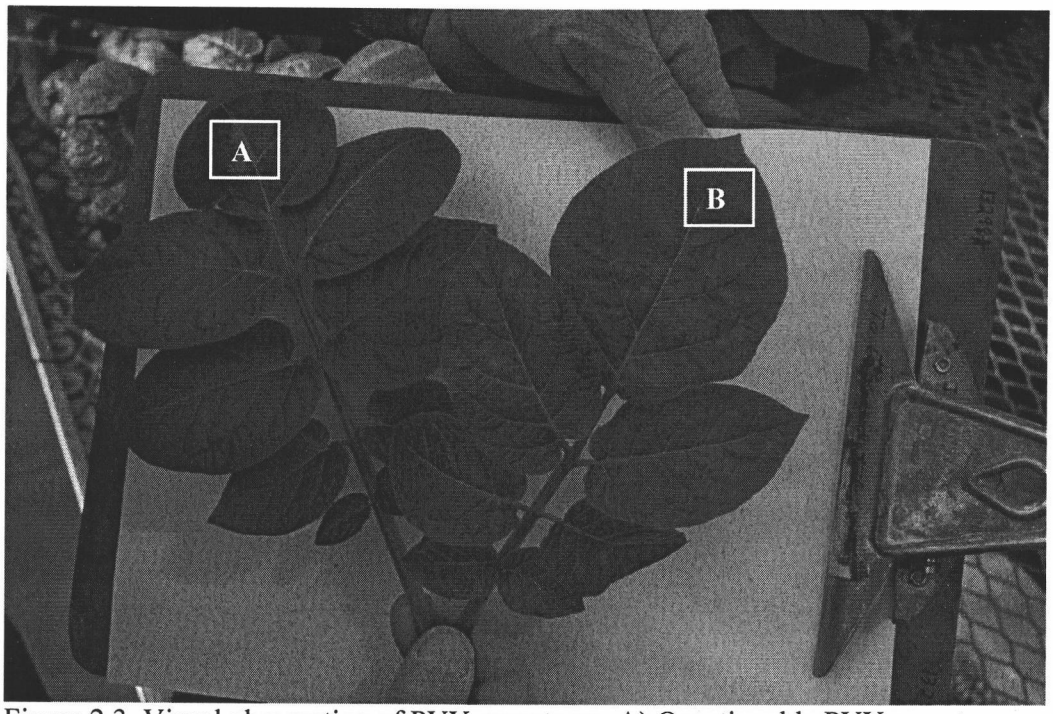

Figure 2.3. Visual observation of PVY symptoms. A) Questionable PVY symptom expression, B) Classical PVY symptom expression.

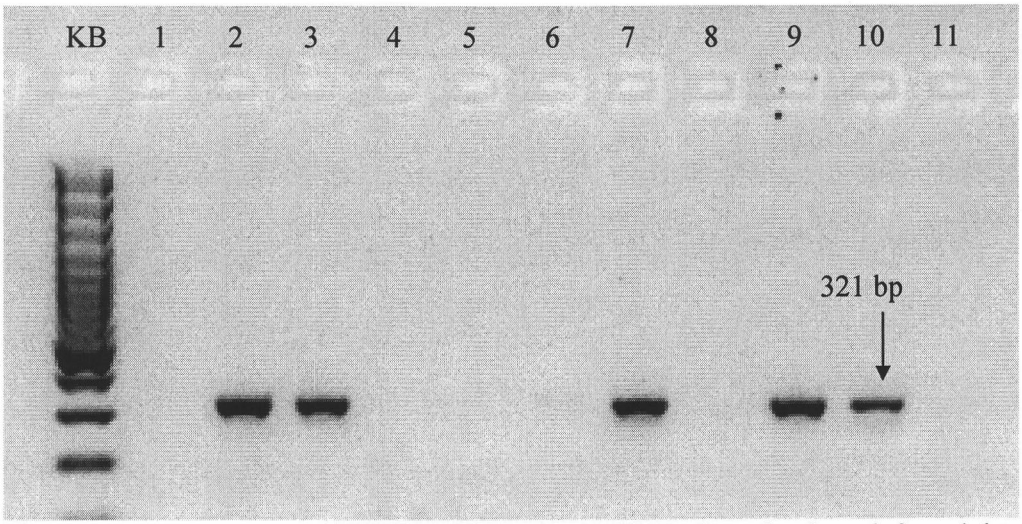

Figure 2.4. Amplification products with marker RYSC3. Samples from left to right: KB: 100 bp ladder, 1: water control, 2: PVY resistant control (AO88628-2), 3: OR00030-1 (PVY resistant parental clone), 4: AO95245-2 (PVY susceptible parental clone), 5-11: subset of the OR05030 population. Presence of a 321 bp product indicates PVY resistance. Absence of the band indicates PVY susceptibility.

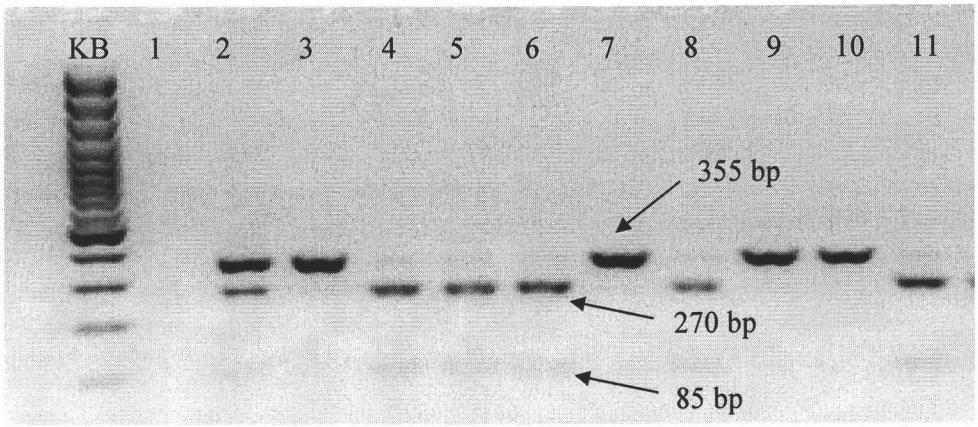

Figure 2.5. Restriction products of marker ADG2 cut with *Bbv*I. Samples from left to right: KB: 100 bp ladder, 1: water control, 2: PVY resistant control (AO88628-2), 3: OR00030-1 (PVY resistant clone), 4: AO95245-2 (PVY susceptible clone), 5-11: subset of the OR05030 population. Presence of an uncut product of 355 bp indicates PVY resistance. Successful digestion resulted in two bands, 270 bp and 85 bp indicating PVY susceptibility.

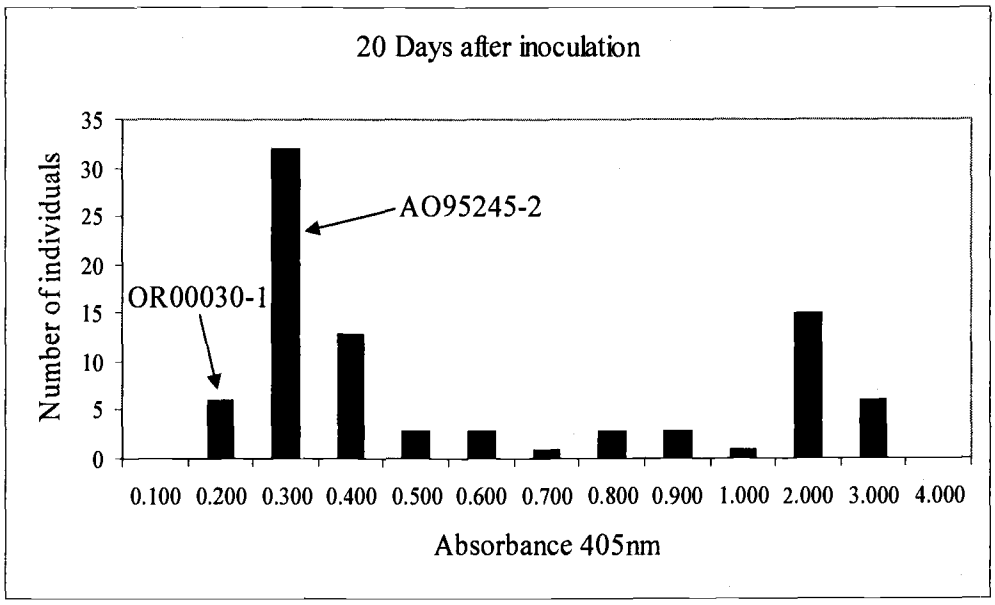

Figure 2.6. Distribution of A405 values for the full-sib population OR05030 at 20 days after inoculation. OR00030-1 (PVY resistant parent, A405: 0.198), AO95245-2 (PVY susceptible parent, A405: 0.263).

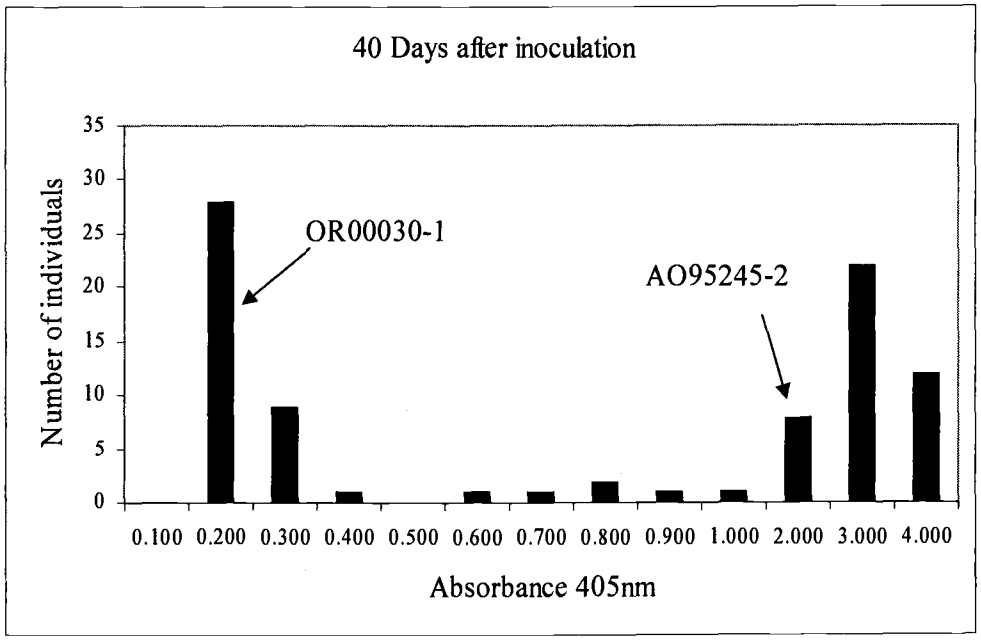

Figure 2.7. Distribution of A405 values for the full-sib population OR05030 at 40 days after inoculation. OR00030-1 (PVY resistant parent, A405: 0.149), AO95245-2 (PVY susceptible parent, A405: 2.120).

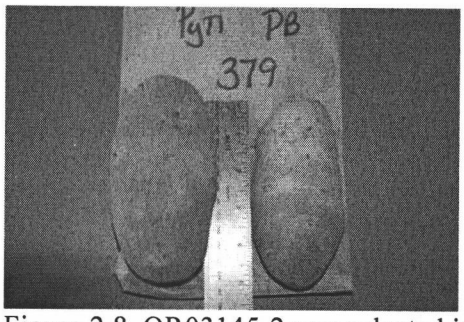

Figure 2.8. OR03145-2 was selected in Powell Butte, Oregon in 2005 based on tuber appearance. Molecular marker evaluations with RYSC3 and ADG2 *Bbv*I indicated the presence of alleles associated with PVY resistance.

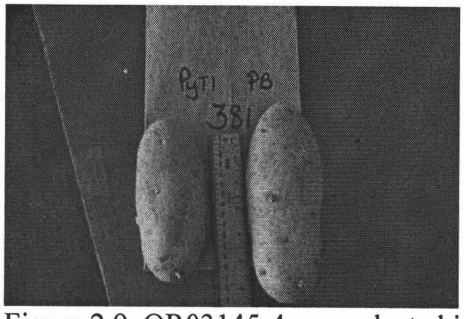

Figure 2.9. OR03145-4 was selected in Powell Butte, Oregon in 2005 based on tuber appearance. Molecular marker evaluations with RYSC3 and ADG2 *Bbv*I indicated the presence of alleles associated with PVY resistance.

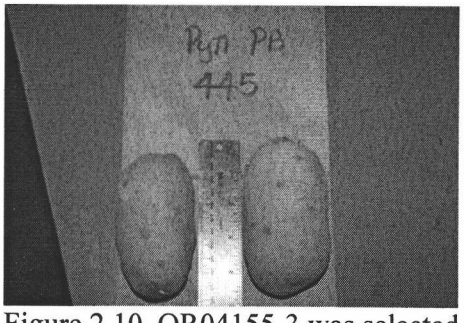

Figure 2.10. OR04155-3 was selected in Powell Butte, Oregon in 2005 based on tuber appearance. Molecular marker evaluations with RYSC3 and ADG2 *Bbv*I indicated the presence of alleles associated with PVY resistance.

