CHARACTERIZING LATE BLIGHT RESISTANCE OF PARENTAL GENOTYPES USED IN THE NORTH DAKOTA STATE UNIVERSITY POTATO BREEDING PROGRAM

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

James Martinson Bjerke

In Partial Fulfillment
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Science

November 2022

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Characterizing Late Blight Resistance of Parental Genotypes Used in the North Dakota State University Potato Breeding Program

By

James Bjerke

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Asunta Thompson
Chair

Gary Secor

Andrew Robinson

Marisol Berti

Approved:

November 21, 2022 — Date

Richard Horsley — Department Chair

ABSTRACT

The potato is an important food crop, and late blight is a potato disease costing growers millions of dollars. Utilizing cultivars with late blight resistance is the longest-term option to manage the disease. This two-part study identified genetic resistance to late blight present in North Dakota State University potato germplasm. More than 750 families were screened using a multiyear detached leaf assay. ND8277B-5, Dakota Trailblazer, EB8109-1, ND028856B-1Russ, and Stirling, were found to be the most successful parents. Additionally, 236 clones were evaluated for six late blight resistance (R) genes: R1, R2, R3, RB, Rpi-smira1, and Rpi-ber1. At least one R gene was found in 136 clones. The R1 gene was most prevalent. R1, R2, R3, and RB genes were present in ND14358AB-1, while three R genes were present in Etb 5-31-3 and J101-K6. These evaluations can guide breeding efforts for R gene stacking, developing a durable resistance to late blight.

ACKNOWLEDGMENTS

I want to thank:

Dr. Asunta Thompson for her incredible knowledge of all-things-potato, and especially her endless patience.

The rest of my thesis committee: Dr. Marisol Berti, Dr. Gary Secor, and Dr. Andy Robinson for giving me their valuable time and advice that I will carry with me forever.

Richard Nilles for his great work across all aspects of the breeding program and the infinite conversation topics that we indulged in while working.

Viviana Rivera for all her hard work over many years conducting the detached leaf assay, without which this project would not exist.

Curt Doetkott for assisting with the statistical analysis.

Felicity Merritt for all her support both academically and emotionally. I'm so proud and lucky to be able to call her my wife in 2023.

All the graduate students that helped me in my time at NDSU. Through many friendships forged, my life has been made better by all of you in ways you can never imagine.

And of course, my amazing family who have always been there for me with never-ending love and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

INTRODUCTION

Solanum tuberosum L., cultivated potato, is one of the most important food crops grown in the world. The plant produces an edible, underground tuber, with leafy foliage above ground. The potato is grown in most countries of the world, with nearly 360 million metric tons produced annually, and is the sixth most widely grown crop (FAOSTAT 2020). China, India, Ukraine, and Russia rank first through fourth, respectively for production. The United States ranked fifth, producing 18.8 million metric tons in 2020 (FAOSTAT 2020).

Late blight is a disease caused by the common oomycete *Phytophthora infestans* (Mont.) de Bary (Kim and Graham 2008). Damage caused each year due to the disease can vary greatly, but associated costs may be up to $210 million in lost revenue, with $77 million spent on fungicidal control in the U.S. alone (Guenthner et al. 2001). The negative economic impact due to late blight in developing countries is over $3.5 billion (White and Shaw 2010). Late blight causes more damage than any other potato disease, with an estimated $6 billion in financial losses per year worldwide (Haverkort et al. 2009).

Management of late blight via the adoption of resistant cultivars is the most durable long-term option available (Nowicki et al. 2012). Understanding what resistance genes are present in the North Dakota State University potato breeding program germplasm is important for focused breeding efforts to overcome late blight.

CHAPTER 1. LITERATURE REVIEW

Potato

Potato (*Solanum tuberosum* L.) is important to the world's food supply, with almost 360 million metric tons produced worldwide, annually (FAOSTAT 2020). In the United States and Canada alone, over 21 million metric tons of potatoes were produced in 2020 (USDA-NASS 2020). The potato contains many vitamins, including C and B_6, nutrients and minerals, including protein and potassium, and high amounts of carbohydrates; in addition, it produces the highest number of calories per hectare of any other staple crop (Ensminger and Ensminger 1993). Primary production is for processing, tablestock, livestock feed, and seed (USDA-NASS 2020). In 2019, over 66% of the potato production in the U.S. went to processors for producing French fries, chips, dehydrated potatoes, and other products (USDA-NASS 2020).

The potato is a member of the Solanaceae family, and is related to tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.), nightshade (*Solanum dulcamara* L.), and pepper (*Capsicum annuum* L.) (Bradeen and Haynes 2011). Potatoes are a perennial plant that produce tubers (swellings of modified stems known as stolons) below ground, and small green fruit above ground (Bradeen and Haynes 2011). The fruits are the product of either cross-fertilization, or, rarely, self-fertilization of the plant's flowers (Bradeen and Haynes 2011). The true seed found within the fruits are primarily used in potato breeding programs to obtain new germplasm from crosses, with the tuber being the primary propagule, and the resulting plant a clone of that tuber (Bradeen and Haynes 2011). When the tuber is cut for planting, each segment is referred to as a seed piece (Bradeen and Haynes 2011). In the United States, potato growers should use certified seed tubers, as they are free of most viral loads accumulated during the clonal propagation process. Tubers are the only part of the potato plant that can be safely eaten

by humans, as the leaves, stems, fruits, and roots all contain toxic glycoalkaloids, including solanine (Bradeen and Haynes 2011). The plant uses these glycoalkaloids as a natural fungicide and insecticide, as well as a defense against various animals (Bradeen and Haynes 2011). Potato plants grow best in loose, well-drained soil, with organic matter present (Bradeen and Haynes 2011). Potatoes are typically planted in the spring and harvested in late summer or early fall in northern growing regions (Leap et al. 2017). For most cultivars, it takes 90 to 120 days after planting for tubers to fully mature; afterwards, harvested tubers can be stored in dark, cool, well-ventilated storages for many months (Leap et al. 2017).

Potato originated in the Andes Mountain region in South America (Bradeen and Haynes 2011). Two main types of wild potato species exist: a "Northern" type from Peru, and a "Southern" type from Bolivia and Argentina (Bradeen and Haynes 2011). Cultivated potato is more closely related to the "Northern" species and Spooner et al. (2005) concluded that it had a single domestication event in southern Peru. In the 16th and 17th centuries, potato was introduced to the rest of the world by European explorers (Bradeen and Haynes 2011). By the 1800's, the potato had achieved widespread acceptance across much of Europe and was likely a major factor in the large population boom of the industrial age (Nunn and Qian 2011).

The potato plant is susceptible to a wide array of diseases and pests, such as early blight (*Alternaria solani* Sorauer), late blight (*Phytophthora infestans* (Mont.) de Bary), common scab (*Streptomyces scabies* Lambert and Loria), various viruses, aphids (Aphididae), the Colorado potato beetle (*Leptinotarsa decemlineata* Say), and leafhoppers (*Empoasca fabae* Harris) (O'Brien and Rich 1976). Chemical and cultural control measures have been developed for most potato diseases and pest problems, although not all are effective or economically viable (Bradeen and Haynes 2011).

Late Blight

Late blight is a disease affecting potatoes caused by the fungal-like oomycete, *Phytophthora infestans* (Mont.) de Bary (Kim and Graham 2008). Late blight is primarily known as the cause of the Irish potato famine in 1845 and 1846, where the country lost two million people to starvation and emigration (Nunn and Qian 2011). *Phytophthora infestans* (Mont.) de Bary, formerly classified as a "water mold", is part of the new kingdom Chromalveolata, and the *Pythiaceae* family, with other economically important oomycotes, such as *Pythium* and *Diasporangium* (Kim and Graham 2008).

Late blight has a significant impact on the food economy. In the late 1990's it was estimated that $287 million ($507 per hectare) in the United States was lost per year to late blight (Guenthner et al. 2001). Yield reductions cost $136 million and $33 million worth of potatoes were lost in storage (Guenthner et al. 2001). The total cost globally is estimated to be ~5.2 billion Euros a year (more than US $6 billion) (Haverkort et al. 2009).

Controlling late blight is achieved through cultural practices, fungicide use, and resistant cultivars (Nowicki et al. 2012). Fungicides cost growers in the United States approximately $77 million a year, about one quarter of the estimated total cost spent on potato production (Guenthner et al. 2001). Fungicides generally do not work well against oomycetes, as oomycetes have a unique cellular composition, with very little chitin in their cell walls (Judelson and Blanco 2005). While fungicides may be effective in some phases of spore growth, most are seen as protectants and used as a preventative measure, prior to infection occurring (Judelson and Blanco 2005; Bohl et al. 2003). Fungicides were applied to 97% of planted potato fields in 2016; chlorothalonil and mancozeb were the most popular, used on 79 and 56% of planted potato hectares, respectively (USDA-NASS 2017). Cultural practices for control also include proper

disposal of infected plant and tuber tissues, irrigation timing, and controlling possible alternate hosts, such as nightshades or tomato (Kirk et al. 2004).

Phytophthora infestans can reproduce and spread in only a few days, making it difficult to control (Nowicki et al. 2012). *Phytophthora infestans* is heterothallic, with two mating types: A1 and A2. The distinction between the two is the release of differing sex hormones (Judelson 1997; Kim et al. 2005). Asexual reproduction occurs when only one of the mating types is present. Conversely, sexual reproduction happens when both types are present (Judelson and Blanco 2005). While the A1 mating type was found globally, the A2 mating type was only present in Mexico until the late 1970's, when it was discovered in Europe (Akino et al. 2014). In the United States, only asexual reproduction had been observed until recently, when some sexual reproduction is believed to have occurred (Danies et al. 2014). Asexual reproduction results in zoospores, multinucleate wall-less spores, specialized for the dispersal of oomycetes (Judelson and Blanco 2005). Zoospores are released in batches after maturing inside the tip of specialized hyphal structures, known as sporangiospores (Haldar et al. 2006).

A differentiating feature from other fungal-like organisms are the two flagella present on zoospores that work in conjunction for mobility, both emerging from the same groove on the outside of the spore (Fry and Grünwald 2010). One flagellum is a posterior facing "whiplash" that pushes away, while the second is an anterior directed "tinsel" flagellum with smaller extruding perpendicular structures, mastigonemes, moving like oars on a boat, pulling the spore forward (Judelson and Blanco 2005). These appendages allow quick and easy movement through fluids, exacerbating the spread of late blight in wet areas (Judelson and Blanco 2005). The zoospores exhibit a "homing" pattern to find plant tissue when swimming in moisture, using specific and non-specific chemoattractants, such as amino acids and isoflavones, exuded by the

host plant (Judelson and Blanco 2005; Haldar et al. 2006). Spores can also recognize the mild electric charge on a plant surface to locate a host site (Haldar et al. 2006). Zoospores are relatively fragile, making them inadequate for spreading the disease after a cold winter (Kirk et al. 2004). In places with milder winters, late blight may survive in piles of culled potatoes, and in volunteers, into the next year (Kirk et al. 2004).

Unlike the United States, Europe and Japan have widespread intermingling populations of A1 and A2 mating types, resulting in continuous sexual reproduction (Lees et al. 2012; Akino et al. 2014). Sexual contact results in oospores instead of the asexual zoospores (Fry and Grünwald 2010). Like zoospores, oospores spread the infection of late blight; however, they have thick cell walls, making them hardy enough to survive through cold winters (Judelson and Blanco 2005; Fry and Grünwald 2010). Both types of spores are light enough to be distributed by the wind (Fry and Grünwald 2010). In the Red River Valley of the North, infections of late blight have to be blown in, or arrive in infected seed tuber tissue to initiate an infection, as there is no known nearby oospore production. Significant infection in the Red River Valley has been occurring sporadically for decades, but it is not a constant annual threat (Secor et al. 2011). In 2009 and 2010, epidemic levels were present in the Red River Valley, believed to be due to the presence of more virulent strains, combined with ideal weather conditions (Secor et al. 2011).

After a *P. infestans* zoospore lands on plant tissue, it will lose its flagella, harden, and form a non-melanized, non-pigmented, appressoria that invades the tissue by producing penetrating intracellular hyphae (Judelson and Blanco 2005). As a hemibiotroph, *P. infestans* uses living host tissue to grow and reproduce, later penetrating and killing the host cells (Birch et al. 2005). After the cells of the host tissue are dead, necrosis sets in, and other necrotrophic fungi attack the plant (Judelson and Blanco 2005). Thousands of zoospores can form in one lesion on a

leaflet (O'Brien and Rich 1976). Each spore may start a new lesion after traveling, repeating the life cycle in another four to seven days (Judelson and Blanco 2005; Nowicki et al. 2012). In just a few days, over 16 hectares of potato plants can be infected with late blight (Fry and Goodwin 1997). The optimal conditions for foliar growth and dissemination of *P. infestans* are between 12 and 18 °C with high humidity; mycelial growth is optimal at a slightly warmer temperature (20 to 24 °C) (Secor et al. 2011). Sporulation can occur in as little as three days from initial infection (Judelson and Blanco 2005).

Strains are genetic variants that propagate; there have been many different strains identified across the globe (Akino et al. 2014). Strains may be of either sexual type (Kim et al. 2012). More recent strains have been shown to be more virulent (Kim et al. 2012). Newer strains, such as US-1 (A1) and US-6 (A1), were introduced to the United States in the 1980s, and late blight has become increasingly more common ever since (Fry and Goodwin 1997). US-23 (A1) displaced US-22 (A2) and US-8 (A2), as the predominant strain found in both the United States and Canada (Hu et al. 2012; USAblight 2014; Kalischuk et al. 2016). The predominant late blight strains change regularly due to the movement of more diverse and aggressive strains (Fry and Goodwin 1997; Goodwin et al. 1998). Due to recombination, *P. infestans* regularly creates new strains in both Europe and Japan, with the latter having at least five different competing strains at any time (Lees et al. 2012; Akino et al. 2014).

Sexual recombination occurs when both A1 and A2 mating type's hyphae interact (Nowicki et al. 2012). After two growing hyphae have detected each other through released hormones, the female type will transform swelling tips of hyphae into balloon-like structures called oogoniums (Fry and Goodwin 1997). The partner forms a collar-like structure called the antheridium that the oogonium then grows through (Judelson and Blanco 2005). A germ tube is

formed, and haploid cells from each structure combine for diploid sex cell formation (Judelson and Blanco 2005). While certain strains may favor a particular sex, A1 or A2 types are not necessarily more likely to exhibit either female or male structures (Judelson and Blanco 2005). Sexual recombination greatly increases genetic variability, making it more difficult to find host plant resistance, or to control the pathogen with fungicides (Fry et al. 1992). Thick-walled oospores cause earlier infection through overwintering spores, provide long survival times in the soil, increase heterogeneity in populations, and result in reoccurring perennial problems of late blight infections; their presence may change the way an infection needs to be handled (Fry and Goodwin 1997; Judelson and Blanco 2005).

Late blight can damage all parts of the potato plant. Foliar infection results in large, dark, circular lesions with lighter edges, which rapidly spread outward through leaf veins (Vleeshouwers et al. 2000). White mycelium and sporulation of the lesion can easily be seen when dew is present on the underside of the leaflet (Bohl et al. 2003). Stems react similarly to the leaves, with large dark lesions, covered with light mycelium (Bohl et al. 2003). Tubers infected with late blight may not exhibit symptoms immediately; the infection site will become dry and firm, and a brown discoloration of the flesh will occur just under the skin (Bohl et al. 2003). Under humid conditions, potato flesh will continue to degrade, and eventually the tuber is left as a rotting mass due to accompanying soft rot (Kirk et al. 2004). Moderate temperatures between 15 and 27 °C are ideal for infection and growth (Secor et al. 2011). Cultivar, temperature, time from initial infection, and storage conditions are all factors determining the extent of late blight tuber rot (Kirk et al. 2004). Once an infection has been established in a tuber, the disease may spread to plants in the field the next season, or to other potatoes in storage (Kirk et al. 2004).

Control and Management

To combat late blight, potato and its wild relatives have evolved a series of resistance (R) genes that follow the gene-for-gene theory of host and parasite (Flor 1971; Marla 2017). These R genes initiate an immune response if the corresponding pathogen's avirulence (Avr) gene's effectors are present (Flor 1971). Single R genes were discovered in *S. demissum*, a wild relative of potato found in Mexico, over one hundred years ago (Ballvora et al. 2002). Twenty-one R genes have been identified in various relatives of potato, with 11 R genes being identified in *S. demissum* alone (Black et al. 1953; Bradeen and Haynes 2011; Marla 2017).

Late blight can damage all parts of the potato plant. Foliar infection results in large, dark, circular lesions with lighter edges, which rapidly spread outward through leaf veins (Vleeshouwers et al. 2000). White mycelium and sporulation of the lesion can easily be seen when dew is present on the underside of the leaflet (Bohl et al. 2003). Stems react similarly to the leaves, with large dark lesions, covered with light mycelium (Bohl et al. 2003). Tubers infected with late blight may not exhibit symptoms immediately; the infection site will become dry and firm, and a brown discoloration of the flesh will occur just under the skin (Bohl et al. 2003). Under humid conditions, potato flesh will continue to degrade, and eventually the tuber is left as a rotting mass due to accompanying soft rot (Kirk et al. 2004). Moderate temperatures between 15 and 27 °C are ideal for infection and growth (Secor et al. 2011). Cultivar, temperature, time from initial infection, and storage conditions are all factors determining the extent of late blight tuber rot (Kirk et al. 2004). Once an infection has been established in a tuber, the disease may spread to plants in the field the next season, or to other potatoes in storage (Kirk et al. 2004).

Proper storage practices limit spore production and the spreading of secondary infections from infected tubers (Bohl et al. 2003). Most importantly, careful inspection and removal of any tubers showing symptoms, but also minimizing bruising, cuts, skinning, and other injuries, along with correctly suberizing the tubers between 10 and 13 °C, reduce the chance of the infection spreading to other tubers during storage (Bohl et al. 2003). It is important that potentially infected tubers are put into storage dry with low relative humidity, to prevent condensation from occurring on stored tubers, causing sporulation and spreading of late blight throughout the pile (Kirk et al. 2004).

Potato breeding programs are continually introgressing late blight resistance genes into their germplasm (Haverkort et al. 2016). However, even with focused breeding efforts, using techniques involving bridge crosses, it can take decades to introduce a single R gene from a wild species to a potato cultivar, as in the case of Rpi-blb2, which took 45 years (Haverkort et al. 2016). Incorporating multiple resistance genes, known as stacking or pyramiding, is currently the most common method of resistance breeding (Jo et al. 2014). Understanding what R genes are present in germplasm is an important first step in any breeding program with the goal of increasing resistance to late blight, as this makes stacking a more targeted process (Jo et al. 2014).

Objectives

The presence and amount of specific resistance genes in the North Dakota State University potato breeding program germplasm is largely unknown. The goal of this research was to investigate and characterize R genes across hundreds of genotypes in the NDSU program. This was done by combining novel analysis of previously conducted screening research and new

laboratory efforts involving DNA extraction and PCR testing. Late blight resistance genes R1, R2, R3, RB, Rpi-smira1, and Rpi-ber1 were specifically targeted. Results are expected to permit targeted breeding efforts through gene stacking.

Literature Cited

Akino, S., D. Takemoto, and K. Hosaka. 2014. *Phytophthora infestans*: A review of past and current studies on potato late blight. *Journal of General Plant Pathology* 80:24-37.

Ballvora, A., M.R. Ercolano, J. Weiß, K. Meksem, C.A. Bormann, P. Oberhagemann, F. Salamini, and C. Gebhardt. 2002. The R1 gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. *The Plant Journal* 30:361-371.

Birch, P.R.J., A.P. Rehmany, L. Pritchard, S. Kamoun, and J.L. Beynon. 2005. Trafficking arms: Oomycete effectors enter host plant cells. *Trends in Microbiology* 14:8-11.

Black W., C. Mastenbroek, W.R. Mills, and L.C. Peterson. 1953. A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. *Euphytica* 2:173-178.

Bohl, W.H., P.B. Hamm, P. Nolte, R.E. Thornton, and D.A. Johnson. 2003. Managing late blight on irrigated potatoes in the Pacific Northwest. Pacific Northwest Extension. http://www.cals.uidaho.edu/edComm/pdf/pnw/pnw0555.pdf. Accessed March 23 2015.

Bradeen, J.M., and K.G. Haynes. 2011. Introduction to Potato. In: Genetics, Genomics, and Breeding of Potato, ed. James M. Bradeen and Chittaranjan Kole, 1-15. Enfield, NH: Science Publishers.

Danies, D., K. Meyers, M.F. Mideros, S. Restrepo, F.N. Martin, D.E.L. Cooke, C.D. Smart, J.B. Ristaino, A.J. Seaman, B.K. Gugino, N.J. Grünwald, and W.E. Fry. 2014. An ephemeral sexual population of *Phytophthora infestans* in the northeastern United States and Canada. *PLoS ONE* 9:12-33.

Ensminger, M.E., and A.H. Ensminger. 1993. Green Revolution. In: Foods & Nutrition Encyclopedia 2nd Edition, ed. Marion Eugene Ensminger and Audrey H. Ensminger, 1102-1105. Boca Raton, FL: CRC Press.

FAOSTAT Database. 2020. faostat3.fao.org. Accessed February 20 2022.

Flor, H.H. 1971. Current status of the gene-for-gene concept. *Annual Review of Phytopathology* 9:275-296.

Fry, W.E., and S.B. Goodwin. 1997. Re-emergence of potato and tomato late blight in the United States. *Plant Disease* 81:1349-1357.

Fry, W.E., S.B. Goodwin, J.M. Matuszak, L.J. Spielman, and M.G. Milgroom. 1992. Population genetics and intercontinental migrations of *Phytophthora infestans*. *Annual Review of Phytopathology* 30:107-130.

Fry, W.E., and N.J. Grünwald. 2010. Introduction to Oomycetes. *The Plant Health Instructor* DOI:10.1094/PHI-I-2010-1207-01.

Goodwin, S.B., C.D. Smart, R.W. Sandrock, K.L. Deahl, Z.K. Punja, and W.E. Fry. 1998. Genetic change within populations of *Phytophthora infestans* in the United States and Canada during 1994 to 1996: Role of migration and recombination. *Phytopathology* 88:939-949.

Guenthner, J.F., K.C. Michael, and P. Nolte. 2001. The economic impact of potato late blight on US growers. *Potato Research* 44:121-125.

Haldar, K., S. Kamoun, N. L. Hiller, S. Bhattacharje, and C.V. Ooij. 2006. Common infection strategies of pathogenic eukaryotes. *Nature Reviews Microbiology* 4:922-931.

Haverkort, A.J., P.M. Boonekamp, R. Hutten, E. Jacobsen, L.A.P. Lotz, G.J.T. Kessel, J.H. Vossen, and R.G.F. Visser. 2016. Durable late blight resistance in potato through dynamic varieties obtained by cisgenesis: Scientific and societal advances in the DuRPh project. *Potato Research* 59:35-66.

Haverkort, A.J., P.C. Struik, R.G.F. Visser, and E. Jacobsen. 2009. Applied biotechnology to combat late blight in potato caused by *Phytophthora infestans*. *Potato Research* 52:249-264.

Hu, C., F.G. Perez, R. Donahoo, A. McLeod, K. Myers, K. Ivors, G. Secor, P.D. Roberts, K. L. Deahl, W.E. Fry, and J.B. Ristaino. 2012. Recent genotypes of *Phytophthora infestans* in the eastern United States reveal clonal populations and reappearance of mefenoxam sensitivity. *Plant Disease* 96:1323-1330.

Jo, K., C. Kim, S. Kim, T. Kim, M. Bergervoet, M.A. Jongsma, R.G.F. Visser, E. Jacobsen, and J.H. Vossen. 2014. Development of late blight resistant potatoes by cisgene stacking. *BMC Biotechnology* 14:50.

Judelson, H. S., and F.A. Blanco. 2005. The spores of *Phytophthora*: Weapons of the plant destroyer. *Nature Reviews Microbiology* 3:47-58.

Judelson, H.S. 1997. The genetics and biology of *Phytophthora infestans*: Modern approaches to a historical challenge. *Fungal Genetics and Biology* 22:65-76.

Kalischuk, M., M. Harding, R. Howard, R.D. Peters, C. Wijekoon, J. LeBoeuf, S. Sabaratnam, D. Waterer, B. Bizimungu, K. Al-Mughrabi, and L. Kawchuk. 2016. Characterization of the *Phytophthora infestans* strains causing late blight provides new disease prevention strategies. *American Journal of Potato Research* 94:211-250.

Kim E., and L.E. Graham. 2008. EEF2 analysis challenges the monophyly of Archaeplastida and Chromalveolata. *PLoS ONE* 3(7): e2621.

Kim, K., S. Eom, S. Lee, H. Jong, S. Kamoun, and Y.S. Lee. 2005. A genetic marker associated with the A1 mating type locus in *Phytophthora infestans. Journal of Microbiology and Biotechnology* 15:502-509.

Kim, H., H. Lee, K. Jo, S.M.M. Mortazavian, D.J. Huigen, B. Evenhuis, G. Kessel, R.G.F. Visser, E. Jacobsen, and J.H. Vossen. 2012. Broad spectrum late blight resistance in potato differential set plants Ma*R8* and Ma*R9* is conferred by multiple stacked *R* genes. *Theoretical and Applied Genetics* 124:923-935.

Kirk, W.W., P. Wharton, R. Hammerschmidt, F.A. Samen, and D. Douches. 2004. Michigan potato diseases late blight. Michigan State University Extension. http://www.potatodiseases.org/lateblight-bulletin.pdf. Accessed 10 April 2015.

Leap, J., D. Wong, O. Martin, and K. Yogg Comerchero. 2017. Organic potato production on California's central coast: A guide for beginning specialty crop growers. UC Santa Cruz: Center for Agroecology and Sustainable Food Systems. https://escholarship.org/uc/item/88p7w7g6. Accessed 1 June 2021.

Lees, A.K., J.A. Stewart, J.S. Lynott, S.F. Carnegie, H. Campbell, and A.M.I. Roberts. 2012. The effect of a dominant *Phytophthora infestans* genotype (13_A2) in Great Britain on host resistance to foliar late blight in commercial potato cultivars. *Potato Research* 55:125-134.

Marla, S.S. 2017. Structural analysis of resistance (R) genes in potato (*Solanum* species) genome. In: The Potato Genome, ed. Kumar Chakrabarti S., Xie C., and Kumar Tiwari J., 269-281. Cham: Springer International Publishing AG.

Nowicki, M., M.R. Foolad, M. Nowakowska, and E.U. Kozik. 2012. Potato and tomato late blight caused by *Phytophthora infestans*: An overview of pathology and resistance breeding. *Plant Disease* 96: 4-17.

Nunn, N., and N. Qian. 2011. The potato's contribution to population and urbanization: Evidence from a historical experiment. *The Quarterly Journal of Economics* 126:593-650.