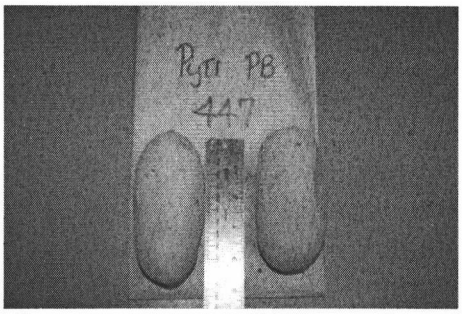

Figure 2.11. OR04155-5 was selected in Powell Butte, Oregon in 2005 based on tuber appearance. Molecular marker evaluations with RYSC3 and ADG2 *Bbv*I indicated the presence of alleles associated with PVY resistance.

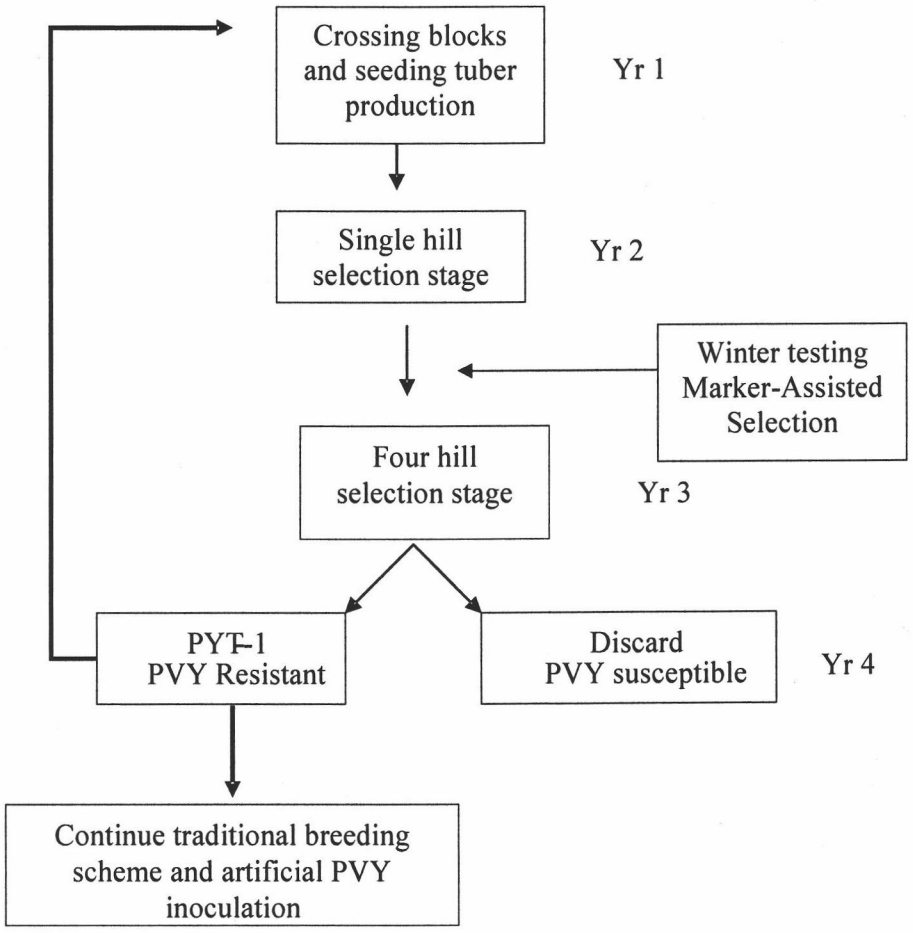

Figure 2.12. Integration of molecular markers associated with Ry_{adg} in a traditional potato breeding program.

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Implementation of Marker-Assisted Selection (MAS) for PVY Resistance (Ry_{sto} gene) in a Potato Breeding Program

Chapter 3

Ryon J. Ottoman

Implementation of Marker-Assisted Selection (MAS) for PVY resistance (Ry_{sto} gene) in a Potato Breeding Program

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Abstract

Potato Virus Y (PVY) seriously impacts the vegetative propagation of potato (*Solanum tuberosum* L). The development of potato varieties with genetic resistance to PVY is one of the best strategies to fight against this disease. Extreme and durable resistance to PVY is desirable for breeding programs. This research explores the use of extreme resistance derived from *Solanum stoloniferum* (Ry_{sto}) mapped to chromosome XII. The usefulness of molecular markers linked to the gene Ry_{sto} for determining the allelic configuration is assessed, providing information as to its benefit in predicting PVY resistance. To achieve this, a full-sib population segregating for Ry_{sto} was screened with molecular markers then inoculated artificially with PVY and tested by ELISA. From the resistant plant population, 77% showed coincidence with molecular marker results confirmed by ELISA at 40 days after inoculation. Undiscovered PVY resistant gene/s may explain the remaining 23% that is unaccounted for. Segregation (resistant: susceptible) for the ELISA and marker results fit a ratio indicating two independent genes segregating for PVY resistance. Results from molecular markers indicated one of the alleles providing resistance is from Ry_{sto} and the other is unknown.

This information was used to screen segregating populations under field conditions. From 622 clones segregating for Ry_{sto}, seventeen (2.7%) were selected at the single hill level based on phenotypic criteria (mainly tuber type and shape). Eight of the 17 elections (47%) contained the marker associated with PVY resistance from Ry_{sto}.

Keywords: Potatoes, PVY, Extreme resistance, Marker-Assisted Selection, ELISA.

Ry_{sto}, *S. stoloniferum.*

Introduction

Virus infections cause serious problems in the vegetative propagation of Potato (*Solanum tuberosum* L). When seed tubersare vegetatively propagated, systemic viruses such as PVY persist from year to year. Viruses causing economic damage in potato are classified into the *Poler (luteo), Poty, Potex,* and *Carla* families (Lawson and Stace-Smith, 2001). *Potyvirus* Y (PVY) is a (+)-sense ssRNA virus of about 8.5-10kb (López-Moya and Garcia, 1999; Shukla et al., 1994). Three common PVY strains of concern in potato are PVY^{o}, PVY^{n}, and PVY^{c} (Brunt, 2001). Additional recombinant PVY strains have been documented including PVY^{ntn}, $PVY^{n}W$ and $PVY^{n:o}$ (Chrzanowska, 1987; Kerlan et al., 1999; McDonald and Singh, 1996 Weidemann, 1988).

Potato Virus Y is vectored by more than 30 species of aphids in a non-persistent manner (deBokx and Piron, 1990; Harrington and Gibson, 1989; Harrington et al., 1986; Hoof, 1977; Hoof, 1980; Katis and Gibson, 1985; Kostiw, 1979; Piron, 1986; Ryden et al., 1979 Sigvald, 1984) thus complicating control. Depending on the cultivar, time of infection and environmental conditions, yield losses associated with PVY range from 10-80% (deBokx and Huttinga, 1981; Rykbost et al., 1999). The symptom expression of PVY infection is highly cultivar dependant. Symptoms typically include vein necrosis, mottling, yellowing of leaflets, leaf-dropping, and premature death (deBokx and Huttinga, 1981). Upon infection in the plant the virus genome is released from the coat protein and (+)-sense ssRNA is translated into viral replicase and replication-associated proteins (Hull, 2002). That assembles into new viral genomes and coat proteins to form new virus particles that then accumulate

within the protoplast of the cell and move to adjacent cells systemically infecting the entire plant.

Plant response to virus infection can be classified as either 'immune' or 'infectible' (Hull, 2002). Immune plants prohibit virus replication in the cell protoplasts. Plants are considered 'infectible' when viruses can replicate in the protoplasts. Examples of infectible plant responses include extreme hypersensitivity (resistant), hypersensitivity (resistant), tolerance, and susceptibility. Extreme hypersensitivity is a resistance response in which virus multiplication is limited to initially infected cells. This response has been observed in potato plants bearing the resistance gene Ry_{sto} (Hinrichs et al., 1998). Hypersensitivity to virus infection involves a necrotic response to virus infection and is regulated by *N* genes that provide strain specific resistance (Barker, 1996). Plants that show little or no response to virus infection are considered tolerant. This situation is classified as a latent response. This latent or tolerant response was observed by Rykbost et al., (1999) while studying yield reduction from seed borne PVY in Russet Norkotah. The authors noted that in a cool short season growing area effects of variable levels of PVY infection on yield were not significantly different. Conversely, in a more stressful environment and a longer growing season PVY infection levels significantly affected yields. For plants considered susceptible, virus replication and systemic movement of the virus occurs without barriers.

To reduce the impact of PVY and other viruses in potato production, seed tuber crops are produced using a limited-generation system (Franc, 2001; Gutbrod and

Mosley, 2001). Problems associated with PVY would be significantly reduced by development of resistant cultivars using known PVY resistant germplasm (Table 1.1).

To facilitate the introgression of PVY resistant genes into breeding programs, molecular maps with markers linked to PVY resistant traits have been developed for Marker-Assisted Selection (MAS) (Flis et al., 2005; Kasai et al., 2000; Song et al., 2005; Sorri et al., 1999; Vidal et al., 2002). Extreme PVY resistance provided by Ry_{sto} has been mapped to the same region as Ry_{adg} on chromosome XI (Brigneti et al., 1997), but the pedigree of the mapping population used was considered unreliable (Gebhardt and Valkonen 2001). Using different germplasm derived from *S. stoloniferum*, the Ry_{sto} gene was remapped to chromosome XII. The RFLP marker GP122 (Gebhardt et al. 2001) was converted into a Cleaved Amplified Polymorphic sequence (CAPs) marker and it was discovered that the gene $Ry\text{-}f_{sto}$ was 1.2 cM from this marker (Flis et al., 2005). In another study by Song et al., (2005) the Ry_{sto} resistance was again localized on chromosome XII but downstream from GP122. Between the probes GP268 and TG28, the PCR based STM0003 primer with a band of 111 base pair was shown to co-segregate with extreme resistance for PVY from Ry_{sto} with a LOD threshold over 3.0 (Song et al., 2005). This marker is a potential candidate for use in MAS for virus resistance potato breeding.

The objectives of this paper are to: A) determine the association of the markers linked to Ry_{sto} with PVY resistance B) demonstrate the application of MAS in an active breeding program and C) screen advanced entries for PVY resistance with markers linked to Ry_{sto}.

Materials and Methods

The plant material used in this study included: *Ry$_{sto}$* resistant and susceptible potato clones, full-sib segregating populations, and advanced breeding selections. OR00002-6, an oblong russet potato clone generated in Oregon (Figure 3.1) was artificially inoculated with PVY and found to be completely resistant. In addition, it was tested with the marker STM0003 and presented patterns associated with resistance from *Ry$_{sto}$*. Norkotah selection #3 is a russet type potato clone with good table market qualities and tolerance to PVY infection (Thompson and Davidson 1999). AO941110-203 and AO96781-4 are russet potato clones being evaluated in Tri-state (Oregon, Idaho, Washington) and Western Regional (OR, ID, WA, TX, CO, CA) field Trails. Performance data is available at (http://www.ars.usda.gov/main/docs.htm?docid=3019).

The experimental population OR05063 and three other populations (OR03151, OR03167 and OR04154) (Table 3.1) were used to implement MAS in the context of an active tetraploid breeding program. The crosses to generate these populations were made between 2003 and 2005 in Oregon State University greenhouses in Corvallis, Oregon. Berries were harvested and seedling tubers were produced from true potato seeds using standard methods. The full-sib population used for genetic studies and corresponding parental lines were planted in 3x3 inch plastic pots containing SUN GRO™ professional blend media in a completely randomized block design with two replications. Greenhouse conditions were set at 18.3°C day and 15.5°C night. Artificial light extended the photoperiod to 16 hrs. Plants were watered and fertilized as needed. The population used for genetic studies was mechanically inoculated using

verified PVY^{o} (PVY) maintained in tobacco tissue that was generously provided by James Crosslin (US Dept. of Agriculture/ARS, Prosser, WA). Infected fresh tobacco tissue (2.5g) was ground in 25ml of cold 1mM potassium phosphate pH8 virus buffer. Two young leaves of each potato plant previously dusted with carborundum were lightly rubbed with cheesecloth dipped in virus buffer. The inoculated leaves were then marked with a hole punch and subsequently monitored for PVY symptoms. Visual PVY symptoms were divided into three classes: typical PVY virus expression, questionable PVY virus expression and no PVY expression. Typical PVY virus expression included leaf mottling and vein burning. Plants with questionable symptoms displayed a slight mottling less distinct than classical PVY leaf mottling. Visual PVY evaluations and ELISA tests were performed at 20, 40, and 60 days after inoculation.

The breeding populations OR03151 (148 progeny), OR03167 (274), and OR04154 (200) were planted as single hill units at the potato research center in Powell Butte, Oregon during the 2005 growing season. Plants were spaced 0.91m between rows and 0.68m within rows. Standard cultural practices for potato production in Powell Butte were used during the growing season. Criteria for selection were based on visual observation of tuber shape and type. Tubers were stored until early spring when an eye (bud) was removed from each selection and planted in the greenhouse in Corvallis. Greenhouse conditions were set at 18.3°C day and 15.5°C night. Plants were watered as needed until plant tissue was available for DNA extraction.

Elite materials evaluated included clones from the 2005 Tri-State (Oregon, Washington, and Idaho) and Western Regional trials (OR, WA, ID, CO, TX, CA) and

entries in the 2005 National Late Blight trial (Table 2.2). Clones from the Tri-State and Western Regional trials were screened for field resistance to PVY at the Hermiston Agricultural Research and Extension Center (Hermiston, OR.). Entries were planted in two ten-hill plots randomized and replicated four times. Each plot was bordered by a spreader row with seedborne PVY and cultural management was conducive to aphid buildup. Twelve tubers were randomly selected from each plot at harvest and 2 tubers from each plot were sprouted and tested for PVY using ELISA.

The Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) with PVY^{O-n} polyclonal anti-bodies (AGDIA Company, Elkhart, IN) was used to determine virus resistance. The third petiole from artificially inoculated plants was collected and ground in a buffer ratio of 1:10 (50mg tissue: 500µl grinding buffer) using a Qiagen/Retsch MM 300 mixer mill (Qiagen Inc, Valencia, CA.) The assay was conducted using directions provided by AGDIA with slight modifications. Two negatives and two positive controls were used in each 96-well ELISA plate. Absorbance values were measured at 405nm (A_{405}nm) using a VERSAmax microplate reader (Molecular Devices Sunnyvale, CA.). The PVY resistant threshold cutoff was established by using two standard deviations from the mean of the positive controls (Sutula et al., 1986).

Genomic DNA was extracted by collecting 30-50mg of young leaf tissue. The leaf samples were cut into four pieces and placed in Qiagen collection tubes (Qiagen Inc, Valencia, CA.) and stored at -80°C until DNA was extracted. The tissue was ground using a Qiagen/Retsch MM 300 mixer mill (Qiagen Inc, Valencia, CA.). DNA was isolated as described by Riera-Lizarazu et al., (2000). DNA concentration and

quality was evaluated on a 1% agarose gel by comparison with lambda DNA of known concentration.

Polymerase Chain Reaction (PCR) amplification of the STM0003 marker linked to Ry_{sto} was carried out using a Techne thermalcycler, (Techne Inc, Burlington NJ.) STM0003 (Table 3.2) developed by Milbourne (1998) and mapped to Ry_{sto} by Song et al., (2005). The PCR reaction volume was 10µl containing 1x taq buffer, 0.03U/µl taq from Qiagen, 2% sucrose in cresol red, 0.1mM dNTPs, , 0.5 µM of each primer and 10 ng template DNA. The PCR was programmed at 94° C for 4 min., followed by 35 cycles of denaturation at 94°C for 45s, primer annealing at 50°C for 45s, and primer extension at 72°C for 30s, followed by a final extension at 72°C for 5 min. Products were then visualized on a 2% agarose gel. Markers RYSC3 (Kasai et al., 2000) and ADG2 *Bbv*I (Sorri et al., 1999) associated with the Ry_{adg} gene were also tested using the PCR conditions described in chapter 2.

The ELISA results obtained at 20, 40, and 60 days after artificial inoculation with PVY were tested for normality using the PROC UNIVARIATE statement of SAS (SAS Institute, 2001). Chi-square tests for homogeneity (Gomez and Gomez, 1983) were conducted to compare the ELISA results and visual PVY symptom scores (phenotype) between 20, 40, and 60 days after inoculation. A Chi-square test using a fixed ratio hypothesis for Chromosome (1:1, 5:1) and Chromatid (0.87:1, 3.7:1) assortment (Allard, 1960) was conducted to fit the marker and ELISA classification of resistant and susceptible plants to the segregation of a single dominant resistant allele (simplex) or two dominant resistant alleles (duplex) in a tetraploid with tetrasomic inheritance model. Also a Chi Square test for two independently assorting PVY

resistant genes was also conducted using the ELISA and STM0003 results. Regressions were performed using the PROC REG statements of SAS (SAS Institute, 2001) to predict PVY visual symptom scores or ELISA results at 20, 40, and 60 days after inoculation based on the marker scores.

Results and Discussion:

The PVY resistant parental line OR00002-6 and the PVY tolerant Norkotah selection #3 were evaluated with the experimental population OR05063. At the genotypic level, a band (111 bp) associated with PVY resistance (Ry_{sto} source) was observed in OR00002-6, but not in Norkotah selection #3 as shown in Figure 3.1. The screening of the parental lines with RYSC3 and ADG2 *Bbv*I showed patterns associated with PVY susceptible alleles at Ry_{adg} (Figures 3.2 and 3.3). None of the parents showed RYSC3 amplification product, and ADG2 product was successfully digested with *Bbv*I resulting in two bands of 270 and 85 bp. OR00002-6 was originally thought to contain the gene Ry_{adg}, but based on molecular marker evaluations performed we concluded that this was not likely. The presence of a band associated with PVY resistance (Ry_{sto} source) at STM0003 indicated that *S. stoloniferum* was the source of PVY resistance in this clone. A careful observation of the pedigree of OR00002-6 (Figure 3.1) also indicated this was possible since the maternal great grandfather PI343201 was derived from *S. stoloniferum* sources. Based on ELISA evaluations of OR00002-6 at 20, 40, and 60 days after inoculation with PVY A_{405}nm values within the resistant class were displayed. Conversely, Norkotah selection #3 was within the susceptible class and the A_{405}nm value slightly increased

at 40 and 60 days after inoculation (Figures 3.5, 3.6, and 3.7). Phenotypic evaluations of Norkotah selection #3 at 20, 40, and 60 days after inoculation showed some light mottling, but not consistent with classical PVY expression. These symptoms are typical of PVY tolerant lines observed under greenhouse conditions (Gutbrod 2006 personal communication). The resistant clone OR00002-6 appeared to be completely free of virus based on visual observation on all three dates except for the first reading (20 days) when poor plant health was confused with PVY infection.

Of the 91 plants in OR05063 (Table 3.3) screened for the gene Ry_{sto}, 48 plants contained a 111 base pair band from the marker STM0003 associated with PVY resistance, while the remaining 43 plants were scored as susceptible. The observed segregation of the marker results fit expected segregation ratios for a single dominant gene (simplex) providing extreme resistance to PVY. Chromosome assortment was shown to be more probable ($\chi^2 = 0.275$, P= 0.6) as shown in Table 3.4. This provides convincing evidence that the PVY resistant parent OR00002-6 has the allelic configuration of a simplex for the gene Ry_{sto} as shown by marker STM0003.

ELISA results for population OR05063 at 20, 40, and 60 days after inoculations (Figures 3.4, 3.5, and 3.6) showed a continuous bi-modal distribution for resistance to PVY. The observed segregation of the ELISA values fit the expected segregation ratio of two dominant alleles (duplex) (Table 3.4) but, based on the segregation of the molecular marker STM0003 as a simplex, this is not likely. Therefore a Chi-square test for two independent genes providing PVY resistance was also conducted. The resulting ($\chi^2 = 3.72$, P= 0.16) shows evidence for two independent genes segregating for PVY resistance. One gene is provided by Ry_{sto} and

the other is of unknown origin. The minor gene/s could be present in the PVY resistant line OR00002-6 or in the susceptible parent (Norkotah selection #3). The genetic basis of the PVY tolerance present in Norkotah selection #3 has not been studied in depth, but there is evidence for uncharacterized PVY resistant genes present. Mollov and Thill (2004) observed that tolerance ("asymptomatic expression of PVY") was observed segregating in a tetraploid population. Tolerance was also shown when the yield of PVY-infected Norkotah was not significantly reduced when grown in a short season and cool climate (Rykbost et al., 1999).

A Chi-square test of homogeneity to compare the numbers of resistant and susceptible plants (based on ELISA and on visual observations of PVY symptoms) between the different evaluation times (20, 40, and 60 days after inoculation) indicated that the ELISA results were significantly different (χ^2=7.55, P=0.006) between 20 and 40 days after inoculation. No significant difference was observed between 40 and 60 days after inoculation (χ^2=0.167, P=0.682) as shown in table 3.5. Visual observations of PVY symptoms were significantly different between 20 and 40 days after inoculation (χ^2=31.83, P=0.0001). More ELISA positives (susceptible) were observed at 40 days than at 20 days. There were also significant differences between symptoms observed at 40 and 60 days after inoculation (χ^2=18.68, P=0.0001). Interestingly fewer plants expressed symptoms of PVY at 60 days than at 40 days. This could be explained by the fact that most plants showing PVY symptoms earlier were dead at 60 days and only the tolerant lines (also considered susceptible based on ELISA) survived. Based on the previous results, we concluded that under the conditions used

in our experiments, 40 days after inoculation seems to be the best time to perform ELISA and visual observations of PVY symptoms.

A regression analysis conducted between the STM0003 marker linked to the gene *Ry_{sto}*, and phenotype class, determined by ELISA, and visual observation at 20, 40, and 60 days after inoculation with PVY (Table 3.6) indicated that the marker could only account for 29-57% (depending on the date of evaluation) of the variation observed by ELISA in OR05063. The largest R^2 (0.57) was observed at 60 days after inoculation. The marker could not precisely predict visual observations, except at 40 days after inoculation ($R^2 = 0.29$). At 20 days after inoculation, 33% (16/49) of the lines declared visually resistant to PVY were susceptible using the marker. Conversely 25% (1/4) of the plants showing PVY mottling were classified as resistant by the marker. At 40 days after inoculation, 23% (14/62) of plants classified by ELISA as resistant were susceptible based on the STM0003 marker. Twenty-four percent of the plants classified as visually resistant at 40 days did not contain the marker band associated with resistance. None of the plants declared resistant based on the marker were susceptible based on ELISA independent of the time of evaluation. Five percent of the plants showing typical PVY symptoms at 40 days were classified as resistant by marker results. At 60 days after inoculations, only 14% (8/56) of plants scored as resistant by ELISA were listed as susceptible by the marker. Twenty-five percent (15/60) of the plants visually virus free at 60 days did not contain the susceptible marker. Norkotah selection #3 and several other cultivars do not express PVY symptoms well and testing by ELISA is necessary to confirm the presence of the virus. Visual observations of PVY symptoms in our experimental population were

complicated by the lack of symptom expression. Some of the lines failed to be declared resistant, based on the presence of the STM0003 marker. This could be due to recombination between the marker and the PVY resistant gene, marker or ELISA errors or the presence of additional PVY resistance genes. The Chi-square test for two independently assorting genes showed evidence (χ^2=3.72, P=0.1.557) that there may be a newly discovered PVY resistant gene segregating in this population.

Three families segregating for PVY resistance were chosen for proof of concept to demonstrate how MAS can be used in a breeding program for PVY resistance. The PVY resistance in families OR03151, OR03167, and OR04154 was provided by the male parent OR00002-6. The female parental lines were AO941110-203, AO96781-4 and Norkotah selection #3. Since OR00002-6 was shown to segregate as a simplex for the gene *Ry$_{sto}$*, four or more clones from each family were selected at the single hill level to ensure recovery of PVY resistant plants. From the 622 seedling tubers planted as single hill units, 17 were selected representing 2.7% of the total population from the crosses shown in table 3.7. Eight of the 17 selections (47%) contained the 111 bp band at marker STM0003 which was previously associated with PVY resistance (*Ry$_{sto}$* source) (Song et al., 2005). The percentage of plants (47%) containing the allele associated with PVY resistance (*Ry$_{sto}$* source) is very close to the 50% expected based on the simplex configuration predicted from the segregation of the STM0003 marker in the experimental population OR05063. The number of selections based on visual observation of tubers combined with MAS for PVY resistance using STM0003 will be increased and further evaluated for yield, quality, and additional disease/pest resistances in replicated trials. Depending on

overall performance, these selections could become new varieties or re-used as parents in multi-trait recurrent selection programs as illustrated in figure 2.12. The materials identified as resistant by molecular markers should be retested using artificial inoculation with PVY and/or field trials with high virus pressure to reconfirm resistance. Based on our previous results using the experimental population OR05063, none of the plants declared resistant based on the marker were susceptible based on ELISA. Some of the lines resistant in the experimental populations were not positive for the STM0003 marker.