O'Brien, M.J., and A.E. Rich. 1976. Potato Diseases, 25-27. *USDA Agriculture Handbook (No. 474)* U.S. Department of Agriculture. Washington, DC.

Secor, G., K. Kinzer, and V. Rivera-Varas. 2011. Plant disease alert – Late blight. NDSU extension. http://www.ndsu.edu/fileadmin/potatopathology/PP1565_Late_Blight_Alert.pdf. Accessed 15 November 2014.

Spooner, D.M., K. McLean, G. Ramsay, R. Waugh, and G.J. Bryan. 2005. A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proceedings of the National Academy of Sciences of the United States of America* 102: 14694-14699.

USAblight Database. 2014. www.usablight.org. Accessed 5 April 2015.

USDA-NASS. 2017. 2016 Agricultural chemical use survey: Fall potatoes. https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/2016_Corn_Potatoes/ChemUseHighlights_FallPotato_2016.pdf . Accessed 9 April 2018.

USDA-NASS. 2020. Statistics by Subject. https://www.nass.usda.gov/Statistics_by_Subject . Accessed 2 June 2021.

Vleeshouwers, V.G., W.V. Dooijeweert, F. Govers, S. Kamoun, and L.T. Colon. 2000. The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. *Planta* 210:853-864.

White S., and D. Shaw. 2010. Breeding for host resistance: The key to sustainable potato production. PPO- Special Report 14:125-132.

CHAPTER 2. DETACHED LEAF ASSAY ANALYSIS USING A DENDROGRAM CLUSTERING METHOD

Abstract

The oomycete, *Phythopthora infestans* (Mont.) de Bary, causes late blight of potato and is one of the major diseases affecting the crop. Resistance genes (R genes) can be an effective way to control the disease. There are two main forms of genetic resistance to late blight in potato: a quantitative resistance that slows, but does not stop, the infection, and a hypersensitive resistance that interacts with a specific late blight strain and causes cell death at the infection location preventing further disease spread. In order to understand the late blight resistance found in the North Dakota State University potato breeding program, more than 750 families were screened using a detached leaf assay from 2002 to 2014. The results of these screenings were analyzed using the dendrogram clustering method, to find families, and therefore parents, with high resistance to late blight. Of the 778 families tested, a majority were susceptible to late blight; however, several clones were identified exhibiting resistance via parental analysis. Thirty parental genotypes exhibited significant late blight resistance more than two times across all years of the detached leaf assay. ND8277B-5, Dakota Trailblazer, EB8109-1, ND028856B-1Russ, and Stirling were the most successful, being parents to 17, 10, eight, eight, and eight progeny families exhibiting resistance, respectively. As this study does not identify what genetic resistance is present in these parents, further research would be necessary to identify what specific resistances these genotypes possess.

Introduction

Late blight is a disease caused by the oomycete *Phythopthora infestans* (Mont.) de Bary that affects potatoes (Kim and Graham 2008). Costing over $6 billion globally each year, late blight has a significant impact on potato production (Haverkort et al. 2009). Several wild potato species near their center of origin in South America, have been found with resistance to late blight, believed to be due to a close coevolution with *P. infestans* (Song et al. 2003). Crossing wild species such as *Solanum bulbocastanum* or *Solanum demissum* with potato has had mixed results (Song et al. 2003). Hybridization may have a negative impact on yield or influence other traits that have been selected for or bred into cultivars over time; in some cases, it may be physically or physiologically impossible to achieve (Song et al. 2003).

The common cultivated potato is autotetraploid (2n = 4x = 48). Over 200 wild potato species have been identified, with a majority being diploid, ranging from diploid to hexaploid, providing wide genetic resources for interploidy breeding (Milbourne et al. 2007; Watanabe 2015). It is more complicated to breed at a tetraploid level than at the diploid level (Carputo and Frusciante 2011). For instance, with a diallelic locus (A and a), there are five possible genotype classes (AAAA, AAAa, AAaa, Aaaa, aaaa), compared with three genotype classes (AA, Aa, aa) for a diploid. Combined with additional alleles, interactions become much more complicated.

There are many techniques for incorporating wild germplasm into cultivated potato, including conventional hybridization, protoplast fusion, embryo rescue, bridging, and ploidy manipulation (Bradeen and Haynes 2011; Watanabe 2015). Favorable parental traits may be lost during hybridization; however, backcrossing, a breeding technique utilized in many crops, is difficult, due to the many deleterious recessive genes in the genome (Bradeen and Haynes 2011). Genetic modification addresses some of these issues, but introduces legal and consumer worries

(de Koeyer et al. 2011). In 2017, the J.R. Simplot Company received approval in the United States and Canada to grow and sell its Innate® Generation 2 lines of cisgenic potato varieties that incorporate late blight resistance (J.R. Simplot Company 2017). Making things more difficult is that tuber and foliar resistance to *P. infestans* are under separate genetic control (Kirk et al. 2009).

Genes with significant qualitative resistance to late blight are termed R genes (Fry and Goodwin 1997). Potato R genes are found in leucine-rich repeat (LRR) domains, like many other plant resistance genes (Ballvora et al. 2002). At least 11 R genes have been identified and incorporated from *S. demissum*, R1 through R11; of these, R1, R2, R3, R4, and R10 have been widely utilized in European potato germplasm (Vleeshouwers et al. 2011; Ballvora et al. 2002). The R genes may only provide temporary resistance, as they may be overcome by newer strains of *P. infestans* in just a few years (Bormann et al. 2004; Fry and Goodwin 1997). Some researchers go so far as calling *P. infestans* an "R gene destroyer" (Vleeshouwers et al. 2011). The most common method of resistance breeding, currently, is to incorporate multiple resistance genes, known as stacking or pyramiding (Jo et al. 2014). It is estimated that about 50% of modern potato cultivars have genes from wild potato species, and only around 15 of the R gene-rich wild species have had any of their genetic material incorporated into modern cultivars (Bradeen and Haynes 2011).

Resistance may be achieved via a defense reaction from the plant, known as a hypersensitive response (Vleeshouwers et al. 2000). Strains of *P. infestans* have avirulence genes (Hammond-Kosack and Jones 1997). When a particular avirulence gene is present with its corresponding R gene in the potato, an incompatible interaction takes place and a hypersensitive

response occurs; explaining why varying *P. infestans* strains will interact with potato cultivars differently (Vleeshouwers et al. 2000).

Interaction between R genes in potato and the avirulence genes in *P. infestans* match Flor's gene-for-gene interaction for plant disease (Ballvora et al. 2002). Effectors are molecules produced by the pathogen that interact with and have specific effects on the host plant (Vleeshouwers and Oliver 2014). Effectors in this case refer to the avirulence proteins expressed by the present avirulence genes in the late blight strain (Vleeshouwers and Oliver 2014). When the avirulence effector from the pathogen is expressed in the plant cells from the invading hyphae without the corresponding resistance protein produced by the R genes, a suppressed immune response will occur, allowing continued growth and spread of the infection (Vleeshouwers and Oliver 2014). However, when the corresponding R proteins are present, the avirulence proteins can be recognized by specific receptors present on the plasma membrane of the plant cells, which signal further cellular processing, causing apoptosis, slowing or stopping the infection (a hypersensitive response) (Gassmann and Bhattacharjee 2012). The pretense of more resistance genes increases the chances of matching virus's specific corresponding avirulence protein and causing the resistance response (Vleeshouwers and Oliver 2014).

In a hypersensitive response, localized cell death occurs within the plant after penetration of the epidermal cells, preventing the spread of the disease (Vleeshouwers et al. 2000). The hypersensitive response on a potato leaf will look like a small black lesion, approximately 5 mm in size (Ballvora et al. 2002). A partial resistance response may occur when more cells are killed by the plant, as it takes a longer period of time for the response to occur, allowing potential spread of the infection (Vleeshouwers et al. 2000). Lesions occurring from a partial response will be slightly larger, around 1 cm in diameter (Ballvora et al. 2002).

Quantitative resistance, also known as field resistance, is generally effective and more durable, but it can be difficult to move the genes from wild species by traditional breeding (Song et al. 2003). For example, Hermsen and Ramanna (1973) relied on two bridge species to move *S. bulbocastanum* resistance genes to *S. tuberosum* (Bradeen and Haynes 2011). Later, a major quantitative resistance locus known as RB was cloned from *S. bulbocastanum* (Song et al. 2003). Quantitative resistance genes decrease the overall effect of an infection of late blight. Field resistance is usually seen as slowing, but not eliminating, the symptoms of late blight, compared with a hypersensitive reaction (Song et al. 2003). This slowing also makes it harder for the pathogen to overcome quantitative resistance genes by mating and evolution (Jo et al. 2014).

Reducing the impact of late blight in potato has been occurring through breeding for over 100 years (Bradeen and Haynes 2011). Stacking is the preferred method of using R genes, as *P. infestans* may rapidly evolve to overcome the resistance provided by only one R gene (Jo et al. 2014). Late blight strains have become more resistant to fungicides; as such, a renewed interest in host resistance in potatoes has occurred (Fry and Goodwin 1997). The first late blight resistance gene to be cloned was R1, from the wild species *S. demissum* (Ballvora et al. 2002). There are over 20 functional R genes cloned from different *Solanum* species, including 11 from *S. demissum* (Kim et al. 2012).

The North Dakota State University potato breeding program has used a dedicated crossing block of late blight resistant parents for many years, with the goal of stacking resistance genes to create new cultivars resistant to a variety of pests and environmental stresses, including late blight. From 2002 to 2014, Viviana Rivera and other members of the NDSU potato improvement team performed a detached leaf assay to assess late blight resistance in progeny families, all the genotypes resulting from a single cross, in the breeding program's potato

germplasm. Late blight resistant progeny have been found, but the resistance genes in the parental genotypes have not been determined. The objective of this study was to understand the resistance present in parental genotypes in order to guide future breeding efforts.

Materials and Methods

Plant Material

Seven hundred seventy-eight progeny families, consisting of 74,015 individuals, were evaluated using a detached leaf assay to detect resistance to late blight over 12 years (Table 2.1). Between 2002 and 2014, families were created in the greenhouse via traditional hybridizations of parental genotypes from the North Dakota State University potato breeding program, the Instituto Nacional de Investigaciones Agropecuarias (INIA) potato breeding program in Chile, and other programs around the world. Progeny families were grown in the greenhouse from true potato seed.

Table 2.1. The number of families and individuals assessed using a detached leaf assay, tested for late blight response, by year (2002 – 2014).

Year	Number of progeny families	Number of individual genotypes evaluated
2002	33	3,752
2003	37	3,734
2005	49	6,125
2006	51	4,651
2007	65	5,433
2008	50	3,857
2009	77	7,141
2010	102	9,040
2011	86	7,857
2012	90	8,829
2013	45	4,225
2014	56	5,259
Total	778	74,015

Detached Leaf Assay

Progeny families were screened for late blight resistance in the greenhouse using a detached leaf assay, from 2002 to 2014 (no evaluations occurred in 2004). Terminal leaflets of seedling plants were collected from fully expanded, mature leaves to use for testing. One leaflet per genotype, a target of 100 per family, were inoculated with 30 µl of late blight zoospores at a concentration of 20,000 zoospores per ml. To keep up with the changing strains, different isolates and cocktails of isolates were used in varying years of the study. The isolates used are shown in Table 2.2.

Table 2.2. The strains and isolates of *Phytophthora infestans* (Mont.) de Bary used to inoculate leaflets for the detached leaf assay, by year, 2002 – 2014.

Year	Strain	Mating type	Isolates used
2002	US-8	A2	693-3, 126-18C
2003	US-8	A2	693-3, 126-18C
2005	US-8	A2	693-3, 711, 714, 481
2006	US-8	A2	693-3, 711, 714, 481
2007	US-8	A2	693-3, 711, 714, 481
2008	US-8	A2	693-3, 711, 714, 481
2009	US-8	A2	693-3, 714
2010	US-8	A2	693-3, 714, 481
2011	US-8	A2	693-3, 714
2012	US-8	A2	693-3, 714
2013	US-24	A1	1044
2014	US-24	A1	1044

Inoculation on the underside of the leaflet was done with a micropipette. Inoculum was prepared by the NDSU Plant Pathology Department. The collected sporangial suspension was maintained at 5 °C for two hours to stimulate zoospore release. A hemacytometer was used to calibrate the zoospore concentration of the isolate cocktails used at 2×10^4 zoospores ml^{-1}. Following inoculation, leaflets were incubated, in sets of ten, on a sterilized plastic mesh in a plastic Rubbermaid® container, approximately 35 cm x 16 cm x 11 cm in size, lined with a moist

paper towel. Tops of the containers were sealed with a piece of plastic wrap held in place by a rubber band. The containers were incubated at 16 to 18 °C and stored with a 12-h light regime to stimulate infection. Similar evaluations were performed by Vleeshouwers et al. (1999) and Goth and Keane (1997), both finding that late blight response on potato leaflets using this method were equivalent to field response.

Five days following the inoculation, the leaflets were evaluated for mycelial growth and lesion size using a rating scale of 0 to 3, where 0 was no growth, 1 was a hypersensitive reaction, 2 a lesion of less than 1 cm with no sporulation, and 3 a large lesion (>1 cm) with sporulation (Figure 2.1). Ratings of 0 and 1 were considered resistant, while ratings of 2 and 3 were considered susceptible. This scoring method was first used by Spielman et al. (1989). The full data set of results can be found in Appendices A1 through A12.

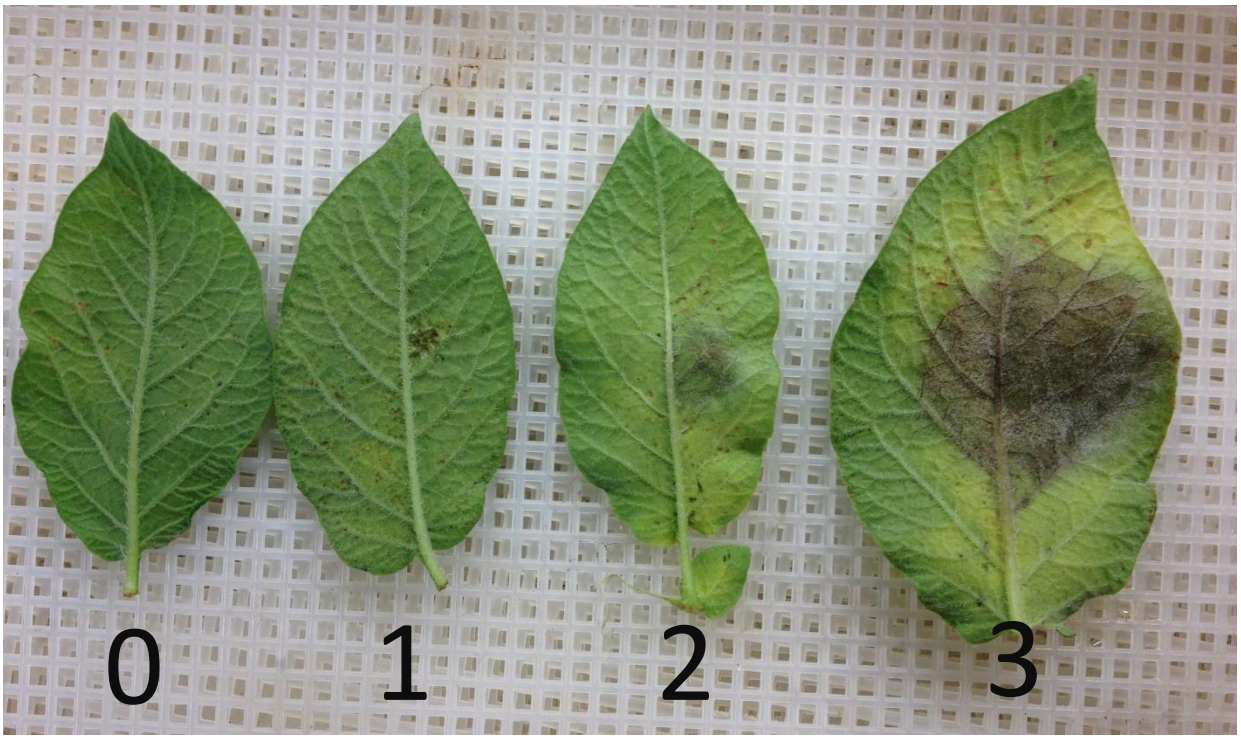

Figure 2.1. Rating scale used to determine severity of late blight infection on potato leaflets. A 0 and 1 score are considered resistant with no reaction, and a hypersensitive reaction, respectively. A score of 2 has a lesion less than 1 cm in diameter, and a 3 indicates a lesion more than 1 cm in diameter with sporulation. Photo courtesy of Viviana Rivera.

Data Analysis

Data from the detached leaf assays from 2002 to 2014 were analyzed using the dendrogram clustering method within the statistical computer program SAS 9.3® (SAS Institute, Cary, NC). The overall resistance rating of each family in one year was mapped onto a plane, the average distance between each cluster calculated, then pooled together with other clusters in succession. Like the commonly used cladogram in biological relatedness studies, the dendrogram separated families with the largest difference relative to the overall average in ratings, far apart spatially from the rest of the families present (SAS Institute, Cary, NC). This process results in a visualization of the family scores relative to each other. The largest distances between clusters of families in the dendrogram would, therefore, be outliers with high late blight resistance. It is important to note that the distance traveled along the x-axis between two families is effectively the illustration of relative distance, not the actual location on the y-axis relative to another entry.

Results And Discussion

Results of the detached leaf assays from 2002 to 2014 indicate there is resistance to late blight caused by *Phythopthora infestans* (Mont.) de Bary, present in the North Dakota State University Potato Breeding germplasm that can be utilized in future breeding efforts; however, susceptibility is the norm. Figure 2.2 exhibits the distribution of the families by the number of individual genotypes that had a resistant response (displayed in five percent increments). Four hundred forty-two families exhibited a resistant response in five percent or less of their genotypes.

As this data set resembles a log normal distribution instead of a normal distribution, a standard average and standard deviation measurement would be skewed due to the strong effect

of the long tail and cut-off important information (Zar 2010). For example, for all years combined, the average family has 11 percent of its genotypes exhibiting a resistant response, with a standard deviation of 17.8, indicating high variation and an abnormal distribution. If this was used, at 95% confidence (two sigma), only families with 47% and above of genotypes exhibiting a resistance response would be considered statistically significant; only 41 families would meet this criterion.

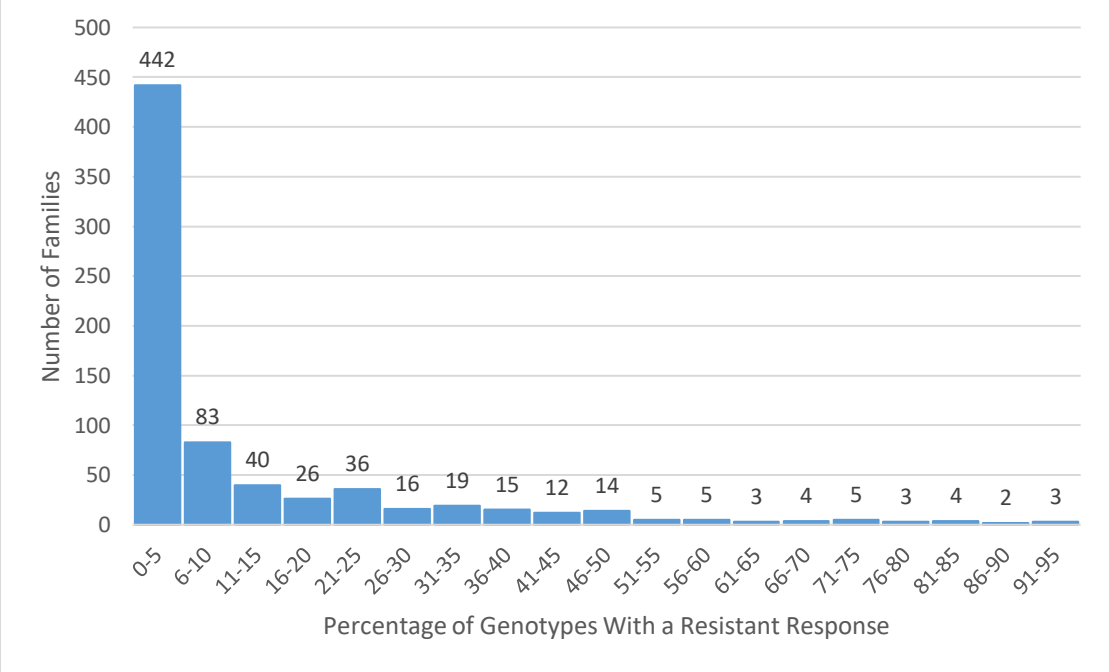

Figure 2.2. The number of families graphed across years of the detached leaf assay, grouped by percentage of crosses exhibiting a resistant response to *P. infestans* inoculation.

To capture the data more accurately, a quantile function was used that works similarly, but instead uses the percent of families relative to the median to establish a point where the resistance response is statistically significant compared with the rest of the families present. This is common in skewed data sets and in many areas of biological research, such as lethality studies and the LD_{50} test (Zar 2010). The median across all years pooled is two, and the third quartile

(the top 25% of data points) begins at 14% of genotypes in a family exhibiting a resistance response. Quartiles were chosen as they are the most common quantile (Zar 2010). Therefore, this research used 14% or higher of genotypes in a family exhibiting a resistance response as the threshold for considering a family significantly resistant compared with the overall dataset. Any reference to significant material has met or passed this threshold.

A dendrogram for each year of the assay was created using SAS® software version 9.3 (SAS Institute, Cary, NC), resulting in 13 different charts. These figures (2.3 to 2.13) accurately grouped similarly scoring families near to each other. It is important to note that spatial distance on the y-axis is not necessarily important. Instead, it is the distance needed to connect entries on the x-axis that shows how similar the score was.

2002 Results

Figure 2.3 consists of the 2002 data evaluating 33 different families. With an average of 12% and a mode of zero percent, most families on the dendrogram exhibited little to no resistance in the 2002 evaluation. Seven families had zero resistance, while a total of 22 families exhibited less than 14% resistance. Eleven families exhibited significant resistance. Two in particular, ND8497B and ND8536B, had a 51% and 49% resistance rating, respectively.

There are several shared parents between the resistant families (Table 2.3). EB8109-1 was the male parent to seven different families that exhibited resistance and a female parent to one. Stirling was the male parent of two families. ND4382-17 was the female parent for two families; however, it is worth noting that both families also had male parents that were shared with the other resistant families. Considering that, ND4382-17 may not have any resistance despite its offspring exhibiting resistance.

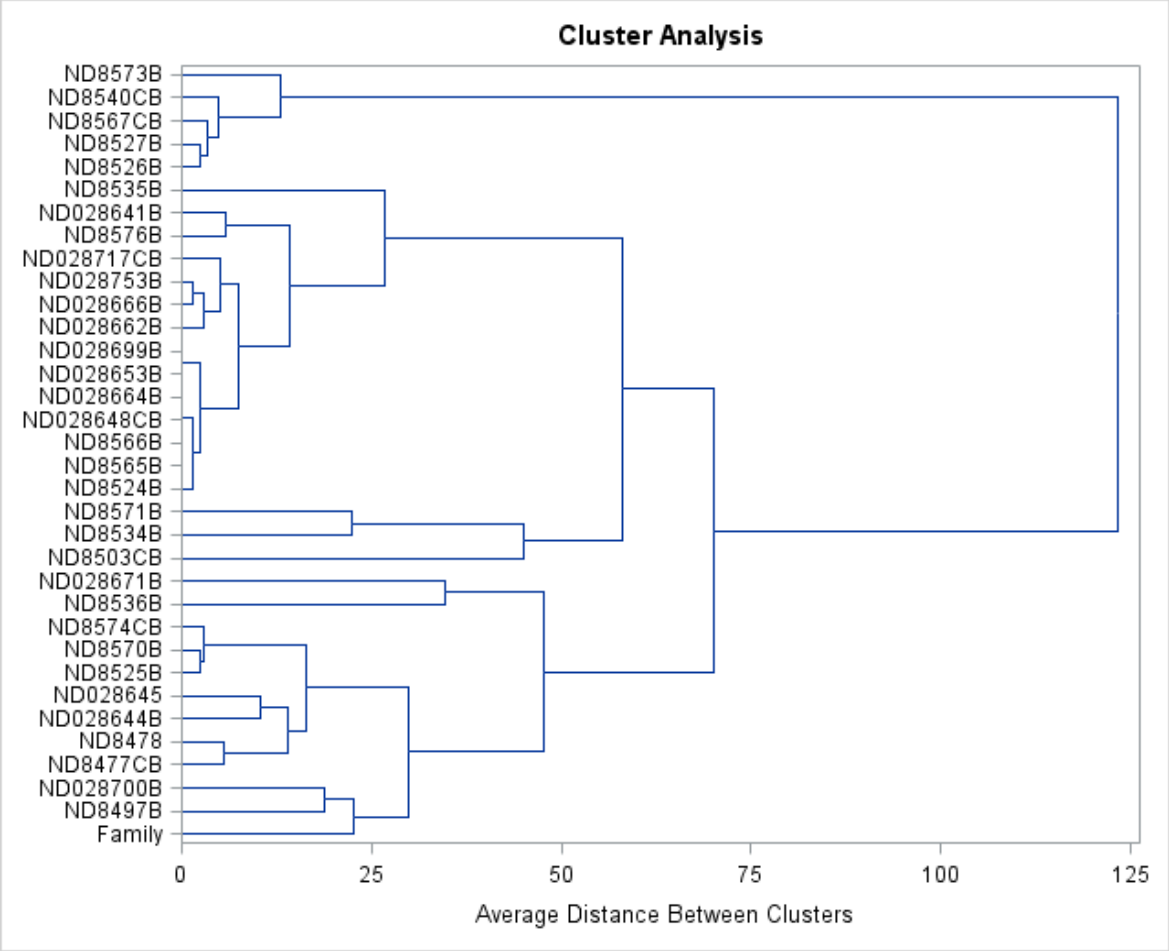

Figure 2.3. Dendrogram exhibiting the clustering distances between the late blight reactions of 33 families screened for resistance to late blight in 2002, using a detached leaf assay.

Table 2.3. Families and their respective parents exhibiting a resistance response to late blight strain US-8 in the 2002 detached leaf assays.

Family	Parentage		% Resistance
	Female	Male	
ND8477CB	ND4382-17	Stirling	24
ND8478	ND4382-17	EB8109-1	28
ND8497B	ND6585B-11	EB8109-1	51
ND8503CB	ND6691CB-3	EB8109-1	39
ND8534B	ND6961B-2	EB8109-1	23
ND8535B	ND6961B-6	EB8109-1	22
ND8536B	ND6962B-23	EB8109-1	49
ND8571B	ND8571B	EB8109-1	25
ND028644B	Tollocan	ND6947B-6	16
ND028671B	AOND98138-4 Russ	Stirling	32
ND028700B	EB8109-1	AND97279-5 Russ	22

2003 Results

The 2003 detached leaf assay evaluations (Figure 2.4) exhibited an average resistance of 6%, a median of 3%, and a mode of 0%. ND039116B exhibited 37% resistance and it, along with families ND039111B, ND039118B, ND039125B, ND039134AB, and ND039173CAb exhibited significant resistance to late blight (Table 2.4).

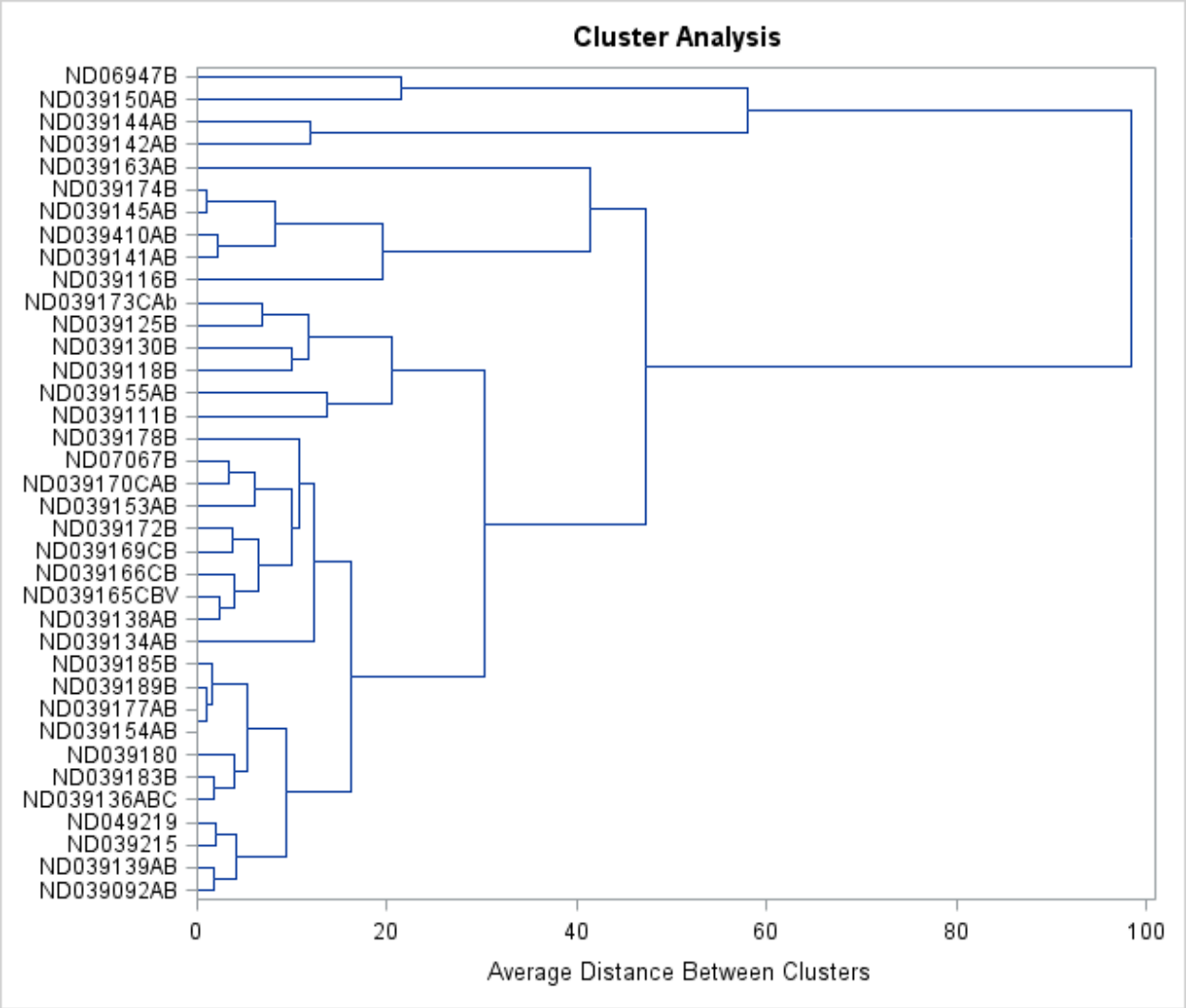

Figure 2.4. Dendrogram exhibiting the clustering distances between the late blight reactions of 37 families screened for resistance to late blight in 2003, using a detached leaf assay.

There are two common parents between the families exhibiting resistance, AND98324-1Russ was the male parent to both ND039111B and ND039118B, and ND6955B-28 was the female parent to ND039116B and ND039118B.

Table 2.4. Families and their respective parents exhibiting a resistance response to late blight strain US-8 in the 2003 detached leaf assays.

Family	Parentage		% Resistance
	Female	Male	
ND039111B	ND6934b-6	AND98324-1Russ	20
ND039116B	ND6955B-28	Dakota Pearl	37
ND039118B	ND6955B-28	AND98324-1Russ	21
ND039125B	ND6961b-1R	ND4659-5R	26
ND039134AB	ND7443Ab-18	LBR8	18
ND039173CAb	ND7799c-1	ND7443Ab-20	18

2005 Results

The average resistance for the 2005 detached leaf assay (Figure 2.5) was 8%, with nine families exhibiting significant resistance (greater than 14%). The families ND049539AB, ND049551B, ND049553B, and ND049554CB exhibited the highest resistances with 41%, 41%, 37%, and 35%, respectively. As in the 2002 results, Stirling is also present as a successful parent.

Table 2.5. Families and their respective parents exhibiting a resistance response to late blight strain US-8 in the 2005 detached leaf assays.

Family	Parentage		% Resistance
	Female	Male	
ND049539AB	ND8165B-1	ND7443Ab-153	41
ND049540CB	ND8165B-1	ND8331CB-2	18
ND049545B	ND8226B-15Russ	Dakota Trailblazer	21
ND049551B	ND8277B-5	Dakota Trailblazer	41
ND049552B	ND8277B-5	ND7519-1	26
ND049553B	ND8281B-3	ND7519-1	37
ND049554CB	ND8281B-3	ND8331CB-2	35
ND059637B	Innovator	Stirling	31
ND059641B	Innovator	Dakota Trailblazer	23

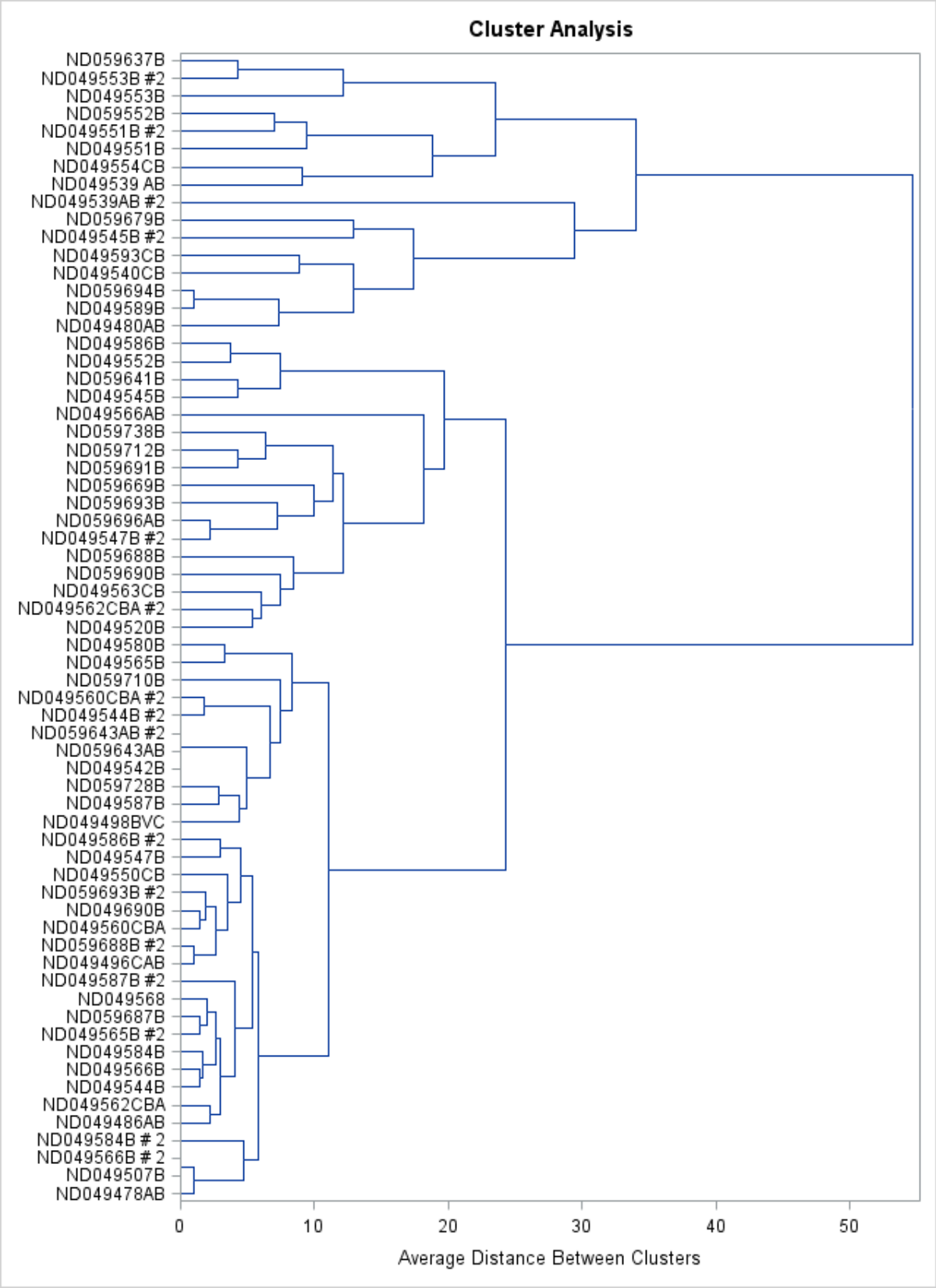

Figure 2.5. Dendrogram exhibiting the clustering distances between the late blight reactions of 49 families screened for resistance to late blight in 2005, using a detached leaf assay.

ND8277B-5 was the female parent of ND049551B and ND049552B. ND7519-1 was the male parent of both ND049552B and ND049553B. ND8281B-3 was the female parent of ND049553B and ND049554CB.

2006 Results

Figure 2.6 shows the late blight detached leaf assay dendrogram for 2006. All the families exhibiting resistance were mapped to the top part of the dendrogram next to each other. Among the families, there was an average resistance of 9% and a mode of 0%. There were 12 families, exhibiting significant resistance and two common parents between them. The highest scoring families were ND050261CB, ND050219B, ND050218B, and ND050216B with percentage resistance of 60, 48, 42, and 42, respectively.

Table 2.6. Families and their respective parents exhibiting a resistance to late blight strain US-8 in the 2006 detached leaf assays.

Family	Parentage		% Resistant
	Female	Male	
ND050216B	ND028651B-3Russ	Innovator	42
ND050218B	ND028651B-3Russ	AOND95292-3Russ	42
ND050219B	ND028651B-3Russ	ND8444b-2Russ	48
ND050255CAB	ND028711BC-1	ND7443Ab-20	17
ND050259CB	ND028770B-4R	ND8506C-6R	23
ND050261CB	ND028770B-4R	ND8512C-17R	60
ND050269CAB	ND028777CB-2	ND7443Ab-20	24
ND050270CAB	ND028799C-2	Stirling	28
ND050280CAB	ND028804CAB-4	NY131	14
ND050282CAB	ND028804CAB-5	ND7443Ab-45	24
ND060410CB	LBR3	ND028804CB-1	18
ND060411B	LBR4	ND8277B-5	32

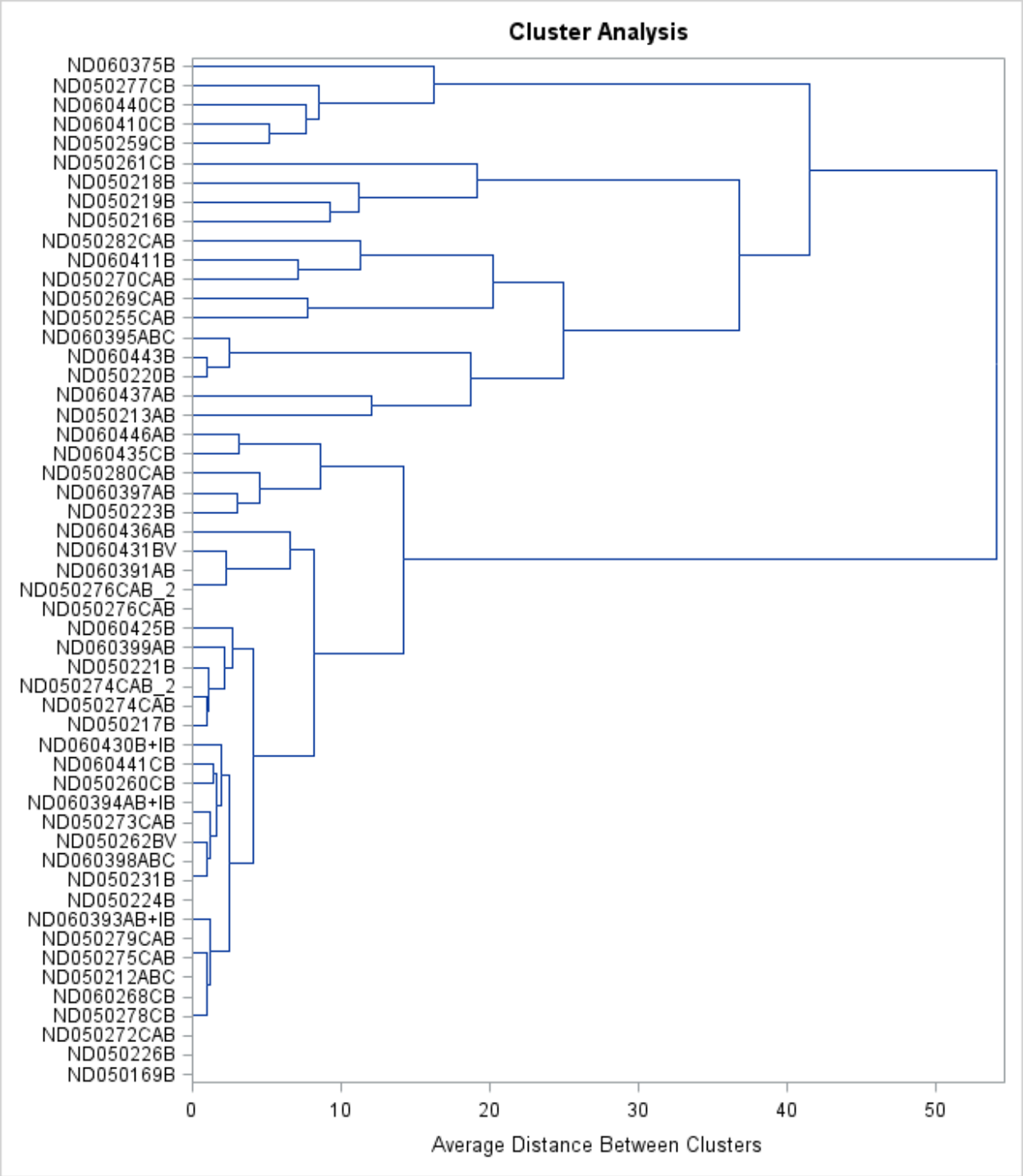

Figure 2.6. Dendrogram exhibiting the clustering distances between the late blight reactions of 53 families screened for resistance to late blight in 2006, using a detached leaf assay.

ND028651B-3Russ was the female parent of three families exhibiting significant late blight resistance, with an average of 44%, and ND028770B-4R was the female parent of two

families that averaged 42% resistance. Stirling and Innovator are also present in several pedigrees.

2007 Results

Figure 2.7 is the 2007 detached leaf assay dendrogram. The average resistance response for this year, exhibited among all families, was 13%. Of the 17 families exhibiting significant resistance to late blight, ND060601CAB, ND060623CB, ND060613B, and ND060572AB had the highest percentage resistance of 85, 75, 72, and 67, respectively (Table 2.7).

Table 2.7. Families and their respective parents exhibiting a resistance response to late blight strain US-8 in the 2007 detached leaf assays.

Family	Parentage		% Resistant
	Female	Male	
ND060566CB	ND6400C-1Russ	ND028856B-1Russ	14
ND060569AB	ND6934b-2	Etb 6-5-5	17
ND060571B	ND6934b-2	ND7192-1	32
ND060572AB	ND6934b-2	ND7443Ab-45	67
ND060574B	ND6947b-20	ND7192-1	19
ND060578B	ND6953b-34	ND7192-1	61
ND060590AB	ND7132-1R	Etb 6-5-5	14
ND060593VB	ND7132-1R	ND039087VB-3R	39
ND060601CAB	ND7192-1	ND028804Ab-1	85
ND060613B	ND7333b-7	ND7632-6	72
ND060619CB	ND7377Cb-1	LBR4	18
ND060620CB	ND7377Cb-1	NY131	15
ND060623CB	ND7377Cb-1	ND5649-1Russ	75
ND060629AB	ND7384Ab-4	R91191-2W/Y	14
ND060630ABC	ND7384Ab-4	ND7377Cb-1	18
ND060631AB	ND7390Ab-10	White Pearl	50
ND060632AB	ND7390Ab-10	ND860-2	15

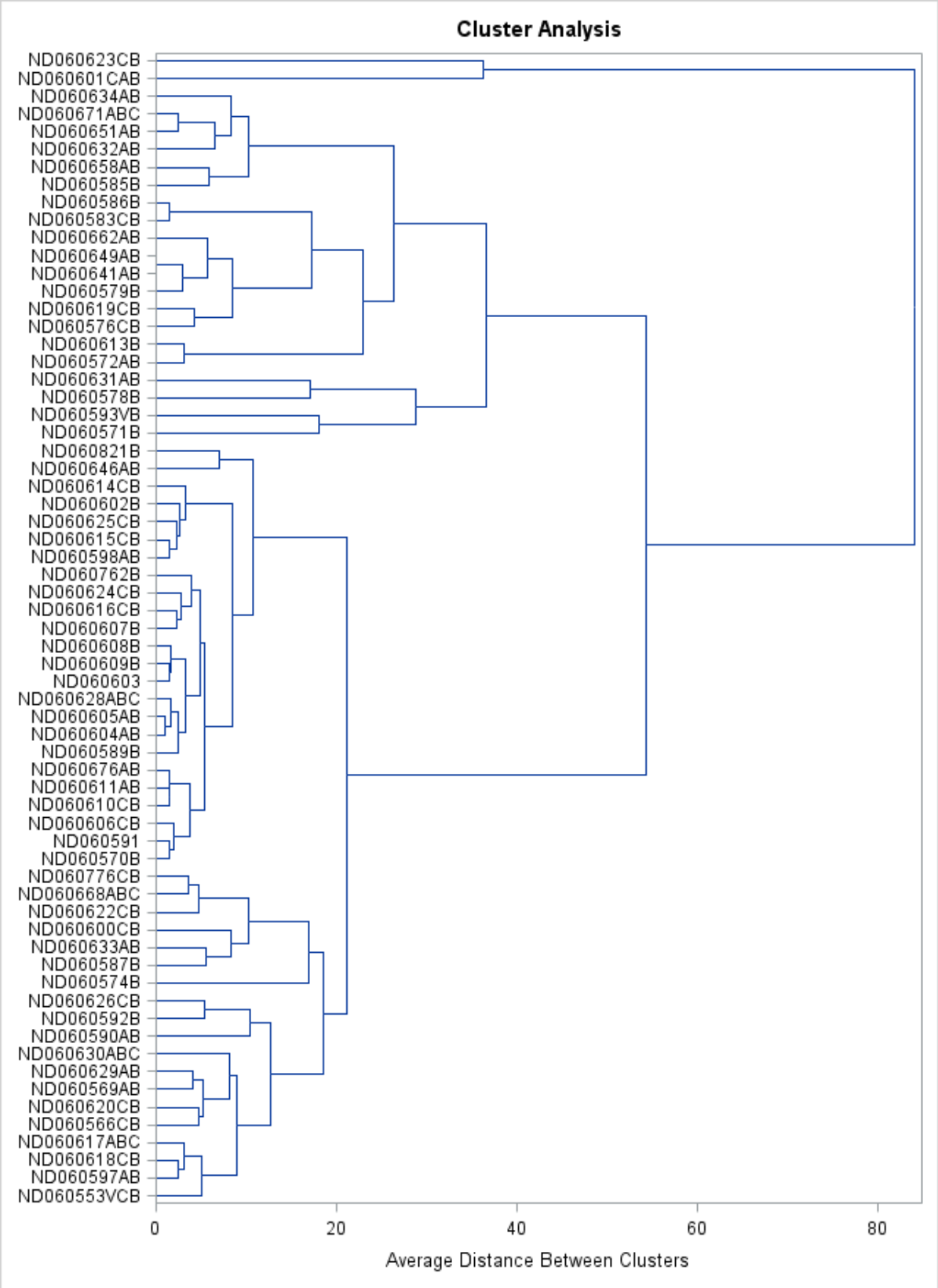

Figure 2.7. Dendrogram exhibiting the clustering distances between the late blight reactions of 65 families screened for resistance to late blight in 2007, using a detached leaf assay.

There were seven shared parents among the families exhibiting significant resistance. ND7192-1 and ND7377Cb-1 were the parents of four families, ND6934b-2 was the female parent to three families, and Etb 6-5-5, ND7384Ab-4, ND7390Ab-10, and ND7132-1R were parents of two families each.

2008 Results

There were 18 families exhibiting resistance in the 2008 detached leaf assays (Figure 2.8). The average percent of resistant genotypes within the families that exhibited resistance was 14%. ND071138B had the highest resistance to late blight at 58%.

Shared parents between the resistant families are present in Table 2.8. ND8277B-5 and ND028856B-1Russ were parents of six families exhibiting significant resistance. Additionally, ND8277B-5 was the parent to ND071138B, ND071425B, and ND071102B, all exhibiting percentage resistance over 40%. Overall, families that had ND8277B-5 as a parent had an average percent resistance of 39; ND8277B-5 was a parent to no susceptible families. ND028888CB-1 was a parent of three families, and ND8527B-94 and ND049351B-5R were the parents of two families each.

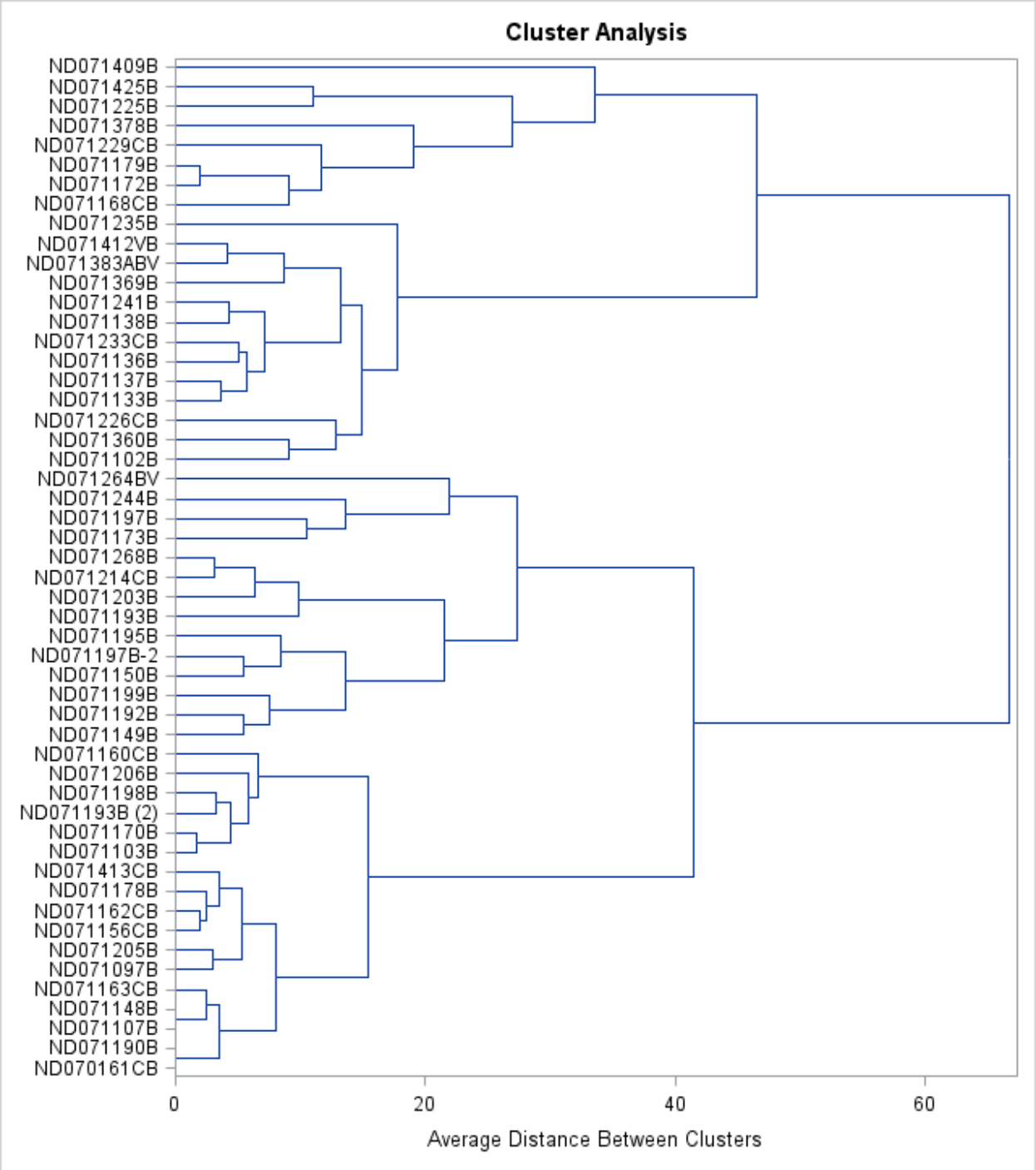

Figure 2.8. Dendrogram exhibiting the clustering distances between the late blight reactions of 50 families screened for resistance to late blight in 2008, using a detached leaf assay.

Table 2.8. Families and their respective parents exhibiting a resistance response to late blight strain US-8 in the 2008 detached leaf assays.