Field resistance and moderate PVY symptom expression has been observed in several advanced breeding lines. In an effort to understand the genetic factors responsible for this, 72 lines (Table 2.2) were selected from potato breeding programs across the nation for evaluation. From the 72 lines, 58 were selected and tested for PVY field resistance under high virus pressure at Hermiston Oregon. Only two (A93157-6LS and A92294-6) showed no PVY infection under field conditions. Six entries showed moderate resistance (25% infection) while all other entries showed much higher levels of PVY infection (Table 2.9). The marker STM0003 linked to Ry_{sto} could not explain the field resistance; neither could markers linked to Ry_{adg} (RYSC3 and ADG2 cut with *BbvI*). Additional sources of resistance, not detected with the marker used, are likely present. The marker STM0003 associated with the Ry_{sto} gene suggested a segregation ratio of 1:1 but it did not account for all of the resistant phenotypes identified by ELISA (Table 3.3) indicating that other PVY resistance genes are present. Other loci for PVY have been found from *S. stoloniferum* on chromosome XII (Flis et al., 2005). This may provide evidence that

resistance to PVY may not fit the single major gene concept. Further, in other *Solanacae* species it is not uncommon that resistance to PVY is provided by Quantitative Trait Loci (QTL) (Caranta et al., 1997). Additional genes for extreme resistance to PVY and /or QTL could explain the resistant phenotypes observed. The marker STM0003 linked to Ry_{sto} can be reliably used to predict PVY resistance (Ry_{sto}) but additional work needs to be done to explore the possibility of having other factors involved in the PVY resistance segregating in populations derived from OR00002-6 and the tolerance observed in Norkotah selection #3

Table 3.1. Crosses derived from the PVY resistant clone OR00002-6 and size of resulting full-sib families.

	Parental lines[a]		
Family	Female	Male	Number of individuals
OR05063	*OR00002-6*	Norkotah #3	94
OR03151	AO94110-203	*OR00002-6*	148
OR03167	AO96781-4	*OR00002-6*	274
OR04154	Norkotah #3	*OR00002-6*	200

[a] Italic font indicates the PVY resistant potato clone containing the Ry_{sto} gene

Table 3.2. Forward and reverse primer sequences for the STM0003 DNA-based marker, annealing temperature (Ta), resistant allele PCR product size and chromosome location.

Marker[a]	Primer	Forward and reverse primer sequences (5'-3')	Ta	Product size (bp)[b]	Chromosome	Reference
STM0003	STM0003-F	GGAGAATCATAACAACCAG	50°C	111 (R)	XII	Milbourne et al., 1998
	STM0003-R	AATTGTAACTCTGTGTGTGTG		Other bands		

[a] Name used in this study

[b] R: band associated with PYV resistance

Table 3.3. Segregation of the OR05063 family for resistance to PVY based on DNA-based marker evaluation and ELISA readings at 20, 40 and 60 days after inoculation (DAI).

	No. of plants per genotypic class[a]		ELISA[b] 20 DAI		ELISA[b] 40 DAI		ELISA[b] 60 DAI	
STM0003	N_1	N_0	X_1	X_0	X_1	X_0	X_1	X_0
	48	43	80	11	62	25	56	26

[a] Genotypic classes: N_1: allele associated with resistance, N_0: allele associated with susceptibility

[b] Two phenotypes classes are distinguished by absence (X_1) or presence (X_0) of virus detected by ELISA

Table 3.4. Chi-square test for fit of fixed segregation ratios in full-sib population OR05063 based on ELISA results, visual observation of PVY symptoms and molecular marker STM0003 associated with *Ry_{sto}* resistance.

Test	Allelic configuration	Time (DAI)	Segregation ratio[a] (Resistant: Susceptible)	χ^2	*P*-value
ELISA	Simplex	20	1:1	52.32	0.001
			0.87:1	72.97	0.0001
		40	1:1	15.73	0.0001
			0.87:1	27.322	0.0001
		60	1:1	10.976	0.0009
			0.87:1	20.50	0.0001
	Duplex	20	5:1	0.887	0.346
			3.7:1	10.304	0.0013
		40	5:1	4.149	0.0417
			3.7:1	0.131	0.717
		60	5:1	7.024	0.008
			3.7:1	0.892	0.334
Phenotype	Simplex	20	1:1	35.85	0.0001
			0.87:1	48.99	0.0001
		40	1:1	2.667	0.102
			0.87:1	6.79	0.009
		60	1:1	49.00	0.0001
			0.87:1	65.93	0.0001
	Duplex	20	5:1	3.894	0.0485
			3.7:1	8.651	0.0033
		40	5:1	12.042	0.0005
			3.7:1	3.845	0.049
		60	5:1	7.56	0.006
			3.7:1	14.013	0.0002
STM0003	Simplex		1:1	0.275	0.6
			0.87:1	3.155	0.0757
	Duplex		5:1	42.242	0.0001
			3.7:1	18.861	0.0001

[a] Simplex: 1:1 (chromosome assortment), 0.87:1 (chromatid assortment)
Duplex: 5:1 (chromosome assortment), 3.7:1 (chromatid assortment)

Table 3.5. Chi-square values for homogeneity in OR05063 for ELISA and visual observation (phenotype) of PVY symptom at 20 and 40 days after inoculation (DAI).

	20 vs. 40 DAI		40 vs. 60 DAI		20 vs. 60 DAI	
Test	χ^2	*P*-Value	χ^2	*P*-Value	χ^2	*P*-Value
ELISA	7.55	0.006	0.167	0.682	9.71	0.001
Phenotype	31.83	0.0001	18.68	0.0001	0.376	0.539

Table 3.6. Comparison of molecular marker (STM0003) with ELISA readings and visual observation (phenotype) of PVY symptoms (phenotype) at 20, 40 and 60 days after inoculation (DAI) in population OR05063. Bold font indicates matches between the molecular marker and test.

Test	Time (DAI)	Class[a]	Genotypic class[b]		Regression equation	R^2
			N_1	N_0		
ELISA	20	X_1	**48**	32	Y=0.619+0.380X	0.29
		X_0	0	**11**		
Phenotype	20	E_1	**33**	16	Y=0.857+0.107X	0.046
		E_0	1	**4**		
		$E_?$	14	23		
ELISA	40	X_1	**48**	14	Y=0.476+0.476X	0.307
		X_0	0	**25**		
Phenotype	40	E_1	**25**	8	Y=0.524+0.428X	0.295
		E_0	1	**20**		
		$E_?$	22	11		
ELISA	60	X_1	**48**	8	Y=0.333+0.667X	0.5714
		X_0	0	**26**		
Phenotype	60	E_1	**45**	15	Y=0.809+0.190X	0.156
		E_0	0	**4**		
		$E_?$	3	15		

[a] Classes are distinguished by absence (X_1) or presence (X_0) of virus detected by ELISA and visual observation (phenotype) of virus symptom expression (E_1: no PVY symptoms, E_0: PVY symptoms, $E_?$: questionable symptoms)

[b] Genotypic classes: N_1: allele associated with resistance, N_0: allele associated with susceptibility. Based on molecular marker screening with STM0003

Table 3.7. Number of full-sib individuals evaluated as single hills in Powell Butte, OR, in 2005 under field conditions, number of selections based on general tuber appearance and number of selections containing markers associated with resistance to PVY (Ry_{sto}).

Family	Number of individuals	Number of selections	Number of selections that contain Ry_{sto}
OR03151	148	5	3
OR03167	274	4	2
OR04154	200	8	3
Total	*622*	*17 (2.7%)*	*8 (47.0%)*

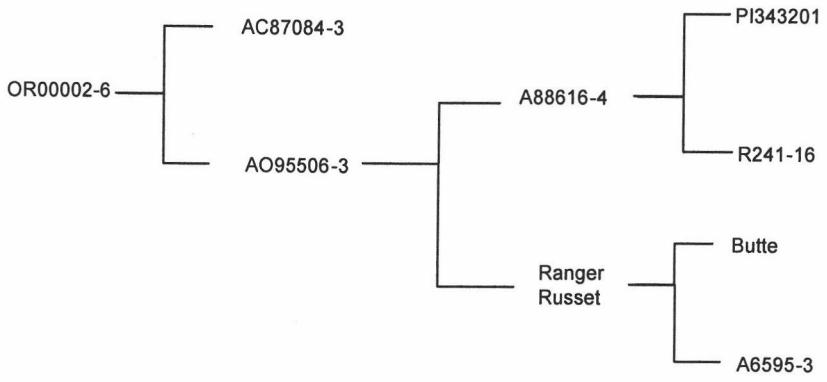

Figure 3.1. Pedigree of the PVY resistant clone OR00002-6

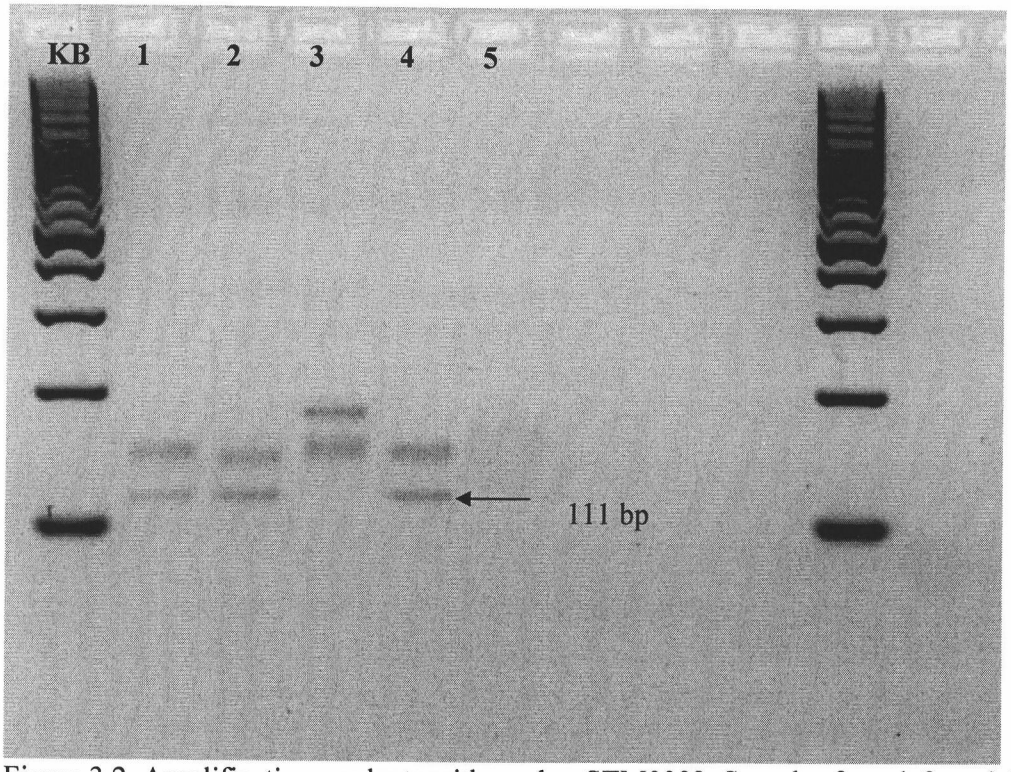

Figure 3.2. Amplification products with marker STM0003. Samples from left to right: KB: 100 bp ladder, 1- 2: members of the full-sib population OR05063, 3: Norkotah selection #3 (PVY susceptible clone), 4: OR00002-6 (PVY resistant clone), 5: water control. Presence of a 111 bp product indicates PVY resistance.

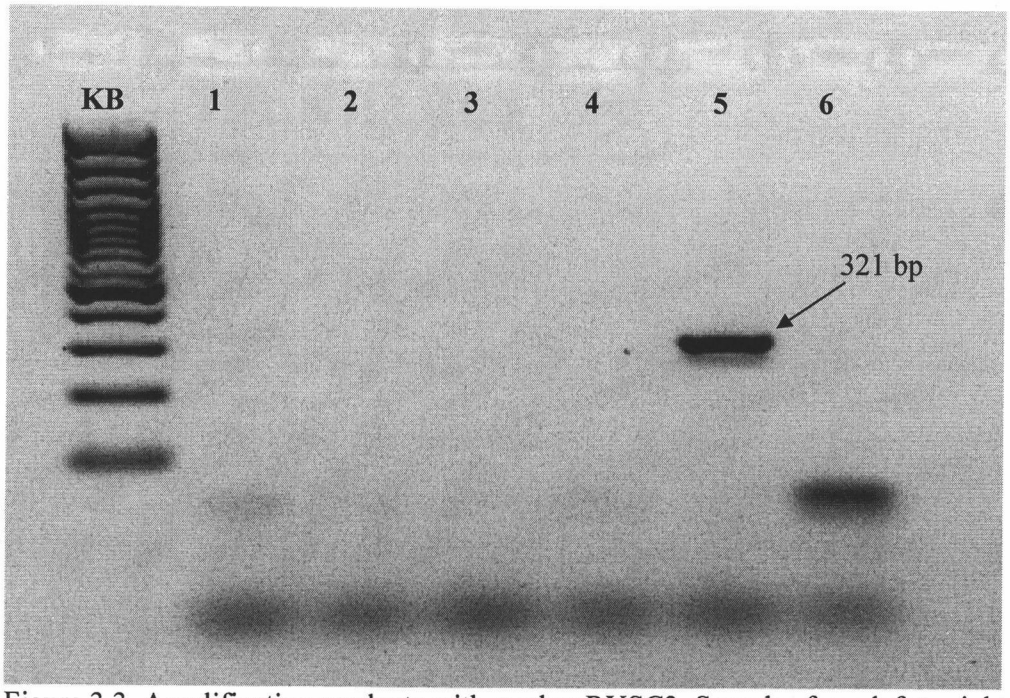

Figure 3.3. Amplification products with marker RYSC3. Samples from left to right: KB: 100 bp ladder, 1- 2: members of the full-sib population OR05063, 3: Norkotah selection #3 (PVY susceptible clone), 4: OR00002-6 (PVY resistant clone), 5 PVY resistant control (AO88628-2) 6: water control. Presence of a 321 bp product indicates PVY resistance. Absence of the band with PVY susceptibility