Family	Parentage		% Resistant
	Female	Male	
ND071102B	ND7333b-7	ND8277B-5	42
ND071136B	ND8277B-5	ND8527B-94	25
ND071137B	ND8277B-5	ND028856B-1Russ	33
ND071138B	ND8277B-5	ND049287B-4	58
ND071168CB	ND8492Cb-2Russ	ND028856B-1Russ	32
ND071172B	ND8527B-94	ND8277B-5	31
ND071179B	Dakota Ruby	ND039036B-2R	31
ND071199B	ND028671B-96	ND028856B-1Russ	17
ND071225B	ND028856B-1Russ	ND049423B-3Russ	48
ND071226CB	ND028888CB-1	AH66-4	49
ND071229CB	ND028888CB-1	ND028856B-1Russ	22
ND071241B	ND028970B-65Russ	ND028856B-1Russ	44
ND071264BV	ND039035B-9R	ND039087BV-3R	17
ND071360B	ND049227B-1	90245.1	25
ND071369B	ND049287B-3	ND028888CB-1	41
ND071409B	ND049351B-5R	ND5858	17
ND071412VB	ND049351B-5R	ND039126VB-2R	16
ND071425B	ND049382B-2	ND8277B-5	47

2009 Results

The dendrogram exhibiting the resistance results from the 2009 leaf assay (Figure 2.9) has the resistant families in the topmost half of the graph. The average percentage resistance across all families in 2009 was 15%. Twenty-nine families were observed to have significant resistance to late blight (Table 2.9).

There were seven total shared parents. The genotype ND049553B-50 was the male parent of ND081575CB, ND081579B, and ND081602CB. ND039173CAB-22 was the male parent of ND081590CAB, ND081607CAB, and ND081681CAB. Both ND081597BV and ND081598B had ND7067B-67R as their female parent. ND039087BV-3R was the female parent of ND081696BVC and ND081697BV. ND039125B-29R was the female parent of four families

that exhibited resistance: ND081702B, ND081703B, ND081704BV, and ND081705B. The progeny families of ND039125B-29R had an average percent resistance of 41%.

Table 2.9. Families and their respective parents exhibiting a resistance response to late blight strain US-8 in the 2009 detached leaf assays.

Family	Parentage		% Resistant
	Female	Male	
ND081572B	ND4659-5R	ND028940B-102R	24
ND081575CB	ND5873-23	ND049553B-50	27
ND081579B	ND6956b-13	ND049553B-50	40
ND081583B	ND6934b-2	Dakota Trailblazer	15
ND081590CAB	ND6947B-136	ND039173CAB-22	57
ND081593B	ND6961B-21PY	Gala	30
ND081597BV	ND7067B-67R	ND039087BV-3R	46
ND081598B	ND7067B-67R	95043.11	36
ND081602CB	ND7377Cb-1	ND049553B-50	49
ND081607CAB	ND7495b-6	ND039173CAB-22	34
ND081611B	ND7818-1Y	Gala	14
ND081624B	Dakota Russet	ND049545B-8Russ	15
ND081626B	Dakota Russet	ND049587B-5Russ	30
ND081630ABC	ND8277B-5	ND028926ABC-78	23
ND081636CB	ND8291C-1Russ	ND059694B-20Russ	67
ND081660B	ND8570B-1Y	Puren	17
ND081680CB	ND028813b-5	ND7377Cb-1	36
ND081681CAB	ND028813b-5	ND039173CAB-22	47
ND081682ABC	ND028926ABC-78	ND039173CAB-22	17
ND081689CBV	ND039036B-2RY	ND039165CBV-70R	48
ND081696BVC	ND039087BV-3R	ND049498BVC-38RY	25
ND081697BV	ND039087BV-3R	ND049565B-64R	21
ND081698V	ND039087BV-3R	95043.11	15
ND081701B	ND039112B-1Russ	ND059669B-2Russ	19
ND081702B	ND039125B-29R	Dakota Ruby	24
ND081703B	ND039125B-29R	ND039036B-2RY	42
ND081704BV	ND039125B-29R	ND039087BV-3R	61
ND081705B	ND039125B-29R	95043.11	36
ND081706AB	ND039134AB-2	ND049552B-42	39
ND081716CBV	ND039165CBV-70R	ND039087BV-3R	23

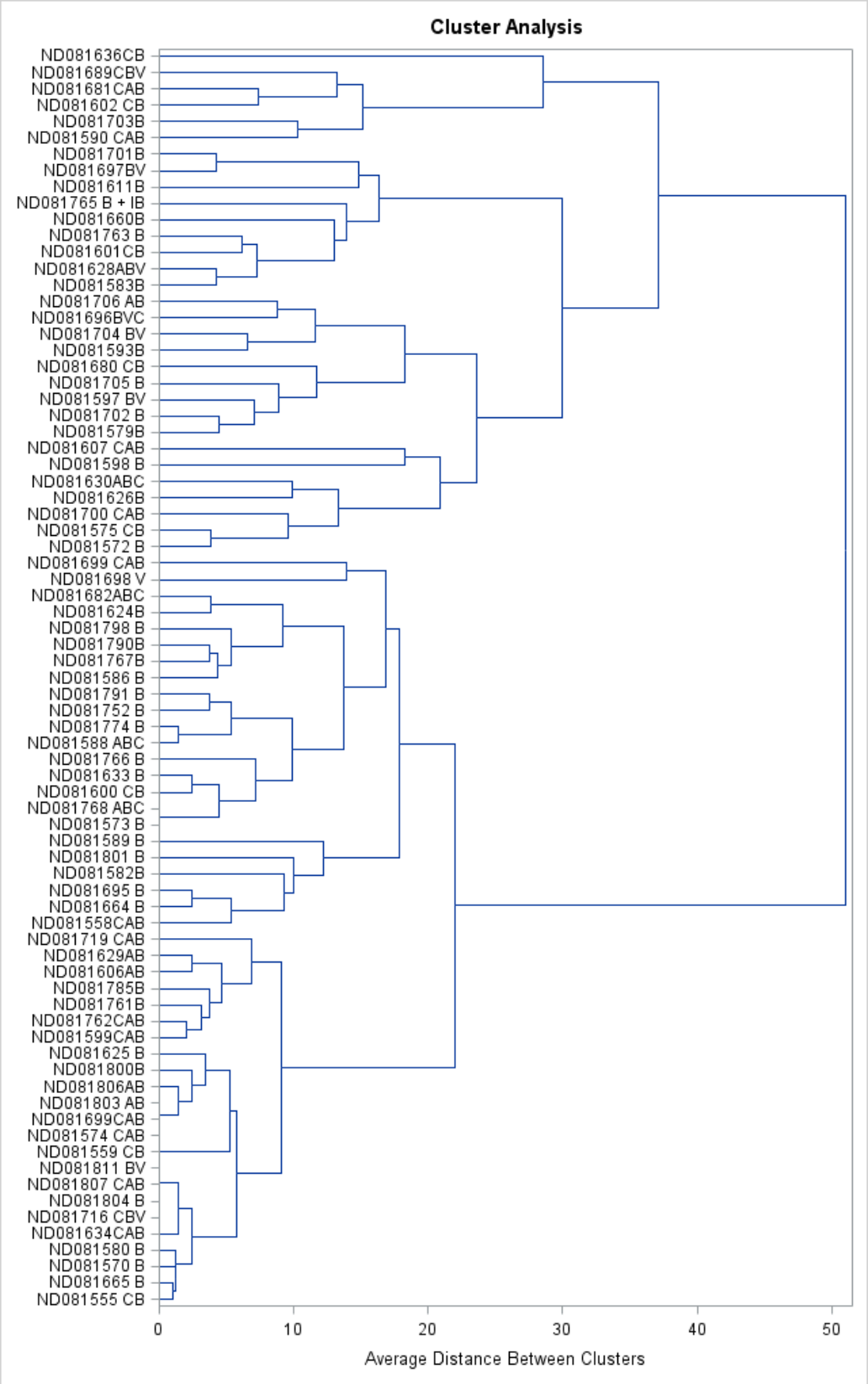

Figure 2.9. Dendrogram exhibiting the clustering distances between the late blight reactions of 77 families screened for resistance to late blight in 2009, using a detached leaf assay.

ND7377Cb-1 was the female parent of ND081602CB and the male parent of ND081680CB; however, the male parent of ND081602CB, ND049553B-50, and the female parent of ND081680CB, ND028813b-5, also exhibited resistance. ND7377Cb-1 was the female parent of two other families with low resistance (ND081600CB had a resistance of 5% and ND081601CB had resistance of 9%). This combined with the lowercase "b" designation by the NDSU potato improvement team believing it to be susceptible to late blight through other tests, makes ND7377Cb-1 an unlikely candidate for harboring the late blight resistance genes that correspond to the avirulence genes in the late blight strains used in this study.

ND028813b-5 was only present as a parent in families where the other parent was one of the shared parents (ND081680CB's male parent was ND7377Cb-1 and ND081681CAB's male parent was ND039173CAB-22). Therefore, ND028813b-5 is also unlikely to carry any late blight resistance genes in contrast with the performance of its progeny.

2010 Results

Figure 2.10 provides the dendrogram representing tiered hierarchical clustering of the 2010 late blight detached leaf assay results conducted by the potato improvement team at NDSU. With an average resistance of 47% among the significantly resistant crosses, 44 significantly resistant crosses out of 101 total, and a 22% average resistance among all crosses, the 2010 late blight detached leaf assay exhibited the most resistance of all the years.

There were 22 total shared parents between the families exhibiting late blight resistance in the 2010 leaf assays (Table 2.10). ND060378B-1 was the parent to seven families with an average resistance of 32%. ND060397AB-20 was the parent to five resistant families, with an average resistance of 42%. ND7443Ab-72Russ, ND049323C-6, ND049474ABC-1Russ,

ND049475ABC-1, King Harry, Dakota Trailblazer, and Patagonia were each parents to three families exhibiting significant late blight resistance.

Table 2.10. Families and their respective parents exhibiting a resistance response to late blight strain US-8 in the 2010 detached leaf assays.

Family	Parentage		% Resistant
	Female	Male	
ND092060ABC	ND6400C-1	ND059787AB-3Russ	39
ND092073ABC	ND7443Ab-72Russ	King Harry	14
ND092074AB	ND7443Ab-72Russ	Dakota Trailblazer	31
ND092075ABC	ND7443Ab-72Russ	ND8291C-2Russ	25
ND092077ABC	ND7443Ab-180	King Harry	59
ND092091AB	ND7519-1	ND060397AB-20	19
ND092119B	ND8277B-5	Patagonia	14
ND092121CB	ND8291C-2Russ	PA99N2-1	22
ND092140B	ND8459-2	ND060378B-1	16
ND092162CB	ND028799C-3	ND060378B-1	16
ND092163CAB	ND028799C-3	ND060397AB-20	34
ND092165CAB	ND028804CAb-5	King Harry	28
ND092178AB	ND028856B-1Russ	ND059787AB-3Russ	89
ND092181CB	ND028888cB-1	ND7799c-1	70
ND092182B	ND028970B-74	LBR8	73
ND092184AB	ND028970B-74	ND7443Ab-44	75
ND092191AB	ND028984B-1	Etb 6-21-4	72
ND092194B	ND039036B-2R	AND00272-1R	87
ND092198CABR	ND039104CAB-3	P99 N2-1	80
ND092200CAB	ND039104CAB-3	ND060397AB-20	91
ND092202B	ND039194-1Russ	Dakota Trailblazer	81
ND092205B	ND049223B-3R	Bison	18
ND092206B	ND049223B-3R	T10-12	24
ND092208B	ND049268-2R	Patagonia	91
ND092217ABC	ND049275-1	ND049475ABC-1	78
ND092220ABC	ND049323C-6	Etb 6-21-4	85
ND092222CB	ND049323C-6	LBR8	94
ND092225CB	ND049323C-6	ND060378B-1	76
ND092227CB	ND049323C-7	Dakota Trailblazer	85

Table 2.10. Families and their respective parents exhibiting a resistance response to late blight strain US-8 in the 2010 detached leaf assays (continued).

Family	Parentage		% Resistant
	Female	Male	
ND092232CAB	ND049326C-2P	Etb 6-5-5	21
ND092244B	ND049423b-1Russ	ND059852b-2Russ	20
ND092245AB	ND049423b-1Russ	ND060389AB-5Russ	61
ND092250ABC	ND049474ABC-1Russ	AOND95292-3Russ	30
ND092254ABC	ND049474ABC-1Russ	ND039194-1Russ	47
ND092257ABC	ND049474ABC-1Russ	ND060487CB-3Russ	37
ND092261ABC	ND049475ABC-1	Etb 6-21-5	31
ND092262ABC	ND049475ABC-1	ND060378B-1	38
ND092265AB	ND049589B-15Russ	Etb 6-5-5	22
ND092281B	ND059734-5R	Patagonia	24
ND092283B	ND059734-5R	ND060378B-1	14
ND092293ABC	ND059804C-10	ND060397AB-20	19
ND092297CAB	ND059809C-2P	ND050167C-3R	37
ND092326AB	ND060378B-1	Etb 6-21-5	21
ND092337AB	ND060397AB-20	ND060378B-1	46

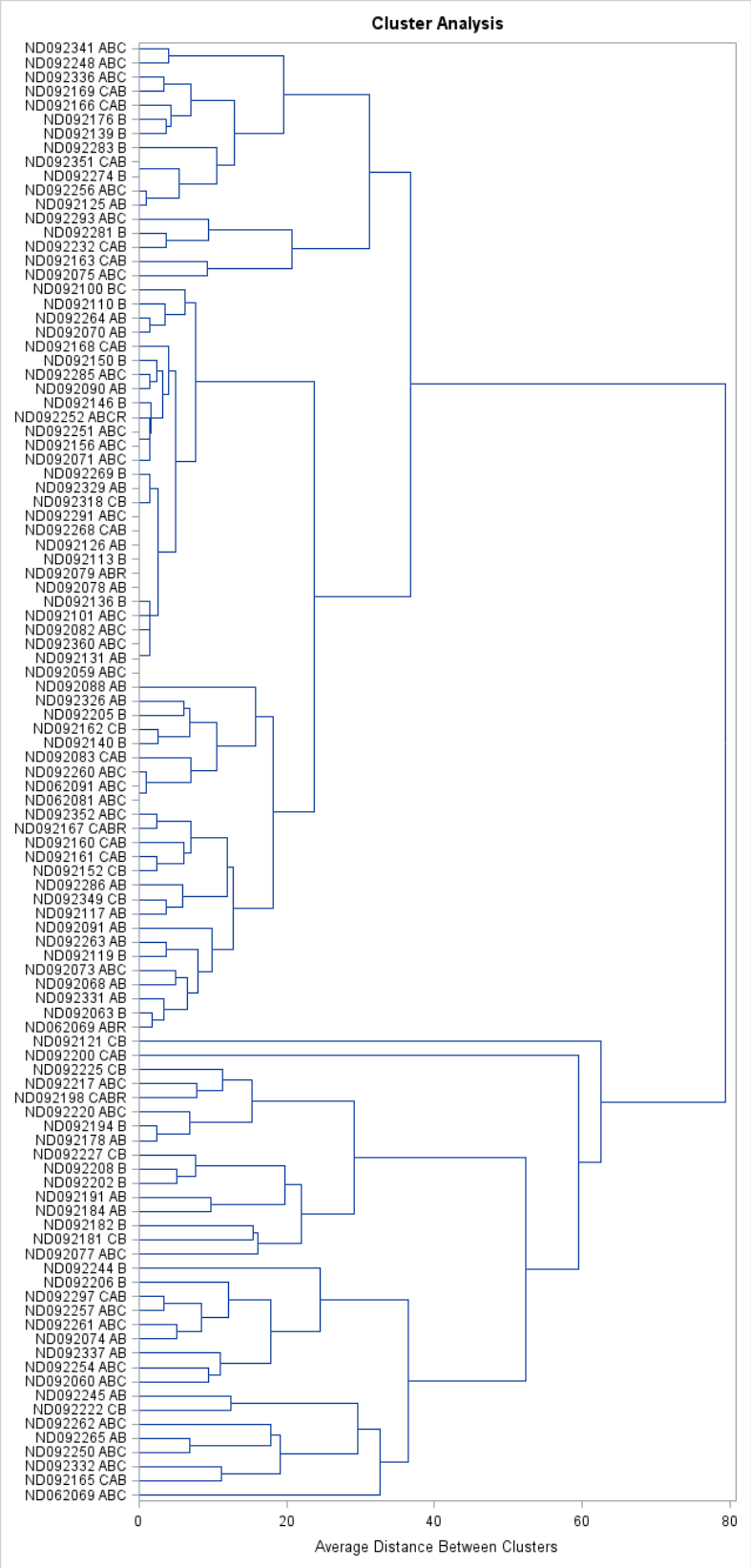

Figure 2.10. Dendrogram exhibiting the clustering distances between the late blight reactions of 102 families screened for resistance to late blight in 2010, using a detached leaf assay.

2011 Results

The 2011 late blight leaf assay families are grouped by resistance in dendrogram form in Figure 2.11, based on their clustering distances. The average resistance among all families was 8%. ND102688B and ND102695CB were the families with the highest resistance, with 52% each.

There was only one shared parent among the 15 families exhibiting significant late blight resistance (Table 2.11). ND8277B-5 was the female parent of ND102650CB and the male parent of ND102652B and ND102724B. LBR8, Patagonia, and Dakota Russet were also common parents, and their crosses have exhibited late blight resistance in 2003, 2009, and 2010 of the late blight leaf assay project. Stirling was also a parent of one of the families exhibiting a resistance response.

Table 2.11. Families and their respective parents exhibiting a resistance response to late blight strain US-8 in the 2011 detached leaf assays.

Family	Parentage		% Resistant
	Female	Male	
ND102598B	ND6934b-6	ND039194-1Russ	32
ND102600B	ND6934b-6	ND050032-4Russ	21
ND102609AB	ND7384Ab-4	ND039194-1Russ	41
ND102614B	ND7403B-5	ND070927-5Russ	32
ND102641CB	ND7799c-1	LBR8	42
ND102648B	Dakota Russet	ND060607B-4	47
ND102650CB	ND8277B-5	ND060618CB-3	38
ND102652B	ND8304-2	ND8277B-5	29
ND102661B	ND8527B-94	Stirling	19
ND102688B	ND049223B-3R	Patagonia	52
ND102695CB	ND049326C-2P	RA90213-60	52
ND102697CB	ND049326C-2P	ND049223B-3R	14
ND102700CB	ND049326C-2P	ND050174B-5R	16
ND102722AB	ND049589B-5Russ	ND7443Ab-72Russ	57
ND102724B	ND050005-1P	ND8277B-5	35

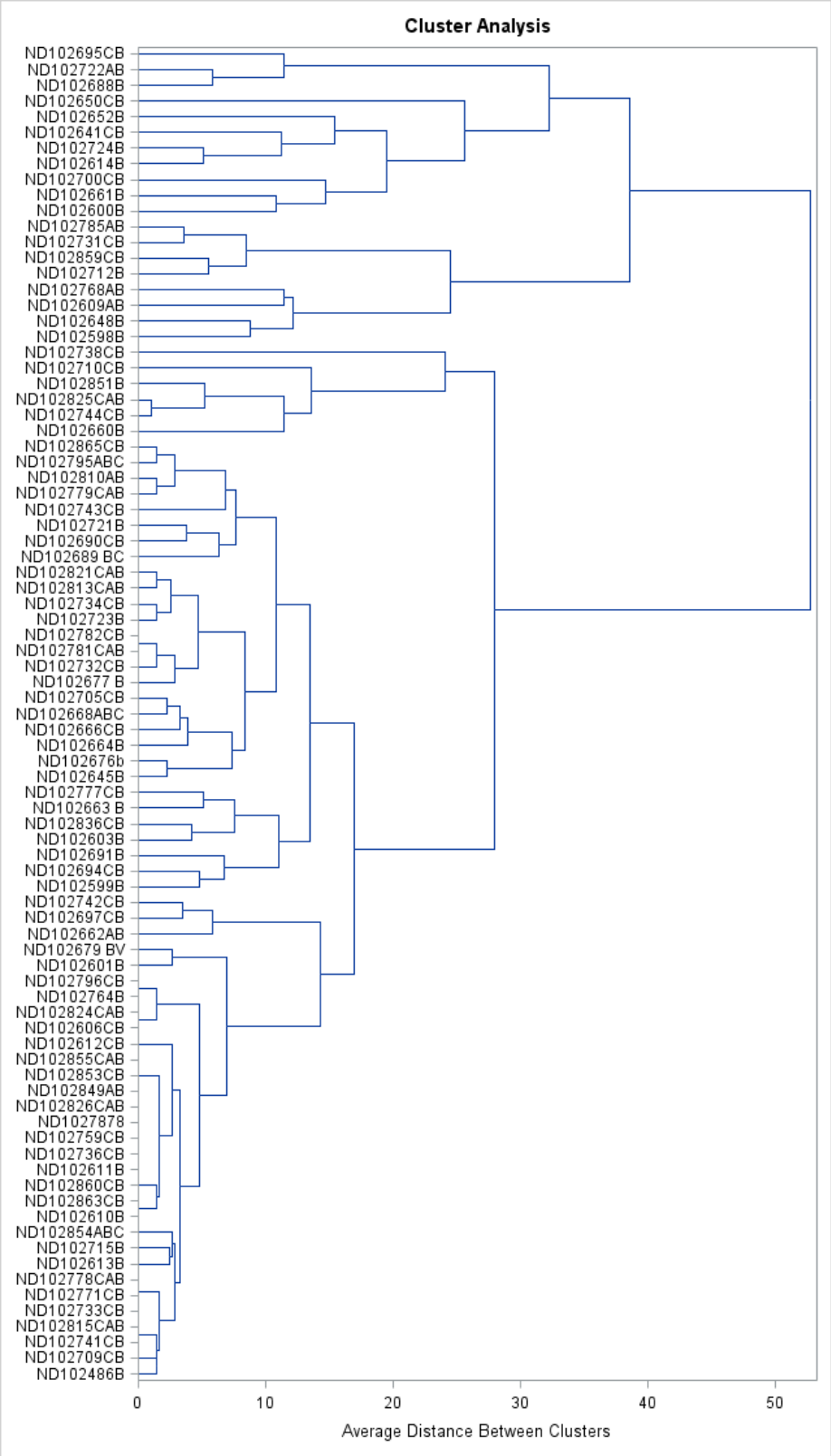

Figure 2.11. Dendrogram exhibiting the clustering distances between the late blight reactions of 90 families screened for resistance to late blight in 2011, using a detached leaf assay.

2012 Results

Figure 2.12 shows the results of the 2012 late blight screening detached leaf assay. Seventeen families, of the 90 tested, exhibited significant late blight resistance, and among those, the average resistance was 24% (Table 2.12).

Despite the lowercase "b" designation, ND050067cb-1R was the female parent of six families exhibiting resistance to late blight: ND113422CB, ND113423CB, ND113424CB, ND113425CB, ND113427CB, and ND113428CB with 26, 26, 24, 24, 26, 24 percent resistance, respectively. Yagana, ND050060Cb-4R, Stirling, Gala, and Patagonia were parents to two families that exhibited a late blight resistant response.

Table 2.12. Families and their respective parents exhibiting a resistance response to late blight strain US-8 in the 2012 detached leaf assays.

Family	Parentage		% Resistant
	Female	Male	
ND113028CB	Dakota Diamond	Stirling	15
ND113038B	Dakota Trailblazer	Russet Norkotah	25
ND113113B	Yagana	ND028742b-12REY	33
ND113114CAB	Yagana	ND039104CAB-5	21
ND113362ABC	ND028799C-3	ND060873Ab-7	31
ND113414B	ND049589B-5Russ	M7	14
ND113416B	ND050032-4Russ	Stirling	14
ND113417CB	ND050060Cb-4R	Gala	24
ND113421CB	ND050060Cb-4R	95043.11	25
ND113422CB	ND050067cb-1R	Gala	26
ND113423CB	ND050067cb-1R	Patagonia	26
ND113424CB	ND050067cb-1R	Romanze	24
ND113425CB	ND050067cb-1R	AND00272-1R	24
ND113427CB	ND050067cb-1R	ND4659-5R	26
ND113428CB	ND050067cb-1R	ND060822CB-2p	24
ND113443CB	ND059624C-4	LBR8	38
ND113448CB	ND059809C-1P	Patagonia	23

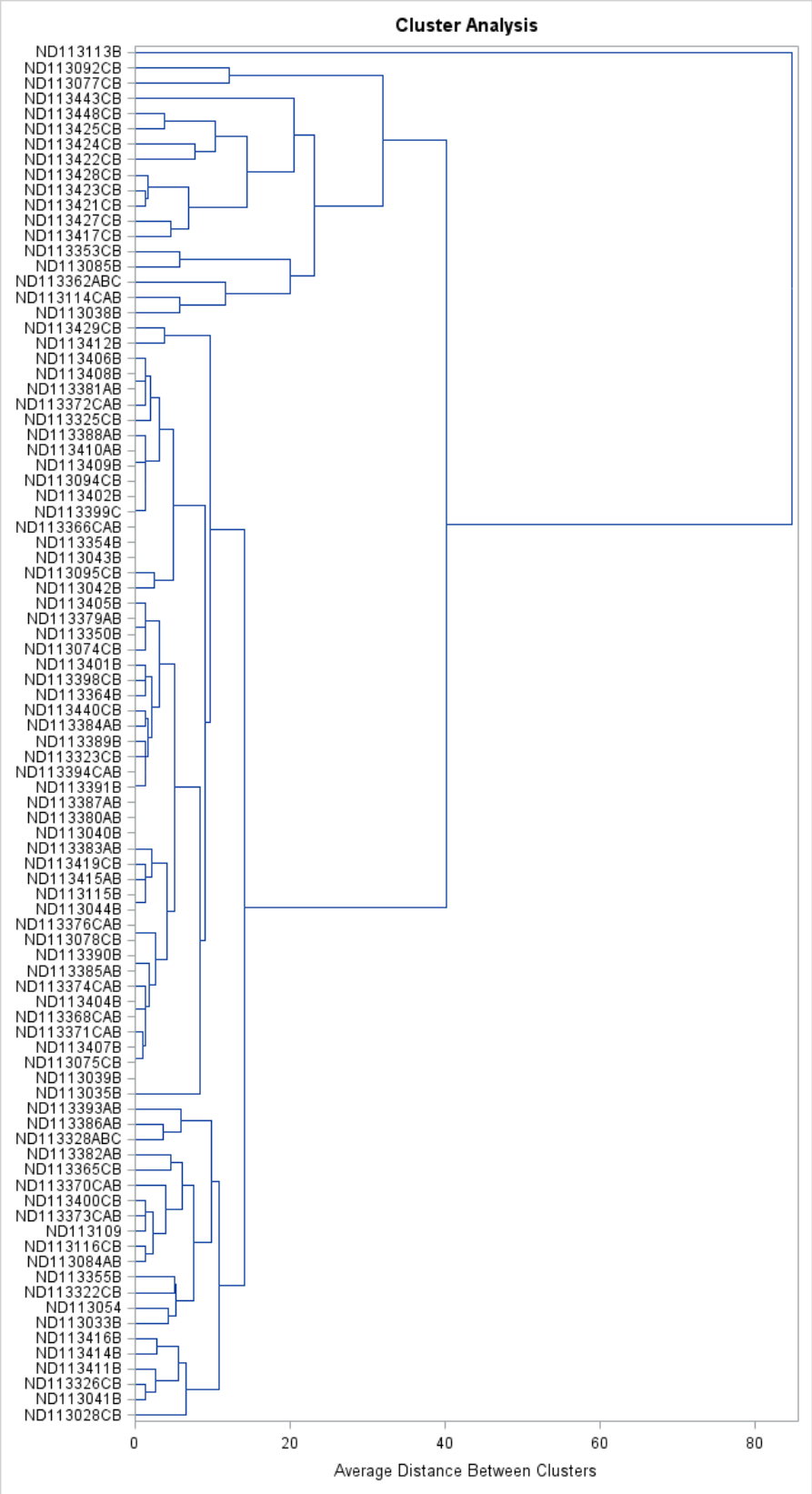

Figure 2.12. Dendrogram exhibiting the clustering distances between the late blight reactions of 90 families screened for resistance to late blight in 2012, using a detached leaf assay.

2013 Results

Analysis of the 2013 detached leaf assay identified possessing significant resistance to the tested late blight strains. Therefore, a dendrogram was not created for this year.

2014 Results

The dendrogram from 2014's detached leaf assays for late blight screening is shown in Figure 2.13. Ten families, of the 56 tested, exhibited a significant resistance response with an average of 42%. Overall, among all families evaluated, the average resistance was 9%.

There were five shared parents among the families exhibiting resistance. ND071127-1Russ was the parent of three families, while Dakota Trailblazer, ND081555CB-2Russ, ND081626B-48Russ, and ND113089B-1 were parents to two families each.

Table 2.13. Families and their respective parents exhibiting a resistance response to late blight strain US-24 in the 2013 detached leaf assays.

Family	Parentage		% Resistant
	Female	Male	
ND12212B	ND070927-2Russ	Dakota Trailblazer	55
ND12214CB	ND071127-1Russ	ND081555CB-2Russ	45
ND12215B	ND071127-1Russ	ND081761b-9Russ	41
ND12216CB	ND071239b-1Russ	ND081555CB-2Russ	66
ND12219B	ND081476B-8Russ	ND071127-1Russ	32
ND12227B	ND081626B-48Russ	ND049251B-9Russ	27
ND12234B	ND113089B-1	ND5858	29
ND12235B	ND113089B-1	ND081752B-6R	54
ND12241YB	90245.1	Dakota Trailblazer	47
ND1315TB	87HM12-16	ND081626B-48Russ	28

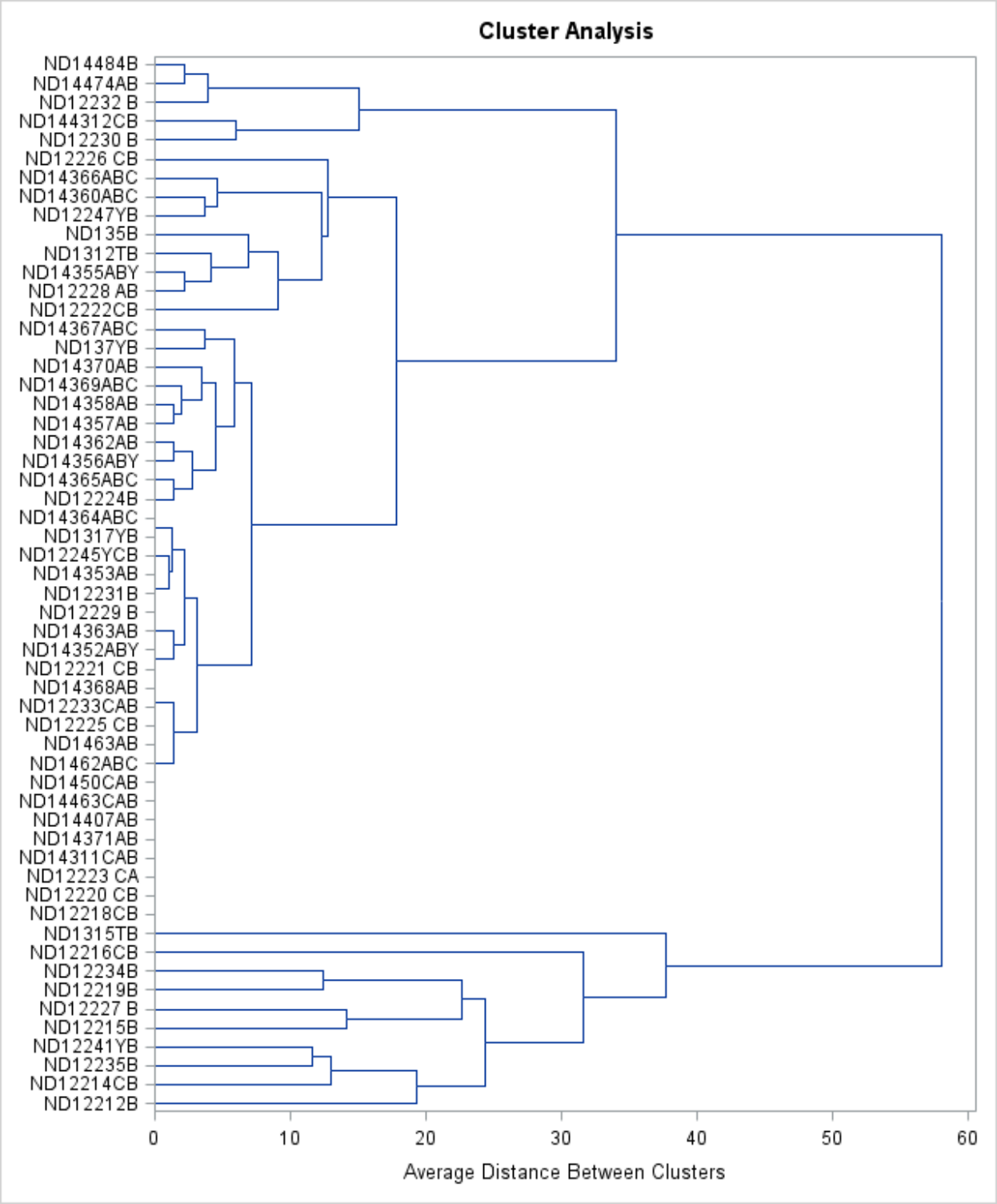

Figure 2.13. Dendrogram exhibiting the clustering distances between the late blight reactions of 56 families screened for resistance to late blight in 2014, using a detached leaf assay.

Overall Results

Table 2.14 is a list of 30 parental genotypes of families exhibiting significant late blight resistance more than two times across all years of the detached leaf assay, in order of the number of families parented. Of the 438 parents crossed over all years, this represents just 7% of the tested parental germplasm that potentially possessed late blight resistance.

Table 2.14. Parents whose progeny families exhibited significant late blight resistance in detached leaf assays from 2002 – 2014 utilizing strains US-8 and US-24.

Parental genotype	Number of progeny families exhibiting resistance as the female parent	Number of progeny families exhibiting resistance as the male parent	Total number of progeny families exhibiting resistance across years	Total number of progeny families evaluated	Percentage progeny families exhibiting resistance across years
ND8277B-5	8	6	14	15	93%
Dakota Trailblazer	1	9	10	52	19%
EB8109-1	1	7	8	18	44%
ND028856B-1Russ	2	6	8	14	57%
Stirling	0	8	8	22	36%
ND039087BV-3R	3	4	7	12	58%
ND049326C-2P	7	0	7	8	88%
ND060378B-1	1	6	7	8	88%
Patagonia	0	7	7	28	25%
LBR8	0	6	6	15	40%
ND039194-1Russ	1	5	6	6	100%
ND049223B-3R	4	2	6	8	75%
ND050067cb-1R	6	0	6	12	50%
ND7377Cb-1	4	2	6	20	30%
ND060397AB-20	1	4	5	8	63%
ND6934b-6	5	0	5	5	100%
ND7443Ab-72Russ	3	1	4	4	100%
95043.11	0	4	4	9	44%
Dakota Russet	4	0	4	12	33%
Etb 6-5-5	0	4	4	24	17%
Gala	0	4	4	11	36%

Table 2.14. Parents whose progeny families exhibited significant late blight resistance in detached leaf assays from 2002 – 2014 utilizing strains US-8 and US-24 (continued).

Parental genotype	Number of progeny families that exhibited resistance as the female parent	Number of progeny families that exhibited resistance as the male parent	Total number of progeny families that exhibited resistance	Total number of progeny families	Percent of progeny families that exhibited resistance
ND028888CB-1	3	1	4	4	100%
ND039125B-29R	4	0	4	4	100%
ND039173CAB-22	0	4	4	11	36%
ND6934b-2	4	0	4	10	40%
ND7192-1	1	3	4	13	31%
ND7384Ab-4	4	0	4	7	57%
ND7799c-1	3	1	4	5	80%
ND8527B-94	3	1	4	6	67%
Innovator	2	1	3	6	50%
King Harry	0	3	3	4	75%
ND028651B-3Russ	3	0	3	5	60%
ND028799C-3	3	0	3	7	43%
ND049323C-6	3	0	3	3	100%
ND049474ABC-1Russ	3	0	3	11	27%
ND049475ABC-1	2	1	3	9	33%
ND049553B-50	0	3	3	5	60%
ND049589B-5Russ	3	0	3	6	50%
ND050032-4Russ	1	2	3	3	100%
ND071127-1Russ	2	1	3	4	75%
ND4659-5R	1	2	3	8	38%
ND7443Ab-20	0	3	3	10	30%
ND7519-1	1	2	3	10	30%

The most common parental genotype across years was ND8277B-5, which was the parent of 14 families. It had already been identified as late blight resistant and given the upper-case B designation. The female parent of ND8277B-5 is LBR9, and the male parent is Stirling; Stirling was a parent of eight resistant families in this study. LBR9 and LBR8 (also a prominent parent,

Table 2.14) were two late blight differential lines previously found to be highly resistant to late blight (Douches et al. 2004).

Dakota Trailblazer and Stirling were parents to ten and eight families, respectively, exhibiting late blight resistance; both have previously been identified as having a high level of field resistance (Stewart et al. 1992; Bradshaw et al. 1995; North Dakota State University 2009; Brown-Donovan 2020). EB8109-1 was the parent of eight late blight resistant families and has previously been recognized as a parent conferring late blight resistance (Brown-Donovan 2020). Patagonia was the parent of seven late blight resistant families, and has previously been reported as late blight resistant (Porter et al. 2017).

As the potato has perfect flowers, with both stamens and pistils, and no sex chromosomes, it is not surprising that there does not seem to be any correlation between late blight resistance being conferred and being either a female or a male parent (Bethke and Jansky 2021).

It is important to note that this is likely not a comprehensive list of late blight resistant genotypes present in the North Dakota State University potato germplasm. The detached leaf assay only utilized six isolates of the ever-evolving *Phytophthora infestans* (Mont. de Bary), and, as such, large amounts of specific resistance could be overlooked.

Conclusion

To maximize potato breeding efforts to combat *Phytophthora infestans* (Mont.) de Bary, the causal agent of potato late blight, identifying resistance is necessary. By analyzing 13 years of late blight resistance screening using a detached leaf assay, dendrograms were created. While susceptibility was the norm, shared parents of resistant families were identified throughout the

years of the study. The most common parental genotypes were ND8277B-5, Dakota Trailblazer, EB8109-1, ND028856B-1Russ, and Stirling. Further research is required to identify the specific late blight resistance genes present in these genotypes within the North Dakota State University germplasm.

Literature Cited

Ballvora, A., M.R. Ercolano, J. Weiß, K. Meksem, C.A. Bormann, P. Oberhagemann, F. Salamini, and C. Gebhardt. 2002. The R1 gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. *The Plant Journal* 30:361-371.

Bethke, P.C. and S.H. Jansky. 2021. Genetic and environmental factors contributing to reproductive success and failure in potato. *American Journal of Potato Research* 98:24-41.

Bormann, C.A., A.M. Rickert, R.A.C. Ruiz, J. Paal, J. Lübeck, J. Strahwald, K. Buhr, and C. Gebhardt. 2004. Tagging quantitative trait loci for maturity-corrected late blight resistance in tetraploid potato with PCR-based candidate gene markers. *Molecular Plant-Microbe Interactions* 17:1126-1138.

Bradeen, J.M. and K.G. Haynes. 2011. Introduction to Potato. In Genetics, Genomics, and Breeding of Potato, ed. James M. Bradeen and Chittaranjan Kole, 1-19. Enfield, NH: Science Publishers.

Bradshaw, J.E., H.E. Stewart, R.L. Wastie, M.F.B. Dale, and M.S. Phillips. 1995. Use of seedling progeny tests for genetical studies as part of a potato (*Solanum tuberosum* subsp. tuberosum) breeding programme. *Theoretical and Applied Genetics* 90:899-905.

Brown-Donovan, K.M. 2020. Pyramiding approaches for potato disease resistance breeding. Dissertation, University of Maine.

Carputo, D. and L. Frusciante. 2011. Classical genetics and traditional breeding. In: Genetics, Genomics, and Breeding of Potato, ed. James M. Bradeen and Chittaranjan Kole, 20-40. Enfield, NH: Science Publishers.

de Koeyer, D., H. Chen, and V. Gustafson. 2011. Molecular Breeding for Potato Improvement. In: Genetics, Genomics, and Breeding of Potato, ed. James M. Bradeen and Chittaranjan Kole, 41-67. Enfield, NH: Science Publishers.

Douches, D., J. Coombs, K. Felcher, and W. Kirk. 2004. Foliar reaction to *Phytophthora infestans* in inoculated potato field trials in Michigan. *American Journal of Potato Research* 81:443-448.

Fry, W.E. and S.B. Goodwin. 1997. Re-emergence of potato and tomato late blight in the United States. *Plant Disease* 81:1349-1357.

Gassmann, W. and S. Bhattacharjee. 2012. Effector-triggered immunity signaling: from gene-for-gene pathways to protein-protein interaction networks. *Molecular Plant-Microbe Interactions* 25:862-868.

Goth, R.W. and J. Keane. 1997. A detached-leaf method to evaluate late blight resistance in potato and tomato. *American Potato Journal* 74:347-352.

Hammond-Kosack, K.E. and J.D.G. Jones. 1997. Plant disease resistance genes. *Annual Review of Plant Physiology and Plant Molecular Biology* 48:575-607.

Haverkort, A.J., P.C. Struik, R.G.F. Visser, and E. Jacobsen. 2009. Applied biotechnology to combat late blight in potato caused by *Phytophthora infestans*. *Potato Research* 52:249-264.

Hermsen, J.G.T., and M.S. Ramanna. 1973. Double-bridge hybrids of *Solanum bulbocastanum* and cultivars of *Solanum tuberosum*. *Euphytica* 22:457-466.

J.R. Simplot Company. 2017. Innate® second generation potatoes with late blight protection receive EPA and FDA clearances. Retrived from https://spudman.com/news/innate-gen-2-receives-epa-fda-clearances/. Accessed 2 June 2021.

Jo, K., C. Kim, S. Kim, T. Kim, M. Bergervoet, M.A. Jongsma, R.G.F. Visser, E. Jacobsen, and J.H. Vossen. 2014. Development of late blight resistant potatoes by cisgene stacking. *BMC Biotechnology* 14:50.

Kim E., and L.E. Graham. 2008. EEF2 Analysis challenges the monophyly of Archaeplastida and Chromalveolata. *PLoS ONE* 3(7): e2621.

Kim, H., H. Lee, K. Jo, S.M.M. Mortazavian, D.J. Huigen, B. Evenhuis, G. Kessel, R.G.F. Visser, E. Jacobsen, and J.H. Vossen. 2012. Broad spectrum late blight resistance in potato differential set plants Ma*R8* and Ma*R9* is conferred by multiple stacked *R* genes. *Theoretical and Applied Genetics* 124:923-935.

Kirk, W.W., F.A. Samen, P. Tumbalam, P. Wharton, D. Douches, C.A. Thill, and A. Thompson. 2009. Impact of different US genotypes of *Phytophthora infestans* on potato seed tuber rot and plant emergence in a range of cultivars and advanced breeding lines. *Potato Research* 52:121-140.

Milbourne, D., B. Pande, and G.J. Bryan. 2007. Potato. In: Genome Mapping and Molecular Breeding in Plants, Volume 3: Pulses, Sugar and Tuber Crops, ed. C. Kole, 205-217. Berlin: Springer-Verlag Berlin Heidelberg.

North Dakota State University. 2009. Dakota Trailblazer Potato Release. https://www.ag.ndsu.edu/plantsciences/research/releases/potato/dakota-trailblazer. Accessed 3 March 2021.

Nowicki, M., M.R. Foolad, M. Nowakowska, and E.U. Kozik. 2012. Potato and tomato late blight caused by *Phytophthora infestans*: An overview of pathology and resistance breeding. *Plant Disease* 96: 4-17.

Porter, L.D., C.R. Brown, S.H. Jansky, D.A. Johnson, and J.K.S. Dung. 2017. Tuber resistance and slow-rotting characteristics of potato clones associated with the Solanaceae coordinated agricultural project to the US-24 clonal lineage of *Phytophthora infestans*. *American Journal of Potato Research* 94:160-172.

SAS Institute. 2010. SAS/STAT® 9.3 User's Guide. Version 9.3. SAS Institute, Cary, NC.

Song, J., J.M. Bradeen, S.K. Naess, J.A. Raasch, S.M. Wielgus, G.T. Haberlach, J. Liu, H. Kuang, S. Austin-Phillips, C.R. Buell, J.P. Helgeson, and J. Jiang. 2003. Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. *Proceedings of the National Academy of Sciences* 100:9128-9133

Spielman, L.J., B.J. McMaster, and W.E. Fry. 1989. Dominance and recessiveness at loci for virulence against potato and tomato in *Phytophthora infestans. Theoretical Applied Genetics* 77:832-838.

Stewart, H.E., R.L. Wastie, J.E. Bradshaw, and J. Brown. 1992. Inheritance of resistance to late blight in foliage and tubers of progenies from parents differing in resistance. *Potato Research* 35:313-319.

Vleeshouwers, V.G., W.V. Dooijeweert, F. Govers, S. Kamoun, and L.T. Colon. 2000. The hypersensitive response is associated with host and nonhost resistance to *Phytophthora infestans*. *Planta* 210:853-864.

Vleeshouwers, V.G., W.V. Dooijeweert, L.C.P. Keizer, L. Sijpkes, F. Govers, and L.T. Colon. 1999. A laboratory assay for *Phytophthora infestans* resistance in various *Solanum* species reflects the field situation. *European Journal of Plant Pathology* 105:241-250.

Vleeshouwers, V.G., and R.P. Oliver. 2014. Effectors as tools in disease resistance breeding against biotrophic, hemibiotrophic, and necrotrophic plant pathogens. *Molecular Plant-Microbe Interactions* 27:196-206.

Vleeshouwers, V.G., S. Raffaele, J.H. Vossen, N. Champouret, R. Oliva, M.E. Segretin, H. Rietman, L.M. Cano, A. Lokossou, G. Kessel, M.A. Pel, and S. Kamoun. 2011.

Understanding and exploiting late blight resistance in the age of effectors. *Annual Review of Phytopathology* 49:507-531.

Watanabe, K. 2015. Potato genetics, genomics, and applications. *Breeding Science* 65:53-68.

Zar, J.H. 2010. Biostatistical Analysis (5th Edition). Upper Saddle River: Prentice Hall.

CHAPTER 3. IDENTIFICATION OF SPECIFIC LATE BLIGHT RESISTANCE GENES IN NDSU POTATO BREEDING PROGRAM GERMPLASM

Abstract

The potato, *Solanum tuberosum* L., is an important, nutritious crop, planted worldwide. *Phythopthora infestans* (Mont.) de Bary is an oomycete causing late blight, the cause of the Irish potato famine, which killed over a million people. To combat late blight more effectively, durable genetic resistance needs to be incorporated into and utilized in improved potato cultivars. Two hundred thirty-six potato genotypes were evaluated for six late blight resistance (R) genes: R1, R2, R3, RB, Rpi-smira1, and Rpi-ber1 using PCR. One hundred thirty-six of the clones exhibited at least one product associated with an R gene. The R1 gene, conferring qualitative, specific resistance, was the most prevalent in the NDSU germplasm, present in 85 genotypes. The R3 gene was present in 39 genotypes, and the R2 gene appeared in 37 genotypes; both genes confer specific, qualitative resistance. The RB gene, conferring quantitative, broad resistance, was found in 12 genotypes. Thirty-nine genotypes exhibited positive bands for at least two of the R gene markers. ND14358AB-1 had R1, R2, R3, and RB present, with Etb 5-31-3 and J101-K6 each having three R genes present. Other genotypes exhibiting the presence of one or more R genes included 95043.11, LBR8, ND039194-1Russ, ND7799C-1, ND4659-5R, ND7519-1, and Stirling. These genotypes were previously identified as parents of progeny families exhibiting significant late blight resistance. The results of this evaluation can guide focused breeding efforts, particularly efficient R gene stacking, as a means of developing durable, long-term resistance to late blight.

Introduction

Solanum tuberosum L., cultivated potato, is a vital and nutritious crop, with nearly 360 million metric tons produced annually worldwide (FAOSTAT 2020). Not only does the potato have the highest caloric density per hectare of all staple crops, but it also contains many nutrients, including vitamins, minerals, and high amounts of carbohydrates (Ensminger and Ensminger 1993).

Phythopthora infestans (Mont.) de Bary is an oomycete causing late blight of potato (Kim and Graham 2008). Late blight has a significant impact on potato production, annually costing over $6 billion globally, due to losses and the cost to control (Haverkort et al. 2009).

R genes are genes with significant qualitative resistance to late blight (Fry and Goodwin 1997). At least 11 R genes (R1 – R11) have been identified from *S. demissum* alone (Black et al. 1953; Bradeen 2011). Many of these have been introgressed into European potato breeding program germplasm (Vleeshouwers et al. 2011; Ballvora et al. 2002).

The R genes in potato and the avirulence genes in *P. infestans* interact the same way as Flor's gene-for-gene interaction for plant disease (Ballvora et al. 2002). The molecules produced by pathogens that interact with and have specific effects on the host plant are called effectors (Vleeshouwers and Oliver 2014). When the effector from the pathogen is expressed in the plant cells from invading hyphae, and the corresponding resistance protein produced by the plant's R genes is not present, continued growth and spread of the infection will occur due to a suppressed immune response (Vleeshouwers and Oliver 2014). The avirulence proteins can be recognized by specific receptors on the plasma membrane of plant cells when the corresponding R proteins are present, which can signal apoptosis (cell death), slowing or even stopping the progression of the infection (Gassmann and Bhattacharjee 2012). This is referred to as a hypersensitive response

(Gassmann and Bhattacharjee 2012). Therefore, increasing the amount of R genes present, increases the chance of matching a pathogen's specific corresponding avirulence protein, thus resulting in a resistance response (Vleeshouwers and Oliver 2014).

Quantitative resistance, or field resistance, is considered effective and more durable, as it slows, but unlike a hypersensitive reaction does not eliminate, the overall symptoms of late blight (Song et al. 2003). A widespread example of a quantitative resistance gene in potato that reacts to *P. infestans* is RB, which was cloned from *S. bulbocastanum* (Song et al. 2003). By slowing pathogen growth, it makes it more difficult for mating and evolution; thus, overcoming the quantitative resistance is more difficult (Jo et al. 2014).

For over 100 years, people have been reducing the impact of late blight in potato through breeding (Bradeen and Haynes 2011). *Phythopthora infestans* rapidly evolves and overcomes the resistance provided from only one R gene, thus, having more than one in a cultivar, known as stacking, can provide more durable and long-term resistance (Jo et al. 2014). As late blight strains have become more resistant to fungicides, a renewed interest in host resistance in potato has developed (Fry and Goodwin 1997). Many functional R genes have been cloned from *Solanum* species, including 11 from *S. demissum* (Kim et al. 2012).

The R gene markers selected for this experiment were from various sources, in order to capture a wide range of potential resistance in the North Dakota State University (NDSU) potato germplasm collection and due to the reliability of their PCR product sequences. R1, R2, R3, and Rpi-smira1 are all specific, narrow spectrum genes, interacting with specific avirulence genes in different late blight strains, providing a hypersensitive and controlling response (Ballvora et al. 2002; Mori et al. 2011; Rietman 2011; Tomczyńska et al. 2014). The RB and Rpi-ber1 genes are broad spectrum quantitative resistance genes, slowing, but not eliminating, an infection

regardless of the specific strain of late blight (Song et al. 2003, Rauscher et al. 2010). Specific resistance is very effective, but easily overcome, by the ever-evolving *P. infestans*, so to have durable long-lasting resistance to late blight, potato breeders have to stack or pyramid both types of R genes (Tomczyńska et al. 2014).

Within the NDSU breeding program germplasm, there is likely many late blight resistance genes present, as resistance has been observed (Chapter 2). The objective of this study is to evaluate common parental genotypes utilized in the NDSU breeding program with genetic markers for late blight resistance genes to determine which specific R genes are present. This research was performed entirely in the Potato Research Laboratory in the Plant Sciences Department at NDSU. It was expected that varying genes would be exhibited as the pedigrees of genotypes incorporate multiple sources. Results of this research will help efficient pyramiding/stacking of important R genes in future potato cultivar releases, benefitting the potato industry, consumers, and the environment.

Materials and Methods

Plant Material

Two hundred thirty-six genotypes from the NDSU potato breeding program were evaluated (Table 3.3). Leaf tissue was harvested from potato plants produced at the Agricultural Experiment Station Research Greenhouse Complex, on the NDSU campus, in 2017. Tissue was collected from three fully expanded, mature leaflets, per genotype.

DNA Extraction

DNA extraction used a cetrimonium bromide (CTAB) procedure as described in Rogers and Bendich (1994). Briefly, leaflet tissue of approximately 30 mg was inserted into a sterile 1.5

ml microfuge tube (Eppendorf Corporation, Hamburg, Germany) and 650 µL of 2% cetrimonium bromide (CTAB) extraction buffer was added for cell lysis. The tissue was homogenized using sterile plungers, and the remaining liquid was pipetted into another 1.5 ml tube. The tubule was then incubated for one hour in a water bath at 65 °C and inverted at the half hour mark. After removing from the water bath, 650 µL of a 24:1 chloroform and isoamyl alcohol solution was added and tubes were repeatedly inverted for thorough mixing. Samples were then centrifuged for 10 minutes at 14,000 rpm. The supernatant was collected and put in a new 1.5 ml tube with 300 µL of -20 °C isopropanol; the tube was mixed by inversion for 1 minute. The tube was centrifuged a second time for 1 minute at 10,000 rpm. The supernatant was carefully poured out as waste, retaining the DNA pellet at the bottom of the tube. The pellet was washed twice with 500 µL of -20 °C 75% ethanol and centrifuged for 1 minute at 10,000 rpm after each wash. The sample was left to dry overnight, resuspended in 100 µL of ddH_2O, and stored at -20 °C until analysis.