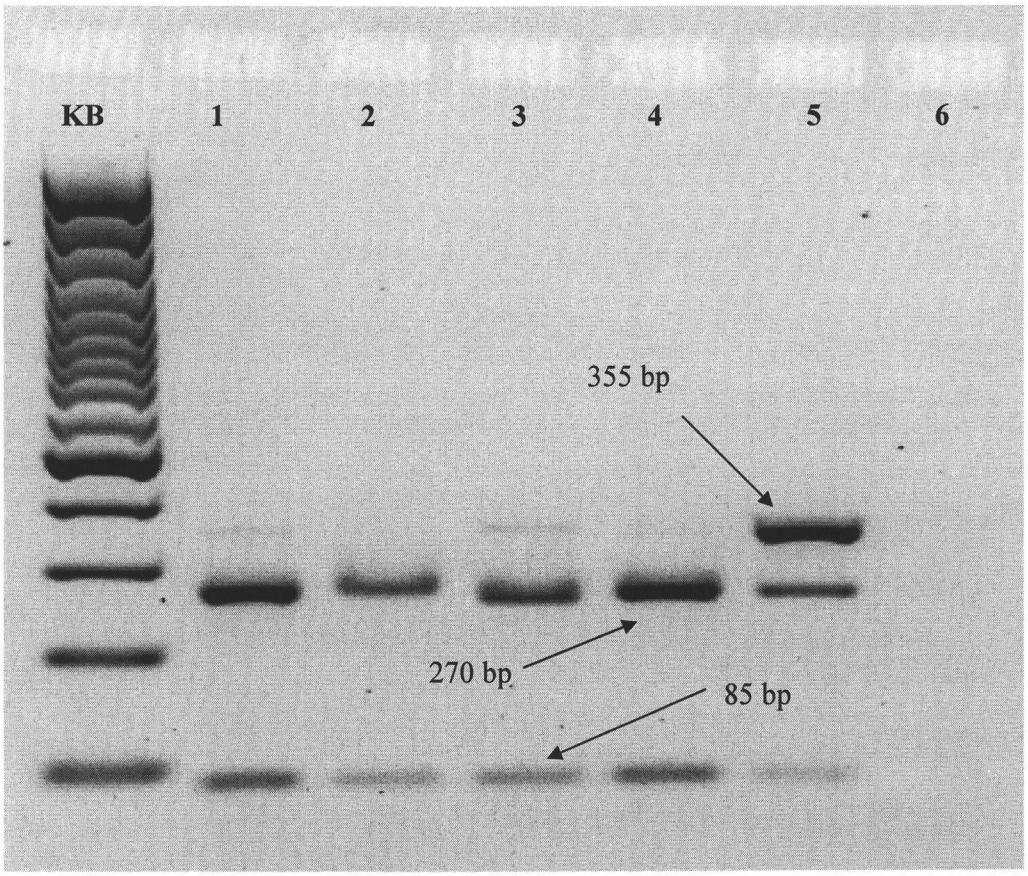

Figure 3.4. Amplification products with marker STM0003. Samples from left to right: KB: 100 bp ladder, 1- 2: members of the full-sib population OR05063, 3: Norkotah selection #3 (PVY susceptible clone), 4: OR00002-6 (PVY resistant clone), 5 PVY resistant control (AO88628-2), 6: water control. Presence of an uncut product of 355 bp product indicates PVY resistance. Successful digestion resulted in two bands, 270 and 85 bp and indicates PVY susceptibility.

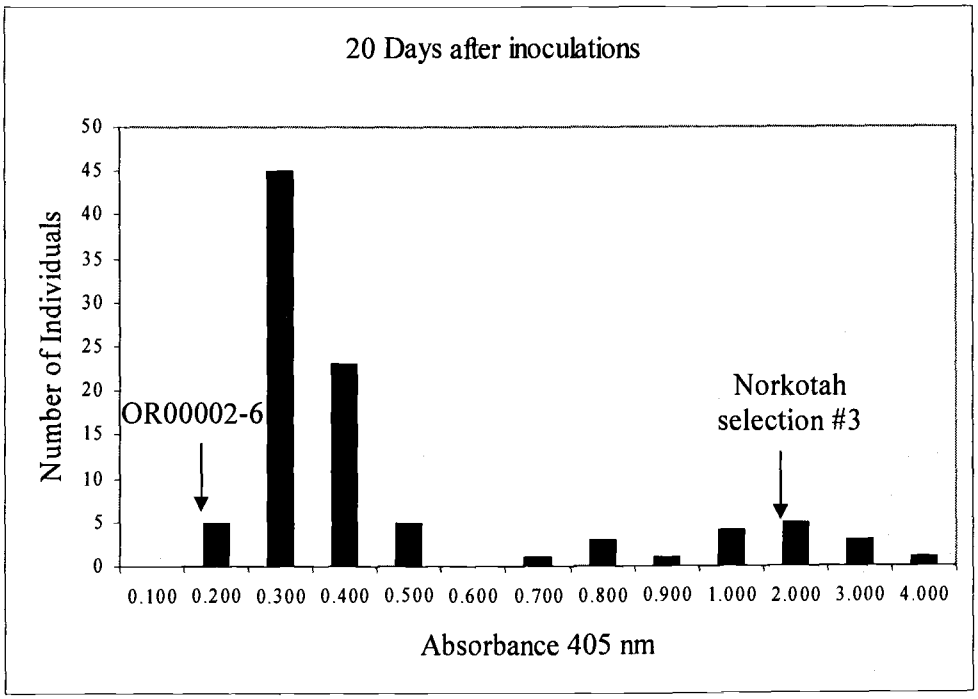

Figure 3.5. Distribution of A405 values for the full-sib population OR05063, OR00002-6 (PVY resistant parent, A405: 0.177) and Norkotah selection #3 (PVY susceptible parent, A405: 2.002) at 20 days after PVY inoculation.

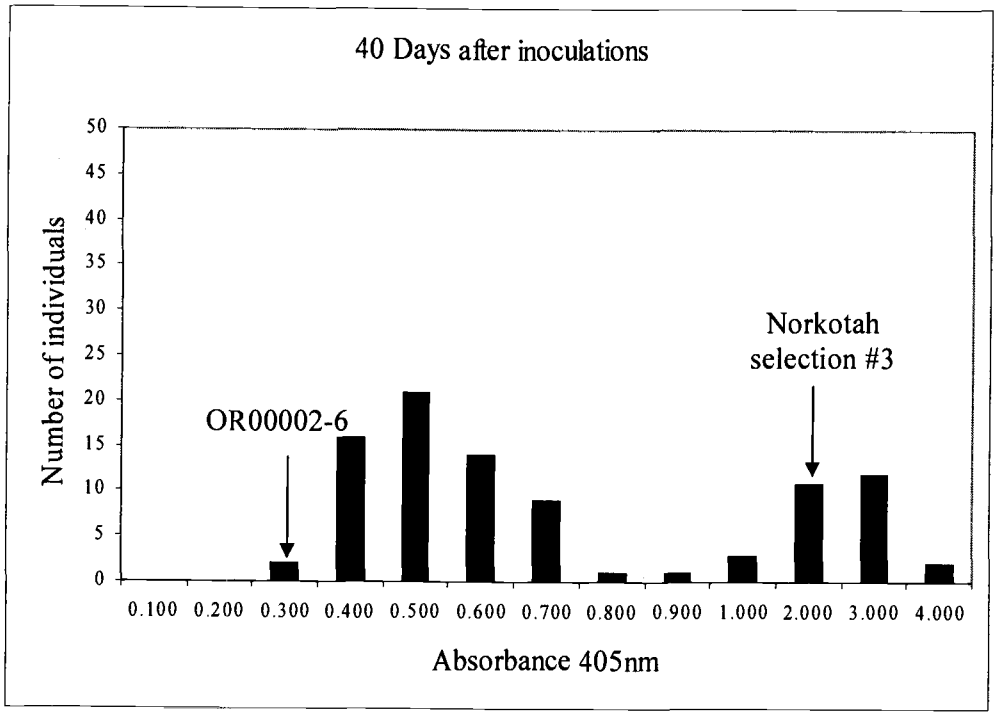

Figure 3.6. Distribution of A405 values for the full-sib population OR05063, OR00002-6 (PVY resistant parent, A405: 0.368) and Norkotah selection #3 (PVY susceptible parent, A405: 2.195) at 40 days after PVY inoculation.

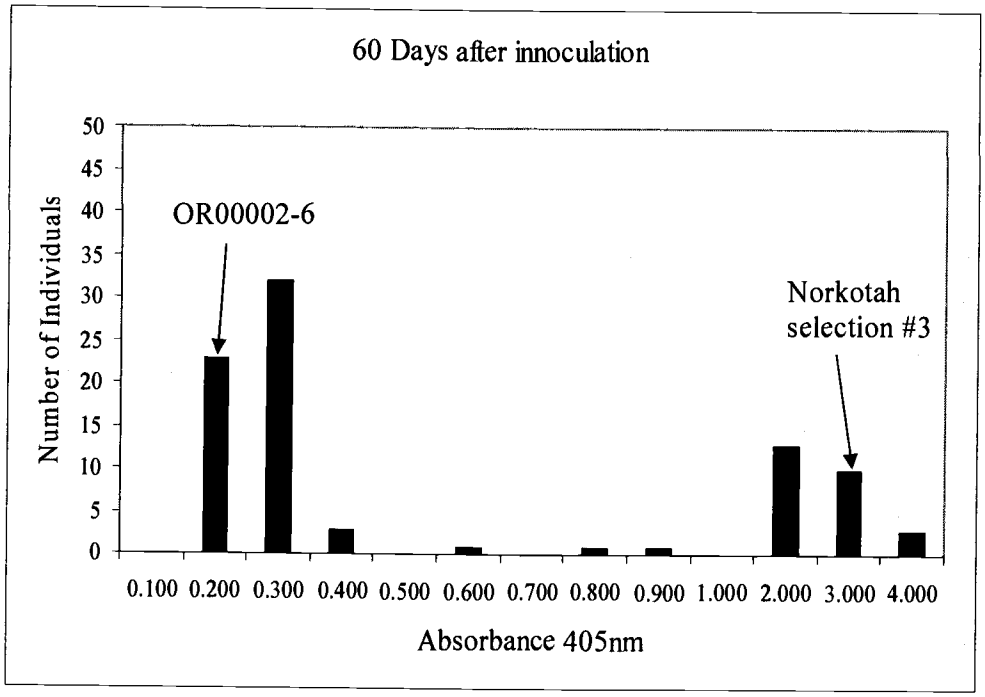

Figure 3.7. Distribution of A405 values for the full-sib population OR05063, OR00002-6 (PVY resistant parent, A405: 0.168), Norkotah selection #3 (PVY susceptible parent, A405: 2.849) at 60 days after PVY inoculation.

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Marker-Assisted Selection for Resistance to Potato Virus Y

Chapter 4

General Conclusions

Ryon J. Ottoman

The objectives of this research were to: A) determine the association of molecular markers for PVY resistance developed from *Solanum tuberosum ssp andigena* and *Solanum stoloniferum* with PVY resistance confirmed by ELISA and visual observations; B) to demonstrate the application of Marker-Assisted Selection in an active breeding program; and C) to screen advanced entries from the Tri-State, Western Regional and National Late Blight trial for PVY resistance using the same markers associated with resistance provided by Ry_{adg} and Ry_{sto} genes.

The PVY resistant parental lines used in this study (OR00030-1 and OR00002-6) are tetraploid potato clones with excellent cultivar potential. OR00030-1 has already been evaluated in yield trials (chapter 2) and appears to have good varietal potential. Based on molecular marker evaluations, it was found that both lines have the allelic configuration of a simplex for extreme resistance provided by Ry_{adg} and Ry_{sto}. Introgression of PVY resistance into breeding germplasm from the parental lines used in this study (chapter 2 and 3) would be much easier than from non-adapted or wild forms of PVY resistance. Also, information about the allelic configuration of the parental lines will help the selection process. The traditional method of artificially inoculating plants with PVY followed by ELISA is very accurate but time consuming. Results presented in chapters 2 and 3 showed a time requirement of 40 days after inoculation to confirm virus expression using ELISA and visual observations. The use of molecular markers will provide the same results much faster (around two weeks).