Molecular Markers

Several markers were chosen from previous research (Ballvora et al. 2002, Mori et al. 2011, Colton et al. 2006, Rietman 2011, Tomczyńska et al. 2014, Tan et al. 2010) to identify R genes present in samples. Table 3.1 lists the primers, their sequences, and cited research they were obtained from. Table 3.2 lists the primers, their fragment sizes, and their target R gene.

All primers had a molecular weight of over 5,000 µg/µmol and were obtained dry, custom made by Invitrogen™ (Thermo-Fisher Scientific, Waltham, MA). Primers were resuspended to 100 pm/µL in ddH_2O and then made into a working stock by further diluting 1:10 with ddH_2O in a separate vial.

Table 3.1. Primers, their sequences, and source of discovery, used to detect late blight resistance genes in the NDSU potato breeding program germplasm.

Primer	Sequence (5'-3')	Source
76-2sf2	CACTCGTGACATATCCTCACTA	Ballvora et al. 2002
76-2SR	CAACCCTGGCATGCCACG	Ballvora et al. 2002
R2SP-S7	TACTAACCTTTTCCTAGATG	Mori et al. 2011
R2SP-A9	AGAACTTTCTCACAGCTTTT	Mori et al. 2011
CT88 1	CACGAGTGCCCTTTTCTGAC	Colton et al. 2006
CT88 1'	ACAATTGAATTTTTAGACTT	Colton et al. 2006
R3bF4	GTCGATGAATGCTATGTTTCTCGAGA	Rietman 2011
R3bR5	ACCAGTTTCTTGCAATTCCAGATTG	Rietman 2011
45/XI	AGAGAGGTTGTTTCCGATAGACC	Tomczyńska et al. 2014
	TCGTTGTAGTTGTCATTCCACAC	Tomczyńska et al. 2014
Q133F	ATCATCTCCTCAAAGAATCAAG	Tan et al. 2010
Q133R2	ATCTCCCCATTGACAACCAA	Tan et al. 2010

Table 3.2. Primer combinations, DNA fragment size, and the R gene it is targeting when added to DNA samples from the NDSU potato breeding program, along with the type of resistance the gene confers.

Primer combination	Size	Target R gene	Type of Resistance
76-2sf2 and 76-2SR	1400 bp*	R1	Specific
R2SP-S7 and R2SP-A9	800 bp*	R2	Specific
CT88 1 and CT88 1'	213 bp	RB	Broad
R3bF4 and R3bR5	378 bp	R3	Specific
45/XI	1000 bp*	Rpi-smira1	Specific
Q133F and Q133R2	504 bp	Rpi-ber1	Broad

* Approximate size

PCR and Gel Electrophoresis

Polymerase chain reaction (PCR) is a well understood and widely used procedure. By controlling thermal cycles and adding Taq polymerase, short DNA fragments known as primers, the DNA sample, and dNTPs, a chain reaction occurs amplifying the wanted specific areas of DNA (Lodge et al. 2007).

Promega GoTaq Green master mix (Promega Corporation, Madison, WI) was used as the polymerase, $MgCl_2$, buffers, and dNTPs required for the amplification of DNA by PCR. Each

well was loaded using 7 μL DNA sample, 12.5 μL master mix, .5 μL of the 1:10 working stock for each primer being run, and sterile ddH2O to reach 25 μL. The thermocycler program was 93 °C for 9 minutes, followed by 35 cycles of 94 °C for 45 seconds, 55 °C for another 45 seconds, then 72 °C for one minute. The cycle ended on a holding temperature of 4 °C.

One liter of 10x buffer stock was made from 48.4 g of Tris base, 11.4 ml acetic acid, 20 ml .5 M EDTA stock, and ddH_20 to 1L. Gels were made from 1.5% agarose and solidified in a tray approximately 13 cm by 9 cm. The comb used provided 30 wells for amplified product. Gels ran for 45 minutes at 80 volts with a standard TAE buffer.

The gels included 8 μL of 100 bp ladder made by Invitrogen™ in the first lane for accurate product size reads. All other lanes were loaded with 15 μL of experimental samples, each sample repeated once in a consecutive lane.

Gels were stained with 6 μL ethidium bromide (EtBr) in 100 mL of millipore water and rocked for 10 minutes. The EtBr and water solution was then drained, and the gel rinsed with another 100 mL of millipore water and rocked for 10 minutes. Following rinsing, gels were photographed using an AlphaImager HP (ProteinSimple, Minneapolis, MN) system utilizing an ultraviolet camera at 312 nm absorbance. Images were processed using the AlphaView Software (ProteinSimple, Minneapolis, MN).

Data Analysis

Photographs were analyzed visually for bands corresponding to the GeneRuler 100 bp Plus DNA Ladder (Thermo-Fisher Scientific, Waltham, MA) that was run on the same gel. The presence or absence of a band of the approximate size of the targeted R gene was recorded in a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, WA) for further analysis.

Results and Discussion

Table 3.3 presents the results of the gels and PCR products across the genotypes tested. A band appearing in the designated size range is noted with a + sign. One hundred thirty-six, of the 236, genotypes exhibited at least one PCR product associated with a late blight resistance gene (Table 3.3). The PCR product most prevalent in this evaluation was associated with R1, appearing in 85 of the 236 genotypes based on the 76-2sf2 and 76-2SR primer combination (Table 3.2; Ballvora et al. 2002). The second most prevalent R gene marker was R3, with 39 appearances based on the R3bF4 and R3bR5 primer combination (Table 3.2; Rietman 2011). R2 appeared in 37 of the 236 genotypes based on the R2SP-S7 and R2SP-A9 primer combination (Table 3.2; Mori et al. 2011). The RB gene, providing broad resistance to late blight, appeared in 12 of the genotypes based on the CT88 1 and CT88 1' primer combination (Table 3.2; Colton et al. 2006). Rpi-ber1 appeared seven times based on the Q133F and Q133R2 primer combination (Table 3.2; Tan et al. 2010). Rpi-smira1 was absent in the North Dakota State University potato germplasm collection based on the 45/XI primer combination (Table 3.2; Tomczyńska et al. 2014). This is not surprising, as this gene was recently identified in the Hungarian variety Sarpo Mira, and has only recently been introduced into European breeding germplasm (Rietman et al. 2012). One hundred genotypes exhibited no PCR products, and thus were presumed to possess none of the resistance genes evaluated by this study.

Table 3.3. Comparison of PCR products of R genes across genotypes from the North Dakota State University potato breeding program. A + designates that a PCR product was produced that matches the size range of the corresponding R gene. A – designates that no PCR product was produced that matches the size range of that R gene.

Genotype	Resistance gene presence					
	R1	R2	RB	R3	Rpi-smira1	Rpi-ber1
463-4	-	-	-	-	-	-
793101.3	-	-	-	-	-	-
90215.1	+	-	-	-	-	-
93057.1	-	-	-	-	-	-
95043.11	+	-	-	-	-	-
Atzimba	-	-	-	+	-	-
Austrian Crescent	+	-	-	-	-	-
Crystal	-	-	-	-	-	-
Dakchip	-	-	-	-	-	-
Dakota Crisp	-	-	-	-	-	-
Dakota Diamond	-	-	-	+	-	-
Dakota Pearl	-	-	-	-	-	-
Dakota Rose	+	-	-	-	-	-
Dakota Ruby	+	-	-	-	-	-
Dakota Russet	-	-	-	-	-	-
Dakota Trailblazer	-	-	-	-	-	-
French Fingerling	+	-	-	-	-	-
Ivory Crisp	-	-	-	-	-	-
NorKing Russet	-	-	-	-	-	-
Norland	-	-	-	-	-	-
NorValley	+	-	-	-	-	-
Ranger Russet	-	-	-	-	-	-
Red LaSoda (NY)	-	-	-	-	-	-
Red Norland	-	-	-	-	-	-
Red Pontiac	-	-	-	-	-	-
Russet Burbank	-	-	-	-	-	-
Russet Norkotah	-	-	-	-	-	-
Snowflake	-	-	-	-	-	-
Stirling	+	-	-	+	-	-
Umatilla Russet	-	-	-	-	-	-
AND00272-1R	+	+	-	-	-	-
AND97279-5Russ	-	-	-	-	-	-
ATND99331-2PintoY	+	-	-	-	-	-
DND3375-103pY	-	+	-	-	-	-

Table 3.3. Comparison of PCR products of R genes across genotypes from the North Dakota State University potato breeding program (continued). A + designates that a PCR product was produced that matches the size range of the corresponding R gene. A – designates that no PCR product was produced that matches the size range of that R gene.

Genotype	Resistance gene presence					
	R1	R2	RB	R3	Rpi-smira1	Rpi-ber1
DND3375-1080M	-	-	-	-	-	-
DND3375-112Y	-	-	-	-	-	-
DND3375-115Y	-	-	-	-	-	-
Etb 5-31-2	+	-	-	-	-	+
Etb 5-31-3	+	-	+	-	-	+
Etb 5-31-7	+	-	-	-	-	+
Etb 6-5-3	+	-	-	-	-	+
Etb 6-5-5	-	-	-	+	-	-
Etb 6-21-1	+	-	-	-	-	-
Etb 6-21-3	-	-	-	+	-	-
Etb 6-21-4	+	-	-	-	-	+
Etb 6-21-6	-	+	-	-	-	-
Etb 6-31-5	-	-	-	-	-	-
I20	-	+	+	-	-	-
J101-K6	-	+	+	-	-	+
J103-K7	-	-	-	-	-	-
J138-A12	+	-	-	-	-	-
LBR8	-	-	-	+	-	-
ND2858-1	-	-	-	-	-	-
ND2861-1	-	-	-	+	-	-
ND4100C-19	-	-	-	-	-	-
ND4659-5R	+	-	-	-	-	+
ND5255-59	-	-	-	-	-	-
ND5873-29	-	-	-	-	-	-
ND6002-1R	-	-	-	-	-	-
ND6953b-34	-	-	-	-	-	-
ND7519-1	+	-	-	-	-	-
ND7763C-2RS	+	-	-	-	-	-
ND7799c-1	+	-	-	-	-	-
ND7818-1Y	-	-	-	-	-	-
ND7834-2P	+	-	-	-	-	-
ND7882b-7Russ	-	-	-	-	-	-
ND8068-5Russ	-	+	-	+	-	-
ND8331Cb-2	-	+	-	-	-	-

Table 3.3. Comparison of PCR products of R genes across genotypes from the North Dakota State University potato breeding program (continued). A + designates that a PCR product was produced that matches the size range of the corresponding R gene. A – designates that no PCR product was produced that matches the size range of that R gene.

Genotype	Resistance gene presence					
	R1	R2	RB	R3	Rpi-smira1	Rpi-ber1
ND039194AB-1Russ	+	+	-	-	-	-
ND049251B-9Russ	+	-	-	-	-	-
ND060735-4Russ	-	-	-	-	-	-
ND060761B-3Russ	+	-	-	-	-	-
ND070927-2Russ	-	-	-	-	-	-
ND071302B-2Russ	-	-	-	-	-	-
ND081557C-5P	+	-	-	-	-	-
ND081571-2R	+	-	-	-	-	-
ND081577-1R	+	-	-	-	-	-
ND081764B-4Russ	-	-	-	-	-	-
ND091831C-8	+	-	-	-	-	-
ND091890-1RR	+	-	-	-	-	-
ND091933ABCR-7Russ	+	-	-	-	-	-
ND091997BT-3Russ	-	-	-	-	-	-
ND092007R-2Russ	-	-	-	-	-	-
ND092018C-2	-	+	-	-	-	-
ND092018C-3	-	-	-	-	-	-
ND092019C-4Russ	-	-	-	-	-	-
ND092024CR-1Russ	-	+	-	+	-	-
ND092150b-5Pinto	+	-	-	+	-	-
ND092355CR-2Russ	-	-	-	-	-	-
ND102631AB-1	+	-	-	-	-	-
ND102642C-2	+	-	-	-	-	-
ND102663B-3R	+	-	-	+	-	-
ND102687AB-1Russ	-	+	-	-	-	-
ND102719B-1Russ	-	+	-	-	-	-
ND102858CB-4	+	-	-	+	-	-
ND102917C-1	+	-	-	+	-	-
ND102921C-3	-	-	-	-	-	-
ND102922C-3	-	-	-	+	-	-
ND102940B-3R	+	+	-	-	-	-
ND102990B-2R	+	-	-	-	-	-
ND113030C-1	-	-	-	-	-	-
ND113035b-1	-	-	-	+	-	-

Table 3.3. Comparison of PCR products of R genes across genotypes from the North Dakota State University potato breeding program (continued). A + designates that a PCR product was produced that matches the size range of the corresponding R gene. A – designates that no PCR product was produced that matches the size range of that R gene.

Genotype	Resistance gene presence					
	R1	R2	RB	R3	Rpi-smira1	Rpi-ber1
ND113065CB-1Russ	-	-	-	+	-	-
ND113060-1	-	-	-	+	-	-
ND113065CB-2Russ	-	-	+	+	-	-
ND113091B-2RY	+	-	-	+	-	-
ND113096-1Russ	-	+	-	-	-	-
ND113099-2Russ	-	-	-	+	-	-
ND113100-1Russ	-	-	-	-	-	-
ND113113B-1PSY	+	-	-	+	-	-
ND113174B-2Russ	-	-	-	+	-	-
ND113207-1R	-	-	-	-	-	-
ND113224C-3Russ	-	-	-	-	-	-
ND113266C-3	-	-	-	-	-	-
ND113278-3	-	-	-	-	-	-
ND113286B-6	-	-	-	-	-	-
ND113289C-1	+	+	-	-	-	-
ND113307C-3	+	-	-	+	-	-
ND113330-1Russ	-	-	-	-	-	-
ND113338C-3R	+	-	-	+	-	-
ND113356B-2PEY	+	-	-	-	-	-
ND113372CAB-5	+	-	+	-	-	-
ND113381AB-6Russ	-	-	+	-	-	-
ND113383Ab-2Russ	-	-	-	-	-	-
ND113386Ab-5	-	+	-	+	-	-
ND113389B-3Russ	-	-	-	+	-	-
ND113390b-2Russ	-	-	-	+	-	-
ND113394CAB-7	-	+	-	+	-	-
ND113421CBY-1R	-	-	-	-	-	-
ND113438CB-1R	+	-	-	-	-	-
ND113460C-3PS	-	+	-	-	-	-
ND113461-1RS	+	-	-	-	-	-
ND113461-2P	-	+	-	+	-	-
ND113503AB-5RussY	+	-	-	-	-	-
ND113508C-4	-	+	-	-	-	-
ND113509C-2	-	-	-	-	-	-

Table 3.3. Comparison of PCR products of R genes across genotypes from the North Dakota State University potato breeding program (continued). A + designates that a PCR product was produced that matches the size range of the corresponding R gene. A – designates that no PCR product was produced that matches the size range of that R gene.

Genotype	Resistance gene presence					
	R1	R2	RB	R3	Rpi-smira1	Rpi-ber1
ND113523CB-3	-	-	-	-	-	-
ND113533ABC-2	-	+	-	-	-	-
ND113545B-2Russ	-	-	-	-	-	-
ND122C-1	-	-	-	-	-	-
ND124C-1	-	+	-	-	-	-
ND127B-1Russ	-	+	-	+	-	-
ND129AB-1Russ	-	+	+	-	-	-
ND1212-1RS	-	-	-	-	-	-
ND1221-1	+	+	-	-	-	-
ND1227b-2Russ	-	+	-	-	-	-
ND1232B-1RY	-	-	-	-	-	-
ND1240-2R	+	+	-	-	-	-
ND1241-1Y	-	-	-	-	-	-
ND1243-1PY	-	-	+	-	-	-
ND1250-2REY	+	-	+	-	-	-
ND12102-1RR	-	-	-	-	-	-
ND12103C-3Russ	-	-	+	-	-	-
ND12107CB-1	-	-	-	-	-	-
ND12108CAb-3Russ	-	-	-	+	-	-
ND12109CB-2Russ	-	+	-	-	-	-
ND12119CB-1Russ	-	+	-	+	-	-
ND12119CB-2Russ	+	-	-	-	-	-
ND12128B-1R	+	-	-	-	-	-
ND12154AB-2Russ	-	-	-	+	-	-
ND12157-3Russ	-	-	-	+	-	-
ND12158CAB-1	+	-	-	-	-	-
ND12162AB-1Russ	-	-	-	-	-	-
ND12163AB-2Russ	-	+	-	-	-	-
ND12180ABC-8	+	+	-	-	-	-
ND12202C-2Russ	+	-	-	+	-	-
ND12209C-3	+	-	-	-	-	-
ND12209C-6	-	-	-	-	-	-
ND12219B-1Russ	-	-	-	-	-	-
ND12219b-3Russ	-	-	-	-	-	-

Table 3.3. Comparison of PCR products of R genes across genotypes from the North Dakota State University potato breeding program (continued). A + designates that a PCR product was produced that matches the size range of the corresponding R gene. A – designates that no PCR product was produced that matches the size range of that R gene.

Genotype	Resistance gene presence					
	R1	R2	RB	R3	Rpi-smira1	Rpi-ber1
ND12225CB-1Russ	-	-	-	-	-	-
ND12229CB-1Russ	-	-	-	+	-	-
ND12237Y-1Russ	-	-	-	-	-	-
ND12239Y-3R	-	-	-	-	-	-
ND12241YB-1Russ	+	-	-	-	-	-
ND12241YB-2Russ	+	-	-	-	-	-
ND12243YC-2	+	-	-	-	-	-
ND12244Y-1R	+	-	-	-	-	-
ND12244Y-2R	+	-	-	-	-	-
ND12247YB-1Russ	-	-	-	-	-	-
ND12248Y-1R	-	-	-	-	-	-
ND12248Y-2R	+	-	-	-	-	-
ND12248Y-5R	+	-	-	-	-	-
ND133-1RR	-	-	-	-	-	-
ND136Y-3Russ	-	-	-	-	-	-
ND1316Y-1	-	-	-	-	-	-
ND1320Y-1	-	-	-	-	-	-
ND1321Y-1	-	-	-	-	-	-
ND1324Y-1	+	-	-	-	-	-
ND1325Y-1	-	-	-	-	-	-
ND1328YABC-1	-	-	-	-	-	-
ND1336-2	-	-	-	-	-	-
ND1336-5	-	-	-	-	-	-
ND1338C-1	+	-	-	-	-	-
ND1338C-3	+	-	-	-	-	-
ND1341Y-1R	+	-	-	-	-	-
ND1344B-1Russ	+	-	-	-	-	-
ND1347-1Russ	-	-	-	-	-	-
ND1350-1	+	-	-	-	-	-
ND1350-2	-	-	-	-	-	-
ND1351ABC-2	+	-	-	-	-	-
ND1353-1Russ	+	-	-	-	-	-
ND1360B-1R	-	-	-	-	-	-
ND1360B-2R	-	-	-	-	-	-

Table 3.3. Comparison of PCR products of R genes across genotypes from the North Dakota State University potato breeding program (continued). A + designates that a PCR product was produced that matches the size range of the corresponding R gene. A – designates that no PCR product was produced that matches the size range of that R gene.

Genotype	Resistance gene presence					
	R1	R2	RB	R3	Rpi-smira1	Rpi-ber1
ND1367B-1Russ	-	-	-	-	-	-
ND1368B-1	+	-	-	-	-	-
ND1378Y-1p	-	-	-	-	-	-
ND1382-2R	+	-	-	-	-	-
ND1382-3R	+	+	-	-	-	-
ND1446CB-5	-	-	-	-	-	-
ND1446CB-8	-	-	-	-	-	-
ND1446CB-9	-	-	-	-	-	-
ND1448CAB-3	-	+	-	-	-	-
ND1450CAb-1	-	-	-	+	-	-
ND1453C-3	-	+	-	-	-	-
ND14217-2R	+	-	-	-	-	-
ND14217-3R	+	-	-	-	-	-
ND14311CAb-3	+	-	-	-	-	-
ND14358AB-1	+	+	+	+	-	-
ND14364ABC-1	-	-	-	-	-	-
ND14367ABC-2	+	-	-	-	-	-
ND14369AbC-1	-	-	-	-	-	-
ND14371Ab-2	+	-	-	-	-	-
ND14424-1R	+	-	-	+	-	-
ND14437CAB-1	-	-	-	-	-	-
ND14437CAB-2	-	-	-	-	-	-
ND14467CAB-1	-	-	-	-	-	-
ND14467CAb-2	-	-	-	-	-	-
ND14474Ab-1	-	-	-	-	-	-
ND14474Ab-2	-	-	-	-	-	-
ND14477C-3	-	+	+	-	-	-
ND14477C-4	-	+	-	-	-	-
ND14477C-5	+	-	-	-	-	-
ND14478C-2	-	-	-	-	-	-
ND14478C-3	+	-	-	-	-	-
P2-4	-	-	-	-	-	-

The Etb genotypes, with the exception of Etb 6-31-5, possessed at least one late blight resistance gene. The Etb genotypes are from a somatic hybridization between *Solanum tuberosum* L. and a wild potato species native to Chile, *Solanum etuberosum* L., reported by Gillen and Novy (2007). Even though the original hybridization was focused on resistance to potato leafroll virus, potato virus Y, potato virus X, and green peach aphids, the presence of late blight resistance genes is unsurprising as they are frequently found in wild species (Gillen and Novy 2007; Kim et al. 2012).

ND14358AB-1 exhibited four positive bands for R1, R2, RB, and R3. Three genotypes had three or more positive bands: Etb 5-31-3, ND14358AB-1, and J101-K6. J101-K6 is the result of a *Solanum bulbocastanum* Dunal fusion backcrossed with Katahdin, and *S. bulbocastanum* has transferred late blight resistant genes as a wild relative to *S. tuberosum* (Helgeson et al. 1998; Rakosy-Tican et al. 2020). Thirty-seven genotypes exhibited positive bands for at least two markers.

Rietman et al. (2012) concluded that a mix of both quantitative and qualitative late blight resistance genes may provide the best durability. The RB gene, from *S. bulbocastanum*, confers quantitative and broad-spectrum resistance, while R1, R2, R3, Rpi-smira1 confer qualitative and specific resistance (Song et al. 2003). Out of the 12 NDSU genotypes exhibiting RB, nine also have a specific resistance gene present.

Stacking three late blight resistance genes is an exciting benchmark per a recent study in Uganda (Ghislain et al. 2019). Over three growing seasons, no isolate of *P. infestans* was found to overcome the stacking effect of three R genes: RB, Rpi-blb2, and Rpi-vnt1.1 (Ghislain et al. 2019).

Results of detached leaf assay evaluations at NDSU from 2003 to 2014 measuring late blight resistance among progeny families was reported in Chapter 2. Several of the most successful parents among progeny families exhibiting significant late blight resistance across all years were evaluated using PCR (Table 3.3). The genotypes 95043.11, LBR8, ND039194-1Russ, ND7799c-1, ND4659-5R, ND7519-1, and Stirling all exhibited at least one R gene and were identified as contributing resistance to progeny families evaluated using the detached leaf assays. Stirling was a common parent in the dedicated crossing block due to its history as a parent with strong late blight resistance (Bradshaw et al. 1995). LBR8 was also a common parent, and is a late blight differential line, previously reported to be highly resistant (Douches et al. 2004).

Dakota Trailblazer has provided field resistance to late blight, was a prevalent parent in the progeny evaluations (Chapter 2), and yet no resistance genes were identified in this study. There could be many reasons for this. There are many resistance genes that exist for late blight, and many have not been found or isolated yet (Yang et al. 2017). This study did not evaluate the germplasm for all known resistance genes, for example a recent study (Brown-Donovan 2020) reported that Dakota Trailblazer may have a resistance gene known as R8. This logic extends to any genotype that exhibited no resistance gene present. It is also important to note that even though replicates were evaluated, scientific experimentation may lead to false results, or human error in interpretation of banding patterns may result. Potato, being tetraploid, has also been known to have complications during the PCR procedure leading to unclear or mixed banding, possibly resulting in mistaken results (Gholami et al. 2012).

Higher copy numbers of R genes have been reported to enhance late blight resistance in potato (Bradeen et al. 2009). For example, Bradeen et al. (2009) reported that the RB gene averaged 2.78 copies across several tested resistant lines. However, the methods in this study are

unable to ascertain how many copies of a gene any genotype may have, as the product is singular and densitometry was not performed. Future research could utilize a quantitative real time PCR method or SNP genotyping in order to estimate copy numbers (Bradeen et al. 2009).

Limitations

This study did not utilize any positive controls to confirm a successful PCR reaction. This means that while the positive results are most likely accurate based on other published results of common genotypes, the negative results may not ensure a lack of that specific resistance gene, as the PCR reaction may have failed. In addition, DNA may have been of low quality, providing poor results, although steps were taken to measure A260/A280 purity and overall concentration using a Thermo Scientific™ NanoDrop™ 2000 spectrophotometer (Thermo-Fisher Scientific, Waltham, MA).

Conclusion

This study found that there is a wide and varied amount of late blight resistance genes present in the North Dakota State University potato germplasm collection. To develop and release late blight resistant cultivars, gene pyramiding efforts need to continue using resistant parents with varying R genes. Several genotypes exhibit gene pyramiding of late blight resistance genes, including ND14358AB-1, Etb 5-31-3, ND14358AB-1, and J101-K6, all expressed more than two resistance genes, providing breeders a head start in gene stacking. These findings will permit focused breeding for late blight resistance, by providing knowledge of R gene presence in the NDSU potato breeding germplasm collection.

Literature Cited

Ballvora, A., M.R. Ercolano, J. Weiß, K. Meksem, C.A. Bormann, P. Oberhagemann, F. Salamini, and C. Gebhardt. 2002. The R1 gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. *The Plant Journal* 30:361-371.

Black W., C. Mastenbroek, W.R. Mills, and L.C. Peterson. 1953. A proposal for an international nomenclature of races of *Phytophthora infestans* and of genes controlling immunity in *Solanum demissum* derivatives. *Euphytica* 2:173-178.

Bradeen, J.M. 2011. Cloning of late blight resistance genes: Strategies and progress. In: Genetics, Genomics, and Breeding of Potato, ed. James M. Bradeen and Chittaranjan Kole, 153-183. Enfield, NH: Science Publishers.

Bradeen, J.M., and K.G. Haynes. 2011. Introduction to Potato. In: Genetics, Genomics, and Breeding of Potato, ed. James M. Bradeen and Chittaranjan Kole, 1-15. Enfield, NH: Science Publishers.