Markers (Kasai et al., 2000; Sorri et al., 1999) for *Solanum tuberosum ssp andigena* PVY resistance used in chapter 2 confirmed ELISA results with 94% accuracy. Further, markers developed for *S. stoloniferum* in chapter 3 showed that

77% of the plants declared resistant at 40 days after inoculation could be explained by the molecular markers. It is reasonable to assume that additional genes can explain 23% of the resistance not accounted for in this population. Then conducting a Chi-square test for two independent there was evidence (χ^2=3.72, P=0.1.557) for two independent genes segregating for resistance to PVY. According to ELISA, the *S. stoloniferum* population segregates 3.7:1 (resistant: susceptible) indicating two genes are responsible for the resistance observed. When the population was screened with molecular markers linked to Ry_{sto}, the segregation ratio fit a 1:1 (resistant: susceptible) indicating a simplex allelic configuration at the Ry_{sto} locus. Knowledge about the allelic configuration of the parental lines (chapters 2 and 3) provides guidance for the selection process by estimating the proper number of selections needed to ensure recovery of PVY resistance. From the material selected in the field approximately 47% contained the marker associated with resistance. By increasing the allelic configuration of the parental lines, the chances of recovering PVY resistant germplasm will be higher so the selection pressure could be increased. Molecular markers used in this study provide an excellent method of tracking the Ry_{adg} and Ry_{sto} sources of resistance which have not been introduced into elite breeding material yet. When combining PVY resistance from Ry_{adg} and Ry_{sto} sources, only molecular markers will confirm successful pyramiding. Selections shown to be resistant to PVY by MAS should be tested using artificial inoculation methods to reconfirm PVY resistance. Resistant selections could either be evaluated further as a potential new variety or they could be used as re-current parents to improve the agronomic status of the PVY resistant parental material.

In chapters 2 and 3 elite lines from the Tri-State breeding program (Oregon, Washington, and Idaho) and entries from Western Regional (Oregon, Washington, Idaho, Colorado, Texas and California) and the 2005 National Late Blight trials were screened for PVY resistance using markers associated with resistance provided by *Solanum tuberosum ssp andigena* and *S. stoloniferum*. As reported in chapter 2, we discovered that the line LBR2 contained both markers RYSC3 and ADG2 *Bbv*I while lines BO718-3 and EGA97061-4 showed a pattern for resistance from the *S. tuberosum andigena* source using the primers ADG2 *Bbv*I, but this could not be confirmed by the primers set RYSC3. Also none of the advanced lines displayed patterns of resistance associated with markers developed for *S. stoloniferum* (chapter 3). Field evaluations showed the elite lines A93157-6LS (Premier Russet) and A92294-6 to be very resistant to PVY. Selections AOA95154-1, CO94183-1R/R, All Blue, CO94165-3P/P, NDA5507-3Y/F, and VC1002-3W/Y seemed to be moderately resistant to PVY, but none of the molecular markers used in this study could account for the resistance observed, indicating that other sources of resistance are likely present.

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Appendix 1 Molecular marker scores (1= PVY resistant); (0= PVY susceptible); with phenotypic (Yes = PVY symptoms); (No = no symptoms); (questionable =?) data for the full-sib family OR05030 at 20 and 40 days after inoculation (DAI).

Entry	RYSC3	ADG2 *Bbv*I	Phenotype **20 DAI** rep 1	Phenotype **20 DAI** rep 2	Phenotype **40 DAI** rep 1	Phenotype **40 DAI** rep 2	Phenotype **20 DAI** Average	Phenotype **40 DAI** Average
OR05030-1	1	1	?	?	?	?	?	?
OR05030-2	1	1	?	no	?	no	?	?
OR05030-3	1	1	?	no	?	no	?	?
OR05030-4	1	1	?	no	?	no	?	?
OR05030-5	1	1	?	no	?	no	?	?
OR05030-6	1	1	?	no	?	?	?	?
OR05030-7	0	0	?	no	yes	yes	?	yes
OR05030-8	1	1	no	no	?	?	no	?
OR05030-9	1	1	?	no	no	no	?	no
OR05030-10	1	1	no	no	?	dead	no	?
OR05030-11	0	0	yes	yes	yes	yes	yes	yes
OR05030-12	0	0	yes	no	yes	?	?	yes
OR05030-13	1	1	?	no	no	no	?	no
OR05030-14	1	1	no	no	no	no	no	no
OR05030-15	0	0	?	no	yes	?	?	yes
OR05030-16	0	0	yes	?	yes	?	yes	yes
OR05030-17	0	0	no	no	yes	yes	no	yes
OR05030-18	0	0	no	yes	yes	yes	?	yes
OR05030-19	0	0	yes	no	yes	yes	?	yes
OR05030-20	0	0	yes	?	yes	no	yes	?
OR05030-21	0	0	no	no	?	?	no	?
OR05030-22	1	1	dead	no	dead	no	no	no
OR05030-23	0	0	no	no	yes	yes	no	yes
OR05030-24	1	1	?	no	?	no	?	?
OR05030-25	1	1	yes	?	no	no	yes	no
OR05030-26	1	1	no	no	?	no	no	?
OR05030-27	1	1	?	no	?	no	?	?
OR05030-28	0	0	yes	no	no	no	?	no
OR05030-29	0	0	yes	yes	yes	?	yes	yes
OR05030-30	0	0	?	dead	yes	dead	?	yes
OR05030-31	0	0	yes	dead	yes	dead	yes	yes
OR05030-32	1	1	no	no	no	no	no	no
OR05030-33	1	1	no	dead	?	dead	no	?
OR05030-34	1	1	yes	no	yes	?	?	yes
OR05030-35	0	0	no	dead	yes	dead	no	yes

Appendix 1 (Continued)

Entry	RYSC3	ADG2 *Bbv*I	Phenotype **20 DAI** rep 1	Phenotype **20 DAI** rep 2	Phenotype **40 DAI** rep 1	Phenotype **40 DAI** rep 2	Phenotype **20 DAI** Average	Phenotype **40 DAI** Average
OR05030-36	0	0	no	no	yes	yes	no	yes
OR05030-37	0	0	no	dead	yes	dead	no	yes
OR05030-38	1	1	?	dead	?	dead	?	?
OR05030-39	1	1	no	no	no	no	no	no
OR05030-40	0	0	?	no	yes	yes	?	yes
OR05030-41	0	0	?	no	yes	yes	?	yes
OR05030-42	0	0	?	yes	yes	yes	yes	yes
OR05030-43	0	0	?	?	yes	yes	?	yes
OR05030-44	0	0	?	no	yes	?	?	yes
OR05030-45	1	1	?	dead	?	dead	?	?
OR05030-46	1	1	?	no	no	no	?	no
OR05030-47	1	1	no	no	yes	no	no	?
OR05030-48	1	1	?	no	no	no	?	no
OR05030-49	1	1	no	dead	no	dead	no	no
OR05030-50	1	1	no	dead	no	dead	no	no
OR05030-51	0	0	yes	?	yes	yes	yes	yes
OR05030-52	0	0	yes	yes	yes	yes	yes	yes
OR05030-53	0	0	?	no	yes	yes	?	yes
OR05030-54	0	0	yes	yes	yes	yes	yes	yes
OR05030-55	1	1	no	dead	no	dead	no	no
OR05030-56	0	0	?	yes	yes	yes	yes	yes
OR05030-57	0	0	yes	no	yes	yes	?	yes
OR05030-58	0	0	yes	no	yes	yes	?	yes
OR05030-59	0	0	?	yes	yes	?	yes	yes
OR05030-60	0	0	yes	no	yes	yes	yes	yes
OR05030-61	0	0	no	no	?	?	no	?
OR05030-62	0	0	no	dead	yes	dead	no	yes
OR05030-63	1	1	no	no	no	no	no	no
OR05030-64	1	1	yes	no	no	?	?	?
OR05030-65	1	1	no	dead	?	dead	no	?
OR05030-66	1	1	?	dead	?	dead	?	?
OR05030-67	0	0	?	no	yes	?	?	yes
OR05030-68	0	0	no	no	yes	yes	no	yes
OR05030-69	0	0	yes	?	?	yes	yes	yes
OR05030-70	0	0	yes	yes	yes	yes	yes	yes
OR05030-71	0	0	yes	yes	yes	?	yes	yes
OR05030-72	0	0	yes	yes	yes	yes	yes	yes

Appendix 1 (Continued)

Entry	RYSC3	ADG2 *Bbv*I	Phenotype **20 DAI** rep 1	Phenotype **20 DAI** rep 2	Phenotype **40 DAI** rep 1	Phenotype **40 DAI** rep 2	Phenotype **20 DAI** Average	Phenotype **40 DAI** Average
OR05030-73	0	0	yes	yes	yes	?	yes	yes
OR05030-74	0	0	yes	?	yes	yes	yes	yes
OR05030-75	0	0	yes	no	yes	yes	?	yes
OR05030-76	1	1	no	no	no	no	no	no
OR05030-77	0	0	?	no	yes	yes	?	yes
OR05030-78	0	0	yes	yes	yes	yes	yes	yes
OR05030-79	1	1	no	dead	no	dead	no	no
OR05030-80	1	1	no	no	no	no	no	no
OR05030-81	0	0	yes	?	yes	yes	yes	yes
OR05030-82	1	1	no	no	no	no	no	no
OR05030-83	0	0	yes	?	yes	yes	yes	yes
OR05030-84	0	0	yes	yes	yes	?	yes	yes
AO95245-2	0	0	no	no	?	?	no	?
OR00030-1	1	1	no	no	no	no	no	no

Appendix 2 Molecular marker scores (1= PVY resistant); (0= PVY susceptible) with ELISA data measured at 405 nm absorbance for the full-sib family OR05030 at 20 and 40 days after inoculation (DAI)

Entry	RYSC3	ADG2 *Bbv*I	ELISA cutoff (0.381) **20 DAI** rep 1	ELISA cutoff (0.424) **20 DAI** rep 2	ELISA cutoff (0.313) **40 DAI** rep 1	ELISA cutoff (0.257) **40 DAI** rep 2	ELISA average cutoff (0.371) **20 DAI**	ELISA average cutoff (0.285) **40 DAI**
OR05030-1	1	1	0.240	0.221	0.146	0.159	0.231	0.153
OR05030-2	1	1	0.329	0.225	0.222	0.082	0.277	0.152
OR05030-3	1	1	0.173	0.236	0.149	0.097	0.204	0.123
OR05030-4	1	1	0.228	0.108	0.114	0.097	0.168	0.105
OR05030-5	1	1	0.320	0.26	0.107	0.132	0.290	0.120
OR05030-6	1	1	0.215	0.384	0.194	0.111	0.299	0.152
OR05030-7	0	0	0.260	0.33	0.793	0.535	0.295	0.664
OR05030-8	1	1	0.273	0.278	0.124	0.475	0.276	0.300
OR05030-9	1	1	0.183	0.263	0.150	0.148	0.223	0.149
OR05030-10	1	1	0.205	0.223	0.166		0.214	0.166
OR05030-11	0	0	1.991	3.199	2.576	3.844	2.595	3.210
OR05030-12	0	0	1.978	0.259	2.563	2.269	1.119	2.416
OR05030-13	1	1	0.307	0.263	0.185	0.103	0.285	0.144
OR05030-14	1	1	0.221	0.143	0.183	0.096	0.182	0.139
OR05030-15	0	0	0.376	0.303	2.577	4	0.340	3.288
OR05030-16	0	0	2.503	2.871	3.079	4	2.687	3.540
OR05030-17	0	0	0.135	0.289	0.716	3.188	0.212	1.952
OR05030-18	0	0	0.248	0.701	1.373	2.847	0.475	2.110
OR05030-19	0	0	0.474	0.156	0.533	0.889	0.315	0.711
OR05030-20	0	0	2.433	0.328	2.012	0.131	1.380	1.072
OR05030-21	0	0	0.385	0.349	4.000	3.434	0.367	3.717
OR05030-22	1	1		0.29		0.131	0.290	0.131
OR05030-23	0	0	0.348	0.298	0.824	0.362	0.323	0.593
OR05030-24	1	1	0.184	0.242	0.179	0.122	0.213	0.151
OR05030-25	1	1	0.172	0.397	0.203	0.21	0.285	0.207
OR05030-26	1	1	0.181	0.182	0.249	0.13	0.182	0.190
OR05030-27	1	1	0.262	0.236	0.192	0.112	0.249	0.152
OR05030-28	0	0	0.291	0.285	0.151	0.149	0.288	0.150
OR05030-29	0	0	0.305	2.596	2.034	2.552	1.451	2.293
OR05030-30	0	0	0.754		1.289		0.754	1.289
OR05030-31	0	0	2.223		3.139		2.223	3.139
OR05030-32	1	1	0.263	0.256	0.276	0.156	0.259	0.216
OR05030-33	1	1	0.195		0.173		0.195	0.173
OR05030-34	1	1	0.930	0.318	2.179	2.489	0.624	2.334

Appendix 2 (Continued)

Entry	RYSC3	ADG2 *Bbv*I	ELISA cutoff (0.381) **20 DAI** rep 1	ELISA cutoff (0.424) **20 DAI** rep 2	ELISA cutoff (0.313) **40 DAI** rep 1	ELISA cutoff (0.257) **40 DAI** rep 2	ELISA average cutoff (0.371) **20 DAI**	ELISA average cutoff (0.285) **40 DAI**
OR05030-35	0	0	0.310		1.248		0.310	1.248
OR05030-36	0	0	0.228	0.793	2.183	3.453	0.511	2.818
OR05030-37	0	0	0.320		3.657		0.320	3.657
OR05030-38	1	1	0.218		0.167		0.218	0.167
OR05030-39	1	1	0.302	0.185	0.203	0.228	0.243	0.215
OR05030-40	0	0	0.796	0.182	2.107	2.393	0.489	2.250
OR05030-41	0	0	1.789	0.296	1.321	3.484	1.043	2.402
OR05030-42	0	0	0.237	1.268	1.778	1.238	0.753	1.508
OR05030-43	0	0	0.437	0.366	2.195	2.399	0.402	2.297
OR05030-44	0	0	0.263	0.219	0.313	0.303	0.241	0.308
OR05030-45	1	1	0.344		0.159		0.344	0.159
OR05030-46	1	1	0.263	0.187	0.129	0.153	0.225	0.141
OR05030-47	1	1	0.270	0.256	0.248	0.082	0.263	0.165
OR05030-48	1	1	0.334	0.215	0.293	0.148	0.275	0.221
OR05030-49	1	1	0.165		0.210		0.165	0.210
OR05030-50	1	1	0.224		0.141		0.224	0.141
OR05030-51	0	0	0.627	0.383	1.465	3.187	0.505	2.326
OR05030-52	0	0	2.018	2.082	1.406	3.253	2.050	2.329
OR05030-53	0	0	1.931	0.763	2.720	3.527	1.347	3.123
OR05030-54	0	0	2.820	1.096	2.383	2.342	1.958	2.362
OR05030-55	1	1	0.298		0.120		0.298	0.120
OR05030-56	0	0	2.428	1.561	2.710	4	1.995	3.355
OR05030-57	0	0	2.825	0.294	1.859	2.99	1.560	2.425
OR05030-58	0	0	1.472	0.253	1.964	3.731	0.862	2.848
OR05030-59	0	0	0.998	1.053	1.788	3.143	1.026	2.465
OR05030-60	0	0	0.331	0.318	1.764	0.782	0.325	1.273
OR05030-61	0	0	0.388	0.314	1.065	0.607	0.351	0.836
OR05030-62	0	0	0.347		0.754		0.347	0.754
OR05030-63	1	1	0.356	0.278	0.161	0.186	0.317	0.174
OR05030-64	1	1	0.381	0.253	0.165	0.198	0.317	0.181
OR05030-65	1	1	0.280		0.195		0.280	0.195
OR05030-66	1	1	0.280		0.223		0.280	0.223
OR05030-67	0	0	3.127	0.255	3.137	1.498	1.691	2.318
OR05030-68	0	0	0.322	1.561	1.367	4	0.941	2.683
OR05030-69	0	0	1.482	0.194	2.644	3.477	0.838	3.061

Appendix 2 (Continued)

Entry	RYSC3	ADG2 *Bbv*I	ELISA cutoff (0.381) **20 DAI** rep 1	ELISA cutoff (0.424) **20 DAI** rep 2	ELISA cutoff (0.313) **40 DAI** rep 1	ELISA cutoff (0.257) **40 DAI** rep 2	ELISA average cutoff (0.371) **20 DAI**	ELISA average cutoff (0.285) **40 DAI**
OR05030-70	0	0	2.639	2.255	2.389	3.67	2.447	3.029
OR05030-71	0	0	1.240	0.385	1.490	1.517	0.812	1.504
OR05030-72	0	0	2.538	0.786	3.064	2.728	1.662	2.896
OR05030-73	0	0	1.358	0.672	1.887	4	1.015	2.944
OR05030-74	0	0	0.377	0.225	2.458	3.477	0.301	2.967
OR05030-75	0	0	2.324	0.282	1.743	3.006	1.303	2.375
OR05030-76	1	1	0.236	0.305	0.319	0.122	0.270	0.221
OR05030-77	0	0	0.840	0.262	1.793	1.414	0.551	1.604
OR05030-78	0	0	2.958	2.727	3.234	3.768	2.842	3.501
OR05030-79	1	1	0.285		0.207		0.285	0.207
OR05030-80	1	1	0.244	0.228	0.182	0.126	0.236	0.154
OR05030-81	0	0	2.000	0.285	1.634	0.204	1.143	0.919
OR05030-82	1	1	0.274	0.278	0.164	0.108	0.276	0.136
OR05030-83	0	0	1.272	0.324	2.440	3.477	0.798	2.958
OR05030-84	0	0	1.743	1.44	2.292	4	1.592	3.146
AO95245-2	0	0	0.278	0.249	0.240	4	0.263	2.120
OR00030-1	1	1	0.218	0.179	0.154	0.144	0.198	0.149

Appendix 3 Molecular marker scores (1= PVY resistant); (0= PVY susceptible); with phenotypic (Yes = PVYsymptoms); (No = no symptoms); (questionable = ?) data for the full-sib Family OR05063 at 20, 40, and 60 days after inoculation (DAI).

Entry	RYSC3	ADG2 *Bbv*I	Ry_{sto} 111bp	Phenotype **20 DAI** rep 1	Phenotype **20 DAI** rep 2	Phenotype **40 DAI** rep 1	Phenotype **40 DAI** rep 2	Phenotype **60 DAI** rep 1	Phenotype **60 DAI** rep 2	Phenotype **20 DAI** Average	Phenotype **40 DAI** Average	Phenotype **60 DAI** Average
OR05063-1	0	0	1	no	no	no	?	no	no	no	?	no
OR05063-2	0	0	0	no	?	yes	yes	?	?	?	yes	?
OR05063-3	0	0	1	no	?	no	?	no	no	?	?	no
OR05063-4	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-5	0	0	0	?	?	yes	yes	yes	yes	?	yes	yes
OR05063-6	0	0	0	yes	yes	yes	yes	?	?	yes	yes	?
OR05063-7	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-8	0	0	0	no	no	?	?	?	no	no	?	?
OR05063-9	0	0	1	dead	no	no	no	dead	no	no	no	no
OR05063-10	0	0	0	yes	?	yes	yes	no	?	yes	yes	?
OR05063-11	0	0	1	yes	no	?	yes	no	?	?	yes	?
OR05063-12	0	0	1	?	?	?	?	?	?	?	?	?
OR05063-13	0	0	0	no	?	?	yes	no	no	?	yes	no
OR05063-14	0	0	0	?	no	yes	?	dead	dead	?	yes	dead
OR05063-15	0	0	0	no	no	yes	?	dead	dead	no	yes	dead
OR05063-16	0	0	0	yes	no	dead	yes	dead	dead	?	yes	dead
OR05063-17	0	0	0	no	no	?	no	no	yes	no	?	?
OR05063-18	0	0	0	no	yes	yes	yes	no	no	?	yes	no
OR05063-19	0	0	1	?	yes	no	no	dead	no	yes	no	no
OR05063-20	0	0	1	no	no	?	no	no	no	no	?	no
OR05063-21	0	0	1	?	no	?	dead	no	no	?	?	no
OR05063-22	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-23	0	0	0	?	no	dead	?	dead	dead	?	?	dead
OR05063-24	0	0	0	no	dead	dead	dead	dead	dead	no	dead	dead
OR05063-25	0	0	1	?	no	no	no	no	no	?	no	no
OR05063-26	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-27	0	0	1	no	no	no	?	no	no	no	?	no
OR05063-28	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-29	0	0	1	no	no	?	no	no	no	no	?	no
OR05063-31	0	0	0	?	no	no	no	dead	no	?	no	no
OR05063-32	0	0	1	no	no	no	yes	no	no	no	?	no
OR05063-33	0	0	.	no	no	?	no	no	no			
OR05063-34	0	0	0	no	?	yes	no	dead	no	?	?	no
OR05063-35	0	0	0	no	no	?	no	dead	no	no	?	no
OR05063-36	0	0	1	no	no	no	?	no	dead	no	?	no
OR05063-37	0	0	1	?	?	?	dead	no	?	?	?	?
OR05063-38	0	0	0	yes	yes	yes	yes	?	dead	yes	yes	?
OR05063-39	0	0	0	dead	no	dead	no	dead	no	no	no	no

Appendix 3 (Continued)

Entry	RYSC3	ADG2 *Bbv*I	Ry_{sto} 111bp	Phenotype **20 DAI** rep 1	Phenotype **20 DAI** rep 2	Phenotype **40 DAI** rep 1	Phenotype **40 DAI** rep 2	Phenotype **60 DAI** rep 1	Phenotype **60 DAI** rep 2	Phenotype **20 DAI** Average	Phenotype **40 DAI** Average	Phenotype **60 DAI** Average
OR05063-40	0	0	1	no	dead	?	dead	no	dead	no	?	no
OR05063-41	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-42	0	0	0	no	no	yes	yes	?	?	no	yes	?
OR05063-43	0	0	0	no	?	no	no	no	no	?	no	no
OR05063-44	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-46	0	0	1	no	no	no	no	no	No	no	no	no
OR05063-47	0	0	0	?	no	yes	?	no	yes	?	yes	?
OR05063-49	0	0	1	no	?	no	?	no	no	?	?	no
OR05063-50	0	0	0	?	?	yes	yes	no	no	?	yes	no
OR05063-51	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-52	0	0	1	no	?	?	?	no	no	?	?	no
OR05063-53	0	0	0	no	?	yes	?	?	no	?	yes	?
OR05063-54	0	0	0	no	no	?	?	no	no	no	?	no
OR05063-55	0	0	1	no	?	no	no	no	no	?	no	no
OR05063-56	0	0	1	no	no	?	no	no	no	no	?	no
OR05063-57	0	0	0	?	no	yes	yes	yes	?	?	yes	yes
OR05063-58	0	0	0	no	no	?	?	no	no	no	?	no
OR05063-59	0	0	.	no	no	no	no	no	no			
OR05063-60	0	0	1	no	no	no	?	no	no	no	?	no
OR05063-61	0	0	0	?	no	dead	dead	dead	dead	?	dead	dead
OR05063-62	0	0	1	no	no	no	?	no	no	no	?	no
OR05063-63	0	0	0	?	no	no	yes	?	yes	?	?	yes
OR05063-64	0	0	1	?	dead	?	dead	no	dead	?	?	no
OR05063-65	0	0	1	no	no	?	no	no	no	no	?	no
OR05063-66	0	0	1	no	?	?	no	dead	no	?	?	no
OR05063-67	0	0	1	no	no	?	no	no	no	no	?	no
OR05063-68	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-69	0	0	1	?	no	no	no	no	no	?	no	no
OR05063-71	0	0	0	?	no	dead	no	dead	no	?	no	no
OR05063-72	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-73	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-74	0	0	0	?	dead	dead	no	dead	dead	?	no	dead
OR05063-75	0	0	.	no	no	?	no	yes	?			
OR05063-76	0	0	0	no	yes	?	yes	no	?	?	yes	?
OR05063-77	0	0	0	no	no	no	yes	?	?	no	?	?
OR05063-78	0	0	1	no	no	no	?	no	no	no	?	no
OR05063-79	0	0	0	no	no	no	no	?	no	no	no	?
OR05063-80	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-82	0	0	0	no	no	?	yes	?	?	no	yes	?