Bradeen, J.M., M. Iorizzo, D.S. Mollov, J. Raasch, L.C. Kramer, B.P. Millett, S. Austin-Phillips, J. Jiang, and D. Carputo. 2009. Higher copy numbers of the potato RB transgene correspond to enhanced transcript and late blight resistance levels. *Molecular Plant-Microbe Interactions* 22:437-446.

Bradshaw, J.E., H.E. Stewart, R.L. Wastie, M.F.B. Dale, and M.S. Phillips. 1995. Use of seedling progeny tests for genetical studies as part of a potato (*Solanum tuberosum* subsp. tuberosum) breeding programme. *Theoretical and Applied Genetics* 90:899-905.

Brown-Donovan, K.M. 2020. Pyramiding approaches for potato disease resistance breeding. Dissertation, University of Maine.

Colton, L. M., H.I. Groza, S.M. Wielgus, and J. Jiang. 2006. Marker-assisted selection for the broad-spectrum potato late blight resistance conferred by gene RB derived from a wild potato species. *Crop Science* 46:589-594.

Douches, D., J. Coombs, K. Felcher, and W. Kirk. 2004. Foliar reaction to *Phytophthora infestans* in inoculated potato field trials in Michigan. *American Journal of Potato Research* 81:443-448.

Ensminger, M.E., and A.H. Ensminger. 1993. Green Revolution. In: Foods & Nutrition Encyclopedia 2nd Edition, ed. Marion Eugene Ensminger and Audrey H. Ensminger, 1102-1105. Boca Raton, FL: CRC Press.

FAOSTAT Database. 2020. faostat3.fao.org. Accessed February 20 2022.

Fry, W.E., and S.B. Goodwin. 1997. Re-emergence of potato and tomato late blight in the United States. *Plant Disease* 81:1349-1357.

Gassmann, W. and S. Bhattacharjee. 2012. Effector-triggered immunity signaling: from gene-for-gene pathways to protein-protein interaction networks. *Molecular Plant-Microbe Interactions* 25:862-868.

Ghislain, M., A.A. Byarugaba, E. Magembe, A. Njoroge, C. Rivera, M.L. Román, J.C. Tovar, S. Gamboa, G.A. Forbes, J.F. Kreuze, A. Barekye, and A. Kiggundu. 2019. Stacking three late blight resistance genes from wild species directly into African highland potato varieties confers complete field resistance to local blight races. *Plant Biotechnology Journal* 17:1119–1129.

Gholami, M., W. A. Bekele, J. Schondelmaier, and R. J. Snowdon. 2012. A tailed PCR procedure for cost-effective, two-order multiplex sequencing of candidate genes in polyploid plants. *Plant Biotechnology Journal* 10:635-645.

Gillen, A.M., and R.G. Novy. 2007. Molecular characterization of the progeny of *Solanum etuberosum* identifies a genomic region associated with resistance to potato leafroll virus. *Euphytica* 155:403-415.

Haverkort, A.J., P.C. Struik, R.G.F. Visser, and E. Jacobsen. 2009. Applied biotechnology to combat late blight in potato caused by *Phytophthora infestans*. *Potato Research* 52:249-264.

Helgeson, J.P., J.D. Pohlman, S. Austin, G.T. Haberlach, S.M. Wielgus, D. Ronis, L. Zambolim, P. Tooley, J.M. McGrath, R.V. James and W.R. Stevenson. 1998. Somatic hybrids between *Solanum bulbocastanum* and potato: A new source of resistance to late blight. *Theoretical and Applied Genetics* 96:738-742.

Jo, K., C. Kim, S. Kim, T. Kim, M. Bergervoet, M.A. Jongsma, R.G.F. Visser, E. Jacobsen, and J.H. Vossen. 2014. Development of late blight resistant potatoes by cisgene stacking. *BMC Biotechnology* 14:50.

Kim E., and L.E. Graham. 2008. EEF2 Analysis challenges the monophyly of Archaeplastida and Chromalveolata. *PLoS ONE* 3(7): e2621.

Kim, H., H. Lee, K. Jo, S.M.M. Mortazavian, D.J. Huigen, B. Evenhuis, G. Kessel, R.G.F. Visser, E. Jacobsen, and J.H. Vossen. 2012. Broad spectrum late blight resistance in potato differential set plants Ma*R8* and Ma*R9* is conferred by multiple stacked *R* genes. *Theoretical and Applied Genetics* 124:923-935.

Lodge, J., P. Lund, and S. Minchin. 2007. Gene Cloning: Principles and Applications 1st Edition, ed. Elizabeth Owen. New York, NY: Taylor & Francis Group.

Mori, K., Y. Sakamoto, N. Mukojima, S. Tamiya, T. Nakao, T. Ishii, and K. Hosaka. 2011. Development of a multiplex PCR method for simultaneous detection of diagnostic DNA markers of five disease and pest resistance genes in potato. *Euphytica* 180:347-355.

Murray, M.G., and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 8:4321-4325.

Rakosy-Tican, E., R. Thieme, J. König, M. Nachtigall, T. Hammann, T. Denes, K. Kruppa, and M. Molnár-Lang. 2020. Introgression of two broad-spectrum late blight resistance genes, Rpi-Blb1 and Rpi-Blb3, from *Solanum bulbocastanum* Dun plus race-specific R genes into potato pre-breeding lines. *Frontiers in Plant Science* 11:699.

Rauscher, G., I. Simko, H. Mayton, M. Bonierbale, C. D. Smart, N. J. Grünwald, A. Greenland, and W. E. Fry. 2010. Quantitative resistance to late blight from *Solanum berthaultii* cosegregates with $R_{Pi\text{-}ber}$: Insights in stability through isolates and environment. *Theoretical and Applied Genetics* 121:1553-1567.

Rietman, H. 2011. Putting the *Phytophthora infestans* genome sequence at work: multiple novel avirulence and potato resistance gene candidates revealed. Ph.D. thesis, Wageningen University, Wageningen, The Netherlands.

Rietman, H., G. Bijsterbosch, L.M. Cano, H. Lee, J.H. Vossen, E. Jacobsen, R.G.F. Visser, S. Kamoun, and V.G.A.A. Vleeshouwers. 2012. Qualitative and quantitative late blight resistance in the potato cultivar Sarpo Mira is determined by the perception of five distinct RXLR effectors. *Molecular Plant-Microbe Interactions* 25:910-919.

Rogers, S.O., and A.J. Bendich. 1994. Extraction of Total Cellular DNA From Plants, Algae and Fungi. In Plant Molecular Biology Manual, ed. Stanton B. Gelvin and Robbert A. Schilperoort 183-190. Dordrecht: Springer.

Song, J., J.M. Bradeen, S.K. Naess, J.A. Raasch, S.M. Wielgus, G.T. Haberlach, J. Liu, H. Kuang, S. Austin-Phillips, C.R. Buell, J.P. Helgeson, and J. Jiang. 2003. Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. *Proceedings of the National Academy of Sciences* 100:9128-9133.

Tan, M. Y. A., R.C.B. Hutten, R.G.F. Visser, and H.J. van Eck. 2010. The effect of pyramiding *Phytophthora infestans* resistance genes $R_{Pi\text{-}mcd1}$ and $R_{Pi\text{-}ber}$ in potato. *Theoretical Applied Genetics* 121:117-125.

Tomczyńska, I, E. Stefanczyk, M. Chmielarz, B. Karasiewicz, P. Kaminski, J.D.G. Jones, A.K. Lees, and J. Silwka. 2014. A locus conferring effective late blight resistance in potato cultivar Sarpo Mira maps to chromosome XI. *Theoretical Applied Genetics* 127:647-657.

Vleeshouwers, V.G., and R.P. Oliver. 2014. Effectors as tools in disease resistance breeding against biotrophic, hemibiotrophic, and necrotrophic plant pathogens. *Molecular Plant-Microbe Interactions* 27:196-206.

Vleeshouwers, V.G., S. Raffaele, J.H. Vossen, N. Champouret, R. Oliva, M.E. Segretin, H. Rietman, L.M. Cano, A. Lokossou, G. Kessel, M.A. Pel, and S. Kamoun. 2011. Understanding and exploiting late blight resistance in the age of effectors. *Annual Review of Phytopathology* 49:507-531.

Yang, L., D. Wang, Y. Xu, H. Zhao, L. Wang, X. Cao, Y. Chen, and Q. Chen. 2017. A new resistance gene against potato late blight originating from *Solanum pinnatisectum* located on potato chromosome 7. *Frontiers in Plant Science* 8:1729.

SUMMARY

The cultivated potato, *Solanum tuberosum* L., is a nutrient-dense vegetable and the sixth most widely grown crop in the world. *Phytophthora infestans* (Mont.) de Bary is an oomycete that causes late blight, a disease that killed millions in the Irish potato famine and today continues to cost an estimated $6 billion in losses each year worldwide. Potato and its wild relatives have evolved a series of resistance genes (R genes) that can trigger an immune response to slow or even stop an infection of late blight. However, these genes follow the gene-for-gene theory of host and parasite, where an R gene will only generate a response when a corresponding avirulence (Avr) gene's effectors are present. As *P. infestans* rapidly evolves, overcoming individual R genes, having multiple different R genes present in one genotype (also known as stacking or pyramiding) is the best way for potato to develop durable long-term resistance to late blight.

The objective of this thesis was to identify potato genotypes present in the North Dakota State University potato breeding program germplasm that may carry late blight resistance genes and to further identify which specific genes are present. This consisted of organizing and analyzing several years of detached leaf assays of progeny families in order to establish parental genotypes that passed on late blight resistance (Chapter 2). Marker assisted selection technology, using PCR was then employed to evaluate many of the parental genotypes, as well as 226 more. Genotypes were evaluated for six late blight resistance gene markers (Chapter 3).

The detached leaf assays from 2002 to 2014 were a form of screening for late blight resistance among progeny families performed by the NDSU potato improvement team. In order to better understand and organize the data, dendrograms were created and a quantile function used to help determine significant resistance. Results indicated susceptibility was widespread,

but several shared parents of resistant families were identified throughout the study. The most common parental genotypes were ND8277B-5, Dakota Trailblazer, EB8109-1, ND028856B-1Russ, and Stirling. Stirling was a common parent selected in the dedicated crossing block as it has been identified as late blight resistant.

To identify specific forms of resistance, 236 potato genotypes were evaluated for six late blight resistance (R) genes: R1, R2, R3, RB, Rpi-smira1, and Rpi-ber1. These markers were selected for their reliability of their PCR product primer sequences and to capture a wide array of possible resistances. One hundred thirty-six of the clones exhibited at least one product associated with an R gene, while several genotypes exhibited existing gene pyramiding of late blight resistance genes. The R1 gene was most prevalent in NDSU germplasm, and identified in 85 genotypes. The R3 gene was identified in 39 genotypes, the R2 gene was present in 37 genotypes, and the RB gene was found in 12 genotypes. The genotypes Etb 5-31-3, ND14358AB-1, and J101-K6, expressed more than two resistance genes, and ND14358AB-1 exhibited four positive bands for R1, R2, R3, and RB.

There are several possible improvements that could be implemented into future experiments similar to this study. The detached leaf assays only utilized limited strains of late blight, leaving several possible sources of resistance unfound. This study could also have utilized better positive controls to ensure successful PCR reactions and further confirm a negative R gene result. The R gene panel selected could be more targeted and specific depending on the germplasm being tested.

These results will be important in decision-making when choosing parents for late blight resistance breeding efforts to achieve targeted R gene stacking. This will allow for durable, long-lasting late blight resistance in future cultivars, potentially preventing millions of dollars of crop

loss and reducing the need for fungicides. Further research will be needed to continue identifying other sources of late blight resistance in the North Dakota State University germplasm, in order to continually combat the ever-evolving *P. infestans*. The results may also provide a frame of reference for future studies utilizing new and advanced genetic techniques such as quantitative real-time PCR.

APPENDIX

Table A1. Families evaluated using a detached leaf assay, with their respective parentages and late blight resistance ratings of progeny, conducted in 2002. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2002, isolates 693-3 and 126-18C of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND8477CB	ND4382-17	Stirling	50	10	2	0	38	24
ND8478	ND4382-17	EB8109-1	50	13	1	2	34	28
ND8497B	ND6585B-11	EB8109-1	39	15	5	3	16	51
ND8503CB	ND6691CB-3	EB8109-1	200	55	22	8	115	39
ND8524B	ND6935B-4R	ND7132-1R	100	1	0	0	99	1
ND8525B	ND6947B-6	Nortena	46	0	0	0	46	0
ND8526B	ND6947B-6	Stirling	200	1	1	2	196	1
ND8527B	ND6947B-6	EB8109-1	200	1	2	0	197	2
ND8534B	ND6961B-2	EB8109-1	200	9	37	19	135	23
ND8535B	ND6961B-6	EB8109-1	100	2	20	2	76	22
ND8536B	ND6962B-23	EB8109-1	100	10	39	1	50	49
ND8540CB	ND7097C-4	Dakota Pearl	200	4	1	1	194	3
ND8565B	ND7227CB-9	EB8109-1	100	0	0	0	100	0
ND8566B	ND7266-3	EB8109-1	50	0	0	0	100	0
ND8567CB	ND7289CB-1	EB8109-1	200	0	0	1	199	0
ND8570B	ND7333B-7	Tollocon	50	2	1	0	47	6
ND8571B	ND8571B	EB8109-1	200	26	24	13	137	25
ND8573B	ND7376B-3	EB8109-1	200	7	6	1	186	7
ND8574CB	ND7377CB-3	Stirling	50	1	0	0	49	2
ND8576B	ND7403B-1	Tollocon	100	4	6	5	85	10
ND028641B	Stirling	ND6948B-7	100	8	5	1	86	13
ND028644B	Tollocon	ND6947B-6	31	0	5	0	26	16
ND028645	Yukon Gold	Dakota Gold	35	0	0	0	35	0
ND028648CB	A91790-13	ND7377CB-16	100	0	0	0	100	0
ND028653B	AND9552-10Russ	ND6948B-7	100	0	1	1	98	1
ND028662B	AOND96198-1Russ	ND7808B-3Russ	100	0	1	4	95	1
ND028664B	AOND96247-1Russ	ND6948B-7	100	0	0	0	100	0
ND028666B	AOND96261-2Russ	Stirling	100	1	3	3	93	4

Table A1. Families evaluated using a detached leaf assay, with their respective parentages and late blight resistance ratings of progeny, conducted in 2002 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2002, isolates 693-3 and 126-18C of P. infestans strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND028671B	AOND98138-4 Russ	Stirling	125	6	34	35	50	32
ND028699B	EB8109-1	Dakota Pearl	100	0	1	1	98	1
ND028700B	EB8109-1	AND97279-5	50	4	7	17	22	22
ND028717CB	MN18767	ND7377CB-17	100	1	6	2	91	7
ND028753B	R89045-35	ND7851b-1	100	0	3	3	94	3

Table A2. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2003. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2003, isolates 693-3 and 126-18C of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND039092AB	ND4778-2	ND7443aB-20	96	0	2	3	87	2
ND039111B	ND6934b-6	AND98324-1Russ	84	4	12	4	62	20
ND039116B	ND6955B-28	Dakota Pearl	42	0	14	1	23	37
ND039118B	ND6955B-28	AND98324-1Russ	100	0	20	12	62	21
ND039125B	ND6961b-1R	ND4659-5R	100	3	22	18	55	26
ND039130B	ND1333b-7	ND6948b-3Russ	100	0	13	17	67	13
ND039134AB	ND7443Ab-18	LBR8	100	6	12	5	75	18
ND039136AB	ND7443Ab-18	ND7469C-1	100	0	0	2	93	0
ND039138AB	ND7443Ab-20	Dakota Pearl	100	0	3	10	81	3
ND039139AB	ND7443Ab-20	A92017-6	100	1	1	4	87	2
ND039141AB	ND7443Ab-20	ND7378b-5Russ	44	0	4	4	36	9
ND039142AB	ND7443Ab-51	LBR8	200	0	2	4	186	1
ND039144AB	ND7443Ab-61	ND6489-34	200	0	0	0	197	0
ND039145AB	ND7443rb-61	ND7428b-6	48	0	0	0	40	0
ND039150AB	ND7443Ab-103	PI583331	200	0	12	39	136	6

Table A2. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2003 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2003, isolates 693-3 and 126-18C of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND039153AB	ND7443Ab-103	ND7486b-1	79	0	0	1	73	0
ND039154AB	ND7443Ab-103	ND7495B-6	100	0	0	0	98	0
ND039155AB	ND7443Ab-115	LBR8	72	0	0	0	65	0
ND039163AB	ND7495b-6	ND7443Ab-20	100	0	10	40	49	10
ND039165CB	ND7525C-2R	MNDO1134-1R	100	1	3	9	83	4
ND039166CB	ND7525C-2R	R89067-84	100	1	6	9	80	7
ND039169CB	ND7684CB-2	AND98324-1Russ	100	0	10	5	81	10
ND039170CA	ND7684cb-2	ND7443aB-103	82	0	2	1	79	2
ND039172B	ND7794b-3	AND95249-1Russ	100	0	7	6	83	7
ND039173CAb	ND7799c-1	ND7443Ab-20	100	0	16	19	56	18
ND039174B	ND7808B-9Russ	ND6954b-11Russ	43	0	0	0	41	0
ND039177AB	ND7851b-7	ND7443Ab-61	100	0	0	0	98	0
ND039178B	ND7887b-9Russ	ND4726-1Russ	96	0	3	13	74	3
ND039180	ND7987-1R	Redsen	100	0	0	5	94	0
ND039183B	ND8050b-8	ND7333B-7	100	1	0	1	92	1
ND039185B	ND8060-3R	R89063-84	100	0	0	0	96	0
ND039189B	ND8089-2R	R89063-84	100	0	0	0	97	0
ND039215	ND7469C-4	R89063-84	100	0	0	0	87	0
ND039410AB	ND7443Ab-20	B0692-4	48	0	4	6	35	9
ND049219	Atlantic	Etb 6-5-5	100	0	0	0	85	0
ND06947B	B0718-3	ND5250-8	200	0	15	23	150	8
ND07067B	Dakota Jewel	Zarevo	100	0	1	4	78	1

Table A3. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2005. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2005, isolates 693-3, 711, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND049466AB	ND7443Ab-102	RX90201-11	100	0	0	0	58	0
ND049478AB	ND7443Ab-186	AH66-4	91	0	0	0	91	0
ND049480AB	ND7443Ab-186	ND7333b-7	100	0	0	0	34	0
ND049486AB	ND7495b-6	ND7443Ab-72	100	0	2	0	95	2
ND049496CA	ND7684CB-2	ND7443Ab-72	100	0	1	3	91	1
ND049498BV	ND7707VC-4R	Patagonia	100	0	2	7	84	2
ND049507B	ND7794b-3Y	ND7331b-4Y	92	0	0	0	92	0
ND049520B	ND7887b-9Russ	ND8444B-2Russ	100	0	2	8	79	2
ND049539AB	ND8165B-1	ND7443Ab-153	122	0	3	19	28	41
ND049540CB	ND8165B-1	ND8331CB-2	39	0	7	5	26	18
ND049542B	ND8180b-1	SM8-12	100	0	3	5	88	3
ND049544B	ND8180b-1	ND8331CB-2	100	0	3	4	179	2
ND049545B	ND8226B-15Russ	Dakota Trailblazer	169	2	3	21	108	21
ND049547B	Dakota Russet	ND8444B-2Russ	200	0	8	15	166	4
ND049550CB	ND8276B-1	ND8331CB-2	100	0	1	7	92	1
ND049551B	ND8277B-5	Dakota Trailblazer	200	7	7	52	60	41
ND049552B	ND8277B-5	ND7519-1	200	0	5	45	101	26
ND049553B	ND8281B-3	ND7519-1	200	8	6	24	94	37
ND049554CB	ND8281B-3	ND8331CB-2	72	0	2	16	28	35
ND049560CB	ND8331CB-2	Etb 6-5-5	100	0	2	5	176	1
ND049562CB	ND8331CB-2	ND7443Ab-102	100	0	7	8	169	4
ND049563CB	ND8331CB-2	ND7794b-3Y	80	0	0	3	76	0
ND049565B	ND8374B-10R	LBR8	100	0	2	17	178	1
ND049566B	ND8374B-10R	Patagonia	100	0	1	1	188	1
ND049568	ND8383-1R	Dakota Jewel	100	0	0	2	95	0
ND049580B	ND8394B-1R	Patagonia	100	0	1	12	85	1
ND049584B	ND8413-2Russ	ND8444B-2Russ	100	0	1	1	184	0
ND049586B	ND8428B-1	ND8226B-15Russ	100	0	2	21	159	10

Table A3. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2005 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2005, isolates 693-3, 711, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND049587B	ND8444b-2Russ	Dakota Trailblazer	100	0	6	8	185	3
ND049589B	ND8444b-2Russ	Dakota Russet	48	0	3	5	38	7
ND049590B	ND8456-1	AH66-4	100	0	1	4	93	1
ND049593CB	ND8456-1	ND8331CB-2	50	0	0	0	24	0
ND059637B	Innovator	Stirling	100	3	2	17	51	31
ND059641B	Innovator	Dakota Trailblazer	100	2	2	13	62	23
ND059643AB	Innovator	ND7443Ab-45	100	0	3	5	88	3
ND059669B	Stirling	ND7495b-6	100	0	1	14	73	13
ND059679B	AND0086-11Russ	ND028594B-11	65	0	3	15	46	5
ND059687B	AND00349B-1Russ	AND01129-1Russ	100	0	1	3	96	1
ND059688B	AND00349B-1Russ	Dakota Trailblazer	200	0	2	5	161	1
ND059690B	AND00349B-1Russ	ND7495b-6	100	1	7	1	75	10
ND059691B	AND00349B-1Russ	ND8444B-2Russ	100	0	4	8	63	5
ND059693B	AND00349B-1Russ	ND8496B-11Russ	200	0	4	24	167	2
ND059694B	AND00349B-1Russ	ND028856B-1Russ	100	0	4	5	38	9
ND059696AB	AND01027-1Russ	ND7386Ab-20	100	0	4	13	74	4
ND059710B	AND9552-10Russ	Dakota Trailblazer	100	1	9	6	83	10
ND059712B	AND9552-13Russ	AND98190-1Russ	80	0	1	8	66	1
ND059728B	AOND96422-3Russ	Stirling	100	0	4	10	86	4
ND059738B	ATND98459-1RY	Patagonia	100	0	7	10	68	8

Table A4. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2006. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2006, isolates 693-3, 711, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND050169B	Dakota Ruby	Patagonia	100	0	0	0	99	0
ND050212AB	ND028615AB-3	Dakota Diamond	100	0	0	0	100	0
ND050213AB	ND028615AB-3	ND7519-1	60	0	9	4	46	15
ND050216B	ND028651B-3Russ	Innovator	100	6	35	7	50	42
ND050217B	ND028651B-3Russ	Dakota Trailblazer	100	0	1	1	95	1
ND050218B	ND028651B-3Russ	AOND95292-3Russ	100	4	37	15	41	42
ND050219B	ND028651B-3Russ	ND8444b-2Russ	100	5	42	7	44	48
ND050220B	ND028651B-3Russ	ND8476B-1Russ	60	0	0	0	60	0
ND050221B	ND028666b-1Russ	Innovator	100	0	2	0	95	2
ND050223B	ND028668B-2Russ	Dakota Trailblazer	100	0	8	4	83	8
ND050224B	ND028672B-4Russ	Innovator	100	0	0	0	97	0
ND050226B	ND028672B-4Russ	Dakota Trailblazer	100	0	0	0	99	0
ND050231B	ND028678-1RY	Patagonia	100	0	0	0	97	0
ND050255CA	ND028711BC-1	ND7443Ab-20	100	2	15	4	77	17
ND050259CB	ND028770B-4R	ND8506C-6R	30	0	7	2	21	23
ND050260CB	ND028770B-4R	ND8512C-5R	100	0	0	1	96	0
ND050261CB	ND028770B-4R	ND8512C-17R	100	16	42	8	31	60
ND050262BV	ND028770B-4R	ND8546V-3R	100	0	0	0	96	0
ND050268CB	ND028777CB-2	NY131	100	0	0	0	99	0
ND050269CA	ND028777CB-2	ND7443Ab-20	100	3	20	1	72	24
ND050270CA	ND028799C-2	Stirling	90	2	23	8	55	28
ND050272CA	ND028801CB-2	ND7443Ab-20	100	0	0	0	99	0
ND050273CA	ND028801CB-2	ND7443Ab-45	100	0	0	0	98	0
ND050274CA	ND028801CB-2	ND7443Ab-68	100	0	2	1	95	2
ND050275CA	ND028801CB-2	ND7443Ab-180	100	0	0	0	100	0
ND050276CA	ND028801CB-2	ND7443Ab-186Russ	94	0	0	0	91	0
ND050277CB	ND028801CB-2	ND8570B-1	30	0	0	0	28	0
ND050278CB	ND028801CB-2	ND028598C-1	100	0	0	0	99	0

Table A4. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2006 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2006, isolates 693-3, 711, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of genotypes evaluated	Frequency of Ratings Within Families				
	Female	Male		0	1	2	3	% Resistant
ND050279CA	ND028801CB-2	ND028615AB-3	100	0	0	0	100	0
ND050280CA	ND028804CAB-4	NY131	100	1	13	3	82	14
ND050282CA	ND028804CAB-5	ND7443Ab-45	100	4	15	3	57	24
ND060375B	B0718-3	ND028856B-1Russ	9	0	0	0	8	0
ND060391AB	Etb 6-5-5	ND6934b-2	91	0	0	0	91	0
ND060393AB	Etb 6-5-5	ND7443Ab-45	100	0	1	0	99	1
ND060394AB	Etb 6-5-5	ND7443Ab-76	100	0	0	0	98	0
ND060395AB	Etb 6-5-5	ND7560C-4	60	0	0	0	58	0
ND060397AB	Etb 6-5-5	ND8277B-5	100	0	10	2	84	10
ND060398AB	Etb 6-5-5	ND8331Cb-2	100	0	0	0	97	0
ND060399AB	Etb 6-5-5	ND8444b-2Russ	100	0	0	0	94	0
ND060410CB	LBR3	ND028804CB-1	33	0	6	1	26	18
ND060411B	LBR4	ND8277B-5	100	8	24	11	57	32
ND060425B	Patagonia	EB8109-1	100	0	1	3	94	1
ND060430B+I	Patagonia	ND039051B-1R	100	1	1	0	98	2
ND060431BV	Patagonia	ND039087BV-3R	90	0	1	0	89	1
ND060435CB	R91191-2W/Y	ND7377Cb-1	100	0	10	0	90	10
ND060436AB	R91191-2W/Y	ND7384Ab-4	85	0	0	1	84	0
ND060437AB	R91191-2W/Y	ND7443Ab-186Russ	44	0	0	0	39	0
ND060440CB	R91191-2W/Y	ND028801CB-1	20	0	1	0	19	5
ND060441CB	Rx91201-11	ND7377Cb-1	100	0	1	0	96	1
ND060443B	R91203-4	EB8109-1	61	0	0	0	61	0
ND060446AB	R91203-4	ND7443Ab-45	100	0	7	0	91	7