Appendix 3 (Continued)

Entry	RYSC3	ADG2 *Bbv*I	Ry_{sto} 111bp	Phenotype **20 DAI** rep 1	Phenotype **20 DAI** rep 2	Phenotype **40 DAI** rep 1	Phenotype **40 DAI** rep 2	Phenotype **60 DAI** rep 1	Phenotype **60 DAI** rep 2	Phenotype **20 DAI** Average	Phenotype **40 DAI** Average	Phenotype **60 DAI** Average
OR05063-83	0	0	1	?	no	no	no	no	no	?	no	no
OR05063-84	0	0	0	?	no	no	?	no	no	?	?	no
OR05063-85	0	0	0	no	no	no	no	no	?	no	no	?
OR05063-86	0	0	1	?	no	no	no	no	no	?	no	no
OR05063-87	0	0	0	?	?	dead	dead	dead	dead	?	dead	dead
OR05063-88	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-89	0	0	0	no	dead	?	dead	no	dead	no	?	no
OR05063-90	0	0	1	no	no	no	no	dead	no	no	no	no
OR05063-91	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-92	0	0	1	no	no	no	yes	no	no	no	?	no
OR05063-93	0	0	0	no	?	no	no	no	dead	?	no	no
OR05063-94	0	0	0	no	no	yes	yes	?	yes	no	yes	yes
OR05063-95	0	0	0	no	no	yes	yes	dead	?	no	yes	?
OR05063-96	0	0	0	no	?	dead	dead	dead	dead	?	dead	dead
OR05063-97	0	0	0	yes	yes	yes	yes	no	no	yes	yes	no
OR05063-98	0	0	1	no	no	no	no	no	no	no	no	no
OR05063-99	0	0	1	no	no	no	no	no	no	no	no	no
NR#3-109	0	0	0	no	?	?	?	no	dead	?	?	no
OR0002-6-110	0	0	1	yes	no	?	no	dead	no	?	?	no

Appendix 4 Molecular marker scores (1= PVY resistant); (0= PVY susceptible) with ELISA data measured at 405 nm absorbance for the full-sib family OR05030 at 20, 40, and 60 days after inoculation (DAI)

Entry	RYSC3	ADG2 *Bbv*I	Ry_{sto} 111bp	ELISA cutoff (0.930) **20 DAI** rep 1	ELISA cutoff (0.963) **20 DAI** rep 2	ELISA cutoff (0.888) **40 DAI** rep 1	ELISA cutoff (0.994) **40 DAI** rep 2	ELISA cutoff (0.551) **60 DAI** rep 1	ELISA cutoff (1.200) **60 DAs** rep 2	ELISA average cutoff (0.946) **20 DAI**	ELISA average cutoff (0.941) **40 DAI**	ELISA average cutoff (0.875) **60 DAI**
OR05063-1	0	0	1	0.281	0.385	0.582	0.714	0.107	0.291	0.333	0.648	0.199
OR05063-2	0	0	0	0.221	0.325	1.335	3.383	1.870	2.181	0.273	2.359	2.026
OR05063-3	0	0	1	0.225	0.347	0.476	0.482	0.183	0.130	0.286	0.479	0.157
OR05063-4	0	0	1	0.189	0.313	0.567	0.193	0.234	0.200	0.251	0.380	0.217
OR05063-5	0	0	0	0.256	0.47	2.108	2.661	2.102	4.000	0.363	2.384	3.051
OR05063-6	0	0	0	2.266	2.902	2.260	3.588	2.160	3.133	2.584	2.924	2.647
OR05063-7	0	0	1	0.288	0.244	0.633	0.418	0.124	0.298	0.266	0.525	0.211
OR05063-8	0	0	0	0.200	1.256	2.994	2.742	2.589	2.979	0.728	2.868	2.784
OR05063-9	0	0	1		0.22	0.380	0.524		0.212	0.220	0.452	0.212
OR05063-10	0	0	0	0.972	0.991	1.911	2.716	1.463	2.411	0.981	2.313	1.937
OR05063-11	0	0	1	0.284	0.436	0.408	0.263	0.269	0.147	0.360	0.335	0.208
OR05063-12	0	0	1	0.185	0.336	0.382	0.303	0.276	0.246	0.261	0.342	0.261
OR05063-13	0	0	0	0.311	0.172	0.619	1.779	1.222	2.335	0.241	1.199	1.778
OR05063-14	0	0	0	0.220	0.495	0.955	0.926			0.357	0.940	
OR05063-15	0	0	0	0.280	0.513	1.498	3.66			0.397	2.579	
OR05063-16	0	0	0	0.523	0.365		1.051			0.444	1.051	
OR05063-17	0	0	0	0.232	0.245	0.431	0.563	1.574	2.929	0.238	0.497	2.252
OR05063-18	0	0	0	0.833	2.981	1.569	3.864	1.544	2.634	1.907	2.717	2.089
OR05063-19	0	0	1	0.232	0.251	0.336	0.339		0.379	0.241	0.337	0.379
OR05063-20	0	0	1	0.350	0.257	0.369	0.452	0.208	0.202	0.303	0.411	0.205
OR05063-21	0	0	1	0.225	0.281	0.424		0.204	0.151	0.253	0.424	0.178
OR05063-22	0	0	1	0.265	0.295	0.474	0.553	0.156	0.251	0.280	0.514	0.204
OR05063-23	0	0	0	0.255	0.355		0.93			0.305	0.930	
OR05063-24	0	0	0	0.772						0.772		
OR05063-25	0	0	1	0.335	0.177	0.432	0.452	0.274	0.158	0.256	0.442	0.216
OR05063-26	0	0	1	0.348	0.315	0.442	0.442	0.242	0.211	0.331	0.442	0.227
OR05063-27	0	0	1	0.448	0.435	0.380	0.532	0.215	0.183	0.441	0.456	0.199
OR05063-28	0	0	1	0.231	0.174	0.381	0.448	0.159	0.183	0.203	0.414	0.171
OR05063-29	0	0	1	0.273	0.199	0.394	0.321	0.144	0.247	0.236	0.358	0.196
OR05063-31	0	0	0	0.556	0.346	0.415	0.537		0.234	0.451	0.476	0.234

Appendix 4 (Continued)

Entry	RYSC3	ADG2 *Bbv*I	Ry_{sto} 111bp	ELISA cutoff (0.930) **20 DAI** rep 1	ELISA cutoff (0.963) **20 DAI** rep 2	ELISA cutoff (0.888) **40 DAI** rep 1	ELISA cutoff (0.994) **40 DAI** rep 2	ELISA cutoff (0.551) **60 DAI** rep 1	ELISA cutoff (1.200) **60 DAI** rep 2	ELISA average cutoff (0.946) **20 DAI**	ELISA average cutoff (0.941) **40 DAI**	ELISA average cutoff (0.875) **60 DAI**
OR05063-32	0	0	1	0.197	0.216	0.503	0.614	0.216	0.183	0.206	0.558	0.199
OR05063-33	0	0	.	0.202	0.289	0.574	0.393	0.210	0.256	0.245	0.484	0.233
OR05063-34	0	0	0	0.337	0.168	0.935	0.563		0.181	0.252	0.749	0.181
OR05063-35	0	0	0	0.204	0.212	0.596	0.67		0.362	0.208	0.633	0.362
OR05063-36	0	0	1	0.287	0.204	0.576	0.628	0.125		0.246	0.602	0.125
OR05063-37	0	0	1	0.232	0.258	0.424		0.166	0.201	0.245	0.424	0.184
OR05063-38	0	0	0	0.805	1.018	2.191	2.887	1.416		0.912	2.539	1.416
OR05063-39	0	0	0		0.244		0.278		0.111	0.244	0.278	0.111
OR05063-40	0	0	1	0.163		0.481		0.236		0.163	0.481	0.236
OR05063-41	0	0	1	0.242	0.213	0.547	0.427	0.173	0.258	0.228	0.487	0.216
OR05063-42	0	0	0	0.246	0.454	1.182	2.595	0.783	3.794	0.350	1.889	2.289
OR05063-43	0	0	0	0.338	0.239	0.442	0.419	0.155	0.134	0.288	0.431	0.144
OR05063-44	0	0	1	0.188	0.236	0.356	0.459	0.163	0.141	0.212	0.408	0.152
OR05063-46	0	0	1	0.191	0.252	0.481	0.649	0.176	0.193	0.222	0.565	0.185
OR05063-47	0	0	0	0.212	0.205	0.944	2.124	0.708	1.956	0.209	1.534	1.332
OR05063-49	0	0	1	0.265	0.43	0.352	0.921	0.196	0.278	0.348	0.637	0.237
OR05063-50	0	0	0	1.222	0.296	2.029	1.477	2.506	2.620	0.759	1.753	2.563
OR05063-51	0	0	1	0.141	0.272	0.696	0.635	0.156	0.170	0.207	0.665	0.163
OR05063-52	0	0	1	0.309	0.34	0.406	0.356	0.238	0.195	0.324	0.381	0.217
OR05063-53	0	0	0	0.353	0.348	1.416	1.237	1.095	2.828	0.350	1.327	1.961
OR05063-54	0	0	0	0.273	0.298	2.296	4	2.426	4.000	0.285	3.148	3.213
OR05063-55	0	0	1	0.179	0.281	0.469	0.474	0.168	0.301	0.230	0.472	0.234
OR05063-56	0	0	1	0.344	0.348	0.425	0.357	0.240	0.297	0.346	0.391	0.269
OR05063-57	0	0	0	0.259	1.796	0.799	2.607	1.074	2.713	1.028	1.703	1.894
OR05063-58	0	0	0	0.380	0.31	1.676	0.854	1.479	0.666	0.345	1.265	1.073
OR05063-59	0	0	.	0.166	0.367	0.583	0.194	0.177	0.122	0.267	0.388	0.150
OR05063-60	0	0	1	0.312	0.594	0.374	0.348	0.168	0.300	0.453	0.361	0.234
OR05063-61	0	0	0	0.582	0.238					0.410		
OR05063-62	0	0	1	0.243	0.242	0.478	0.543	0.200	0.205	0.243	0.510	0.203
OR05063-63	0	0	0	0.259	0.213	0.447	0.892	0.271	2.466	0.236	0.669	1.369
OR05063-64	0	0	1	0.321		0.535		0.239		0.321	0.535	0.239
OR05063-65	0	0	1	0.403	0.317	0.538	0.363	0.161	0.233	0.360	0.450	0.197
OR05063-66	0	0	1	0.166	0.316	0.950	0.411		0.247	0.241	0.680	0.247
OR05063-67	0	0	1	0.241	0.189	0.619	0.746	0.158	0.246	0.215	0.683	0.202
OR05063-68	0	0	1	0.233	0.387	0.339	0.214	0.238	0.085	0.310	0.276	0.161

Appendix 4 (Continued)

Entry	RYSC3	ADG2 *Bbv*I	Ry_{sto} 111bp	ELISA cutoff (0.930) **20 DAI** rep 1	ELISA cutoff (0.963) **20 DAI** rep 2	ELISA cutoff (0.888) **40 DAI** rep 1	ELISA cutoff (0.994) **40 DAI** rep 2	ELISA cutoff (0.551) **60 DAI** rep 1	ELISA cutoff (1.200) **60 DAI** rep 2	ELISA average cutoff (0.946) **20 DAI**	ELISA average cutoff (0.941) **40 DAI**	ELISA average cutoff (0.875) **60 DAI**
OR05063-69	0	0	1	0.240	0.214	0.476	0.214	0.173	0.244	0.227	0.345	0.208
OR05063-71	0	0	0	1.625	0.292		2.994		1.978	0.959	2.994	1.978
OR05063-72	0	0	1	0.320	0.172	0.459	0.231	0.174	0.275	0.246	0.345	0.224
OR05063-73	0	0	1	0.303	0.235	0.641	0.549	0.138	0.284	0.269	0.595	0.211
OR05063-74	0	0	0	0.251			0.347			0.251	0.347	
OR05063-75	0	0	.	0.244	0.449	0.581	0.57	0.445	0.568	0.346	0.576	0.506
OR05063-76	0	0	0	1.785	2	1.558	3.872	1.714	3.241	1.893	2.715	2.477
OR05063-77	0	0	0	0.360	0.382	0.440	1.603	0.813	0.977	0.371	1.022	0.895
OR05063-78	0	0	1	0.241	0.472	0.455	0.294	0.202	0.270	0.356	0.375	0.236
OR05063-79	0	0	0	0.305	1.6	1.434	0.648	0.172	2.209	0.952	1.041	1.191
OR05063-80	0	0	1	0.290	0.41	0.521	0.443	0.215	0.166	0.350	0.482	0.191
OR05063-82	0	0	0	3.281	2.147	2.568	2.792	1.583	1.920	2.714	2.680	1.751
OR05063-83	0	0	1	0.185	0.209	0.578	0.594	0.240	0.263	0.197	0.586	0.251
OR05063-84	0	0	0	0.180	0.302	0.650	0.491	0.151	0.301	0.241	0.571	0.226
OR05063-85	0	0	0	0.202	0.2	0.342	0.309	0.199	1.330	0.201	0.325	0.764
OR05063-86	0	0	1	0.297	0.222	0.505	0.516	0.158	0.236	0.259	0.511	0.197
OR05063-87	0	0	0	0.761	0.923					0.842		
OR05063-88	0	0	1	0.326	0.354	0.739	0.273	0.229	0.269	0.340	0.506	0.249
OR05063-89	0	0	0	0.654		0.611		0.160		0.654	0.611	0.160
OR05063-90	0	0	1	0.368	0.299	0.518	0.264		0.293	0.333	0.391	0.293
OR05063-91	0	0	1	0.129	0.368	0.580	0.537	0.203	0.302	0.249	0.559	0.252
OR05063-92	0	0	1	0.284	0.276	0.621	0.576	0.143	0.191	0.280	0.598	0.167
OR05063-93	0	0	0	1.815	2.047	1.511	1.618	1.640		1.931	1.564	1.640
OR05063-94	0	0	0	0.312	0.244	1.117	0.534	1.281	2.278	0.278	0.825	1.779
OR05063-95	0	0	0	0.167	0.225	1.174	0.8		2.244	0.196	0.987	2.244
OR05063-96	0	0	0	3.264	0.567					1.916		
OR05063-97	0	0	0	3.908	2.189	3.004	3.395	2.935	4.000	3.048	3.199	3.467
OR05063-98	0	0	1	0.180	0.185	0.201	0.653	0.340	0.249	0.182	0.427	0.294
OR05063-99	0	0	1	0.208	0.209	0.219	0.627	0.401	0.314	0.208	0.423	0.357
NR#3-109	0	0	0	1.669	2.334	2.513	1.876	2.849		2.002	2.195	2.849
OR00002-6-110	0	0	1	0.195	0.159	0.275	0.461		0.168	0.177	0.368	0.168