Table A5. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2007. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2007, isolates 693-3, 711, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND060553VC	ND5781-9R	ND028957VCB-1R	100	3	8	5	78	12
ND060566CB	ND6400C-1Russ	ND028856B-1Russ	100	0	14	6	79	14
ND060569AB	ND6934b-2	Etb 6-5-5	100	0	17	7	74	17
ND060570B	ND6934b-2	ND7132-1R	100	0	5	4	89	5
ND060571B	ND6934b-2	ND7192-1	90	6	19	2	50	32
ND060572AB	ND6934b-2	ND7443Ab-45	43	11	18	2	12	67
ND060574B	ND6947b-20	ND7192-1	100	16	2	6	72	19
ND060576CB	ND6947b-20	ND8540Cb-5	50	0	5	1	32	13
ND060578B	ND6953b-34	ND7192-1	100	27	33	4	34	61
ND060579B	ND6953b-34	ND028595b-4Y	29	2	1	0	26	10
ND060583CB	ND6961B-21PY	NY131	20	0	0	0	9	0
ND060585B	ND6961B-21PY	ND5765-9R	45	3	0	1	41	7
ND060586B	ND6961B-21PY	ND7132-1R	11	0	1	0	8	11
ND060587B	ND6961B-21PY	ND028601-4R	85	4	1	3	61	7
ND060589B	ND7132-1R	EB8109-1	100	0	7	1	92	7
ND060590AB	ND7132-1R	Etb 6-5-5	100	1	13	1	67	14
ND060591	ND7132-1R	ND028587-1RY	100	0	6	4	90	6
ND060592B	ND7132-1R	ND039051B-1R	100	0	10	1	76	10
ND060593VB	ND7132-1R	ND039087VB-3R	100	3	35	1	50	39
ND060597AB	ND7192-1	Etb 6-5-5	100	1	10	5	81	11
ND060598AB	ND7192-1	ND7384Ab-4	100	2	0	1	97	2
ND060600CB	ND7192-1	ND028801CB-1	100	1	6	4	65	9
ND060601CA	ND7192-1	ND028804Ab-1	100	54	31	0	15	85
ND060602B	ND7192-1	ND039571B-4	100	0	1	0	99	1
ND060603	ND7192-1	ND860-2	100	0	4	0	92	4
ND060604AB	ND7291b-2Y	Etb 6-5-5	100	0	6	1	90	6
ND060605AB	ND7333b-7	Etb 6-5-5	100	0	5	1	90	5
ND060606CB	ND7333b-7	NY131	100	0	5	5	88	5

Table A5. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2007 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2007, isolates 693-3, 711, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND060607B	ND7333b-7	P2-4	100	1	0	1	90	1
ND060608B	ND7333b-7	ND860-2	100	0	2	0	92	2
ND060609B	ND7333b-7	ND7192-1	100	0	3	0	91	3
ND060610CB	ND7333b-7	ND7377Cb-1	100	2	3	3	88	5
ND060611AB	ND7333b-7	ND7443Ab-76	100	3	4	3	88	7
ND060613B	ND7333b-7	ND7632-6	43	12	16	0	11	72
ND060614CB	ND7377Cb-1	Dakota Diamond	100	0	0	0	95	0
ND060615CB	ND7377Cb-1	White Pearl	100	1	1	1	97	2
ND060616CB	ND7377Cb-1	EB8109-1	100	1	1	1	92	2
ND060617AB	ND7377Cb-1	Etb 6-5-5	100	2	9	5	84	11
ND060618CB	ND7377Cb-1	EGA970614	100	1	9	7	82	10
ND060619CB	ND7377Cb-1	LBR4	48	1	5	0	28	18
ND060620CB	ND7377Cb-1	NY131	100	1	13	4	75	15
ND060622CB	ND7377Cb-1	ND2858-1	100	1	4	0	71	7
ND060623CB	ND7377Cb-1	ND5649-1Russ	100	73	1	3	22	75
ND060624CB	ND7377Cb-1	ND7511C-1	100	0	0	3	92	0
ND060625CB	ND7377Cb-1	Dakota Russet	100	0	0	2	98	0
ND060626CB	ND7377Cb-1	ND028856B-1Russ	100	0	7	1	74	7
ND060628AB	ND7384Ab-4	NY131	100	0	5	0	91	5
ND060629AB	ND7384Ab-4	R91191-2W/Y	100	0	14	9	76	14
ND060630AB	ND7384Ab-4	ND7377Cb-1	100	0	18	1	80	18
ND060631AB	ND7390Ab-10	White Pearl	100	27	17	4	40	50
ND060632AB	ND7390Ab-10	ND860-2	60	1	7	2	45	15
ND060633AB	ND7390Ab-10	ND7192-1	71	0	0	0	59	0
ND060634AB	ND7390Ab-10	ND039057B-1	70	2	4	7	50	10
ND060641AB	ND7443Ab-72	Dakota Crisp	24	0	0	0	24	0
ND060646AB	ND7443Ab-72	ND7333b-7	100	5	5	0	84	11
ND060649AB	ND7443Ab-76	R91203-4	24	0	0	0	24	0

Table A5. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2007 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2007, isolates 693-3, 711, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of genotypes evaluated	Frequency of Ratings Within Families				
	Female	Male		0	1	2	3	% Resistant
ND060651AB	ND7443Ab-76	ND7192-1	50	1	2	0	47	6
ND060658AB	ND7443Ab-180	ND5641-2Russ	43	0	0	0	36	0
ND060662AB	ND7443Ab-180	Dakota Russet	20	0	0	0	19	0
ND060668AB	ND7443Ab-186Russ	ND6400C-1Russ	78	1	0	0	71	1
ND060671AB	ND7443Ab-186Russ	ND8291C-2Russ	48	0	0	0	46	0
ND060676AB	ND7495b-6	ND7443Ab-186Russ	100	3	3	4	88	6
ND060762B	ND8444b-2Russ	AWN86514-2	100	0	1	0	88	1
ND060776CB	ND8492Cb-5Russ	ND028673B-2Russ	86	4	0	0	69	5
ND060821B	ND028770b-4R	LBR4	95	1	0	0	81	1

Table A6. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2008. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2008, isolates 693-3, 711, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of genotypes evaluated	Frequency of Ratings Within Families				
	Female	Male		0	1	2	3	% Resistant
ND071097B	ND7291b-2Y	R91102-2	100	0	0	0	92	0
ND071161CB	ND8478cb-5	ND028970B-65Russ	100	0	0	0	100	0
ND071102B	ND7333b-7	ND8277B-5	43	4	14	11	14	42
ND071103B	ND7333b-7	ND028856B-1Russ	100	0	3	12	84	3
ND071107B	ND7495b-6	ND039004B-2	100	0	0	0	97	0
ND071133B	Dakota Russet	ND028856B-1Russ	11	0	1	3	7	9
ND071136B	ND8277B-5	ND8527B-94	20	0	5	5	10	25
ND071137B	ND8277B-5	ND028856B-1Russ	12	0	4	3	5	33
ND071138B	ND8277B-5	ND049287B-4	20	3	8	3	5	58
ND071148B	ND8444b-2Russ	Dakota Trailblazer	100	0	0	0	97	0
ND071149B	ND8444b-2Russ	ND028970B-65Russ	59	0	3	16	37	5
ND071150B	ND8444b-2Russ	ND049423B-3Russ	41	0	1	5	35	2

Table A6. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2008 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2008, isolates 693-3, 711, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	%
ND071156CB	ND8477CB-21	ND039004B-2	100	0	1	3	91	1
ND071160CB	ND8478Cb-5	ND028970B-65Russ	100	0	1	7	85	1
ND071162CB	ND8478Cb-5	ND049285CB-4	100	0	1	5	91	1
ND071163CB	ND8478Cb-5	ND049287B-3	100	0	1	2	96	1
ND071168CB	ND8492Cb-2Russ	ND028856B-1Russ	100	0	32	36	32	32
ND071170B	ND8527B-94	R91101-2	100	1	3	11	85	4
ND071172B	ND8527B-94	ND8277B-5	100	5	26	41	28	31
ND071173B	ND8527B-94	ND8570B-1	100	0	7	20	73	7
ND071178B	Dakota Ruby	ND039035B-9R	100	0	2	4	93	2
ND071179B	Dakota Ruby	ND039036B-2R	100	4	27	40	29	31
ND071190B	ND028662b-4Russ	Dakota Trailblazer	100	0	0	0	100	0
ND071192B	ND028666b-1Russ	Dakota Trailblazer	62	0	5	15	42	8
ND071193B	ND028666b-1Russ	ND8444b-2Russ	162	0	3	21	133	2
ND071195B	ND028666b-1Russ	ND028970B-65Russ	51	0	2	6	43	4
ND071197B	ND028666b-1Russ	ND049423B-3Russ	176	3	13	19	139	9
ND071198B	ND028671B-96	Dakota Trailblazer	100	0	5	13	80	5
ND071199B	ND028671B-96	ND028856B-1Russ	65	0	11	14	40	17
ND071203B	ND028734B-1	ND028666b-1Russ	64	0	0	2	62	0
ND071205B	ND028734B-1	ND039004B-2	100	0	0	0	89	0
ND071206B	ND028734B-1	ND049287B-3	90	0	3	9	78	3
ND071214CB	ND028801CB-1	R91101-2	71	0	1	8	62	1
ND071225B	ND028856B-1Russ	ND049423B-3Russ	100	6	42	35	17	48
ND071226CB	ND028888CB-1	AH66-4	41	4	16	18	3	49
ND071229CB	ND028888CB-1	ND028856B-1Russ	100	2	19	45	31	22
ND071233CB	ND028946b-1R	ND039166CB-2R	18	0	0	5	11	0
ND071235B	ND028946b-1R	ND049531B-5R	37	0	1	18	18	3
ND071241B	ND028970B-65Russ	ND028856B-1Russ	18	3	5	6	4	44
ND071244B	ND028970B-65Russ	ND049423B-3Russ	100	0	7	30	63	7

Table A6. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2008 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2008, isolates 693-3, 711, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	%
ND071264BV	ND039035B-9R	ND039087BV-3R	90	0	15	26	47	17
ND071268B	ND039035B-9R	95043.11	71	0	0	8	59	0
ND071360B	ND049227B-1	90245.1	32	1	7	14	10	25
ND071369B	ND049287B-3	ND028888CB-1	29	5	7	17	0	41
ND071378B	ND049297B-4	ND049227B-1	100	0	13	45	42	13
ND071383AB	ND049305AB-1	ND039087BV-3R	12	0	0	12	0	0
ND071409B	ND049351B-5R	ND5858	81	1	13	59	8	17
ND071412VB	ND049351B-5R	ND039126VB-2R	19	0	3	14	2	16
ND071413CB	ND049351B-5R	ND039166CB-2R	100	0	1	7	90	1
ND071425B	ND049382B-2	ND8277B-5	100	9	38	42	10	47

Table A7. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2009. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2009, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND081555CB	ND2858-1	Dakota Trailblazer	100	0	0	1	97	0
ND081558CAB	ND2858-1	ND039173CAB-22	72	0	1	1	70	1
ND081559C	ND2858-1	95043.11	100	0	5	0	95	5
ND081570B	ND4659-5R	ND7067B-67R	100	0	0	2	98	0
ND081572B	ND4659-5R	ND028940B-102R	100	0	24	30	46	24
ND081573	ND4659-5R	95043.11	100	0	2	20	78	2
ND081574CAB	ND5873-23	ND039173CAB-22	100	0	1	4	95	1
ND081575CB	ND5873-23	ND049553B-50	100	2	25	29	43	27
ND081579B	ND6956b-13	ND049553B-50	100	3	37	14	46	40
ND081580B	ND6934b-2	Gala	100	0	1	1	98	1
ND081582B	ND6934b-2	AND97279-5Russ	90	1	9	4	69	12

Table A7. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2009 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2009, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND081583B	ND6934b-2	Dakota Trailblazer	70	4	6	2	54	15
ND081586B	ND6934b-2	ND049587B-5Russ	100	1	8	10	81	9
ND081588ABC	ND6947B-136	ND028926ABC-78	100	0	1	14	83	1
ND081589B	ND6947B-136	ND039004B-2Y	80	0	1	0	78	1
ND081590CAB	ND6947B-136	ND039173CAB-22	100	21	36	25	18	57
ND081593B	ND6961B-21PY	Gala	100	6	24	9	60	30
ND081597BV	ND7067B-67R	ND039087BV-3R	100	4	42	11	43	46
ND081598B	ND7067B-67R	95043.11	73	3	23	18	29	36
ND081599CAB	ND7333b-7	ND039173CAB-22	100	0	4	3	91	4
ND081600CB	ND7377Cb-1	ND8331Cb-3	100	0	5	16	79	5
ND081601CB	ND7377Cb-1	ND049552B-98	60	2	3	3	48	9
ND081602CB	ND7377Cb-1	ND049553B-50	100	10	39	27	24	49
ND081606AB	ND7443Ab-	ND049547B-7Russ	100	2	3	2	88	5
ND081607CAB	ND7495b-6	ND039173CAB-22	100	0	34	31	35	34
ND081611B	ND7818-1Y	Gala	50	1	5	3	34	14
ND081624B	Dakota Russet	ND049545B-8Russ	100	3	12	5	77	15
ND081625 B	Dakota Russet	ND049547B-7Russ	100	0	1	6	93	1
ND081626B	Dakota Russet	ND049587B-5Russ	100	10	20	23	47	30
ND081628ABV	ND8266A-1R	ND039087BV-3R	70	1	4	1	56	8
ND081629A	ND8266A-1R	95043.11	100	1	2	2	90	3
ND081630ABC	ND8277B-5	ND028926ABC-78	100	10	13	30	47	23
ND081633CB	ND8291C-1Russ	Dakota Trailblazer	100	0	3	17	80	3
ND081634CAB	ND8291C-1Russ	ND7443Ab-45Russ	100	0	0	1	99	0
ND081636CB	ND8291C-1Russ	ND059694B-20Russ	50	5	25	4	11	67
ND081660B	ND8570B-1Y	Puren	81	6	8	12	55	17
ND081664B	ND8570B-1Y	793101.3	70	0	1	2	64	1
ND081665B	ND8570B-1Y	93057.1	100	0	0	1	98	0
ND081680CB	ND028813b-5	ND7377Cb-1	100	6	30	19	45	36

Table A7. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2009 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2009, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND081681CAB	ND028813b-5	ND039173CAB-22	90	9	33	23	25	47
ND081682ABC	ND028926ABC-78	ND039173CAB-22	100	2	15	7	76	17
ND081689CBV	ND039036B-2RY	ND039165CBV-70R	100	14	34	18	34	48
ND081695B	ND039087BV-3R	ND049326C-2P	70	0	0	3	66	0
ND081696BVC	ND039087BV-3R	ND049498BVC-	100	2	23	19	56	25
ND081697BV	ND039087BV-3R	ND049565B-64R	62	0	13	4	45	21
ND081698V	ND039087BV-3R	95043.11	100	0	15	17	68	15
ND081699CAB	ND039104CAB-3	ND039173CAB-22	100	8	4	17	71	12
ND081700CAB	ND039104CAB-3	ND049552B-42	100	0	1	4	95	1
ND081701B	ND039112B-1Russ	ND059669B-2Russ	100	0	19	28	52	19
ND081702B	ND039125B-29R	Dakota Ruby	70	0	16	7	45	24
ND081703B	ND039125B-29R	ND039036B-2RY	100	6	36	11	47	42
ND081704BV	ND039125B-29R	ND039087BV-3R	100	20	41	17	22	61
ND081705B	ND039125B-29R	95043.11	100	9	27	9	55	36
ND081706AB	ND039134AB-2	ND049552B-42	100	5	34	9	52	39
ND081716CBV	ND039165CBV-70R	ND039087BV-3R	100	0	23	14	63	23
ND081719CAB	ND039173CAB-22	ND049553B-50	100	0	0	0	10	0
ND081752 B	ND049351B-5R	ND7067B-67R	90	0	1	1	85	1
ND081761B	ND049547b-2Russ	Dakota Trailblazer	100	0	0	12	88	0
ND081762CAB	ND049547b-2Russ	ND039173CAB-22	100	0	7	1	92	7
ND081763B	ND049547B-7Russ	Dakota Trailblazer	100	0	6	3	91	6
ND081765B + IB	ND049547B-7Russ	ND049547b-2Russ	52	0	0	0	52	0
ND081766B	ND049547B-7Russ	ND049587B-5Russ	57	0	0	12	45	0
ND081767B	ND049548B-1	ND049552B-98	100	0	1	24	75	1
ND081768ABC	ND049551B-3Russ	ND028926ABC-78	100	0	9	7	84	9
ND081774B	ND049587B-5Russ	Dakota Trailblazer	100	0	2	20	78	2
ND081785B	ND059674B-20Russ	AH66-4	100	0	2	15	83	2
ND081790B	ND059694B-20Russ	ND049547b-2Russ	100	0	6	5	89	6

Table A7. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2009 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2009, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of genotypes evaluated	Frequency of Ratings Within Families				
	Female	Male		0	1	2	3	% Resistant
ND081791B	ND059694B-20Russ	ND049547B-7Russ	100	2	9	6	81	11
ND081798B	ND059961AB-	ND049587B-5Russ	100	0	3	11	86	3
ND081800	793101.3	Gala	100	0	6	8	86	6
ND081801B	793101.3	Granola	100	0	3	2	95	3
ND081803AB	793101.3	ND039163AB-209	84	0	3	11	70	4
ND081804B	Unknown	Dakota Trailblazer	100	0	1	4	95	1
ND081806AB	90245.1	ND049305AB-1	100	0	0	0	10	0
ND081807CAB	93057.1	ND039173CAB-22	100	0	2	3	95	2
ND081811BV	95043.11	ND039087BV-3R	100	0	0	0	10	0
ND081555CB	ND2858-1	Dakota Trailblazer	100	0	0	0	10	0

Table A8. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2010. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2010, isolates 693-3, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of genotypes evaluated	Frequency of Ratings Within Families				
	Female	Male		0	1	2	3	% Resistant
ND092069AB	ND7443Ab-45Russ	PA99N2-1	99	0	7	10	82	7
ND092081CA	ND7443Ab-180	ND8331Cb-2	99	0	10	15	74	10
ND092091AB	ND7515-1	ND060397AB-20	99	0	10	15	74	10
ND092059AB	ND6400C-1	ND049474ABC-1Russ	100	0	0	2	98	0
ND092060AB	ND6400C-1	ND059787AB-3Russ	100	3	36	30	31	39
ND092063B	ND6934B-2	ND860-2	100	1	8	10	81	9
ND092068AB	ND7443Ab-45Russ	Dakota Trailblazer	100	0	13	8	79	13
ND092070AB	ND7443Ab-45Russ	ND860-2	100	0	2	5	91	2
ND092071AB	ND7443Ab-46	ND860-2	100	0	1	4	95	1
ND092073AB	ND7443Ab-72Russ	King Harry	95	2	11	4	78	14
ND092074AB	ND7443Ab-72Russ	Dakota Trailblazer	100	2	29	31	38	31

Table A8. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2010 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2010, isolates 693-3, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of genotypes	Frequency of Ratings Within Families				
	Female	Male	evaluated	0	1	2	3	% Resistant
ND092075AB	ND7443Ab-72Russ	ND8291C-2Russ	100	0	25	8	67	25
ND092077AB	ND7443Ab-180	King Harry	100	0	59	24	17	59
ND092078AB	ND7443Ab-180	Dakota Trailblazer	100	0	0	0	100	0
ND092079AB	ND7443Ab-180	PA99N2-1	100	0	0	0	100	0
ND092082AB	ND7443Ab-180	ND049475ABC-1	100	0	0	1	99	0
ND092083CA	ND7443Ab-180	ND059804C-10	100	2	9	17	68	11
ND092088AB	ND7519-1	Etb 6-5-5	100	0	2	23	75	2
ND092090AB	ND7519-1	ND049475ABC-1	100	1	2	2	95	3
ND092091AB	ND7519-1	ND060397AB-20	100	1	18	4	77	19
ND092100BC	ND7560c-4	ND028984B-1	100	0	5	0	94	5
ND092101AB	ND7560c-4	ND049475ABC-1	100	0	1	1	98	1
ND092110B	ND7982-1R	Romanze	100	0	5	4	91	5
ND092113B	ND8083b-1pY	ND4659-5R	100	0	0	0	100	0
ND092117AB	Dakota Russet	ND060389AB-5Russ	100	0	0	13	85	0
ND092119B	ND8277B-5	Patagonia	100	0	14	2	84	14
ND092121CB	ND8291C-2Russ	PA99N2-1	100	8	14	64	14	22
ND092125AB	ND8304-2	Etb 6-5-5	61	0	0	0	60	0
ND092126AB	ND8304-2	Etb 6-21-5	100	0	0	0	100	0
ND092131AB	ND8305-1	Etb 6-21-5	100	0	0	2	98	0
ND092136B	ND8459-2	J101-K6-A22	100	0	0	1	97	0
ND092139B	ND8459-2	ND028970B-74	54	0	0	3	51	0
ND092140B	ND8459-2	ND060378B-1	100	0	16	15	69	16
ND092146B	Dakota Ruby	ND049223B-3R	100	0	0	3	97	0
ND092150B	ND028742b-12PEY	Dakota Ruby	100	0	2	3	93	2
ND092152CB	ND028742b-12PEY	ND050167C-3R	87	2	2	1	81	5
ND092156AB	ND028799C-3	Etb 6-5-5	100	0	0	4	96	0
ND092160CA	ND028799C-3	ND049461Ab-2	88	0	0	7	81	0
ND092161CA	ND028799C-3	ND049474ABC-1Russ	86	2	0	2	82	2

Table A8. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2010 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2010, isolates 693-3, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of genotypes	Frequency of Ratings Within Families				
	Female	Male	evaluated	0	1	2	3	% Resistant
ND092162CB	ND028799C-3	ND060378B-1	100	1	15	16	67	16
ND092163CA	ND028799C-3	ND060397AB-20	100	3	31	4	62	34
ND092165CA	ND028804CAb-5	King Harry	40	0	11	7	22	28
ND092166CA	ND028804CAb-5	Etb 6-21-5	48	0	0	1	47	0
ND092167CA	ND028804CAb-5	PA99N2-1	79	0	1	2	76	1
ND092168CA	ND028804CAb-5	ND7519-1	100	0	0	0	94	0
ND092169CA	ND028804CAb-5	ND7799c-1	65	1	0	2	55	2
ND092176B	ND028856B-1Russ	Dakota Trailblazer	55	0	0	5	48	0
ND092178AB	ND028856B-1Russ	ND059787AB-3Russ	100	0	89	8	3	89
ND092181CB	ND028888cB-1	ND7799c-1	100	4	66	27	3	70
ND092182B	ND028970B-74	LBR8	100	12	61	17	10	73
ND092184AB	ND028970B-74	ND7443Ab-44	73	12	43	14	4	75
ND092191AB	ND028984B-1	Etb 6-21-4	92	17	49	17	9	72
ND092194B	ND039036B-2R	AND00272-1R	100	0	87	9	4	87
ND092198CA	ND039104CAB-3	P99 N2-1	100	3	77	12	8	80
ND092200CA	ND039104CAB-3	ND060397AB-20	95	50	36	6	3	91
ND092202B	ND039194-1Russ	Dakota Trailblazer	64	0	52	9	3	81
ND092205B	ND049223B-3R	Bison	93	0	17	9	67	18
ND092206B	ND049223B-3R	T10-12	100	0	24	30	46	24
ND092208B	ND049268-2R	Patagonia	56	0	51	5	0	91
ND092217AB	ND049275-1	ND049475ABC-1	100	0	78	18	4	78
ND092220AB	ND049323C-6	Etb 6-21-4	100	0	85	14	1	85
ND092222CB	ND049323C-6	LBR8	34	0	32	1	1	94
ND092225CB	ND049323C-6	ND060378B-1	92	3	67	16	6	76
ND092227CB	ND049323C-7	Dakota Trailblazer	68	0	58	10	0	85
ND092232CA	ND049326C-2P	Etb 6-5-5	100	0	21	25	54	21
ND092244B	ND049423b-1Russ	ND059852b-2Russ	80	0	16	37	27	20
ND092245AB	ND049423b-1Russ	ND060389AB-5Russ	41	0	25	6	10	61

Table A8. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2010 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2010, isolates 693-3, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND092248AB	ND049474ABC-1Russ	ND049474ABC-1Russ	36	0	0	0	36	0
ND092250AB	ND049474ABC-1Russ	AOND95292-3Russ	61	0	18	8	35	30
ND092251AB	ND049474ABC-1Russ	Etb 6-5-5	100	0	0	4	96	0
ND092252AB	ND049474ABC-1Russ	PA99N2-1	100	0	1	3	96	1
ND092254AB	ND049474ABC-1Russ	ND039194-1Russ	100	4	43	24	29	47
ND092256AB	ND049474ABC-1Russ	ND060475C-11Russ	61	0	0	0	61	0
ND092257AB	ND049474ABC-1Russ	ND060487CB-3Russ	95	1	34	23	37	37
ND092260AB	ND049475ABC-1	Etb 6-21-4	100	0	10	15	75	10
ND092261AB	ND049475ABC-1	Etb 6-21-5	100	0	31	28	41	31
ND092262AB	ND049475ABC-1	ND060378B-1	61	0	23	16	22	38
ND092263AB	ND049547B-27Russ	ND059787AB-3Russ	100	0	11	4	85	11
ND092264AB	ND049589B-15Russ	Etb 6-5-5	100	0	2	6	92	2
ND092265AB	ND049589B-15Russ	Etb 6-5-5	65	0	14	12	39	22
ND092268CA	ND049589B-15Russ	ND060476CAB-2	100	0	0	0	10	0
ND092269B	ND050093-9Russ	Dakota Trailblazer	100	0	1	0	99	1
ND092274B	ND059614-7R	Patagonia	66	0	0	0	66	0
ND092281B	ND059734-5R	Patagonia	100	0	24	23	53	24
ND092283B	ND059734-5R	ND060378B-1	73	0	10	1	62	14
ND092285AB	ND059787AB-3Russ	ND049474ABC-1Russ	100	0	3	2	95	3
ND092286AB	ND059787AB-3Russ	ND060389AB-5Russ	100	0	4	9	87	4
ND092291AB	ND059804C-10	ND049475ABC-1	100	0	0	0	100	0
ND092293AB	ND059804C-10	ND060397AB-20	100	0	19	20	61	19
ND092297CA	ND059809C-2P	ND050167C-3R	100	2	35	23	40	37
ND092318CB	ND059852C-2Russ	ND049547B-2Russ	100	0	0	0	100	0
ND092326AB	ND060378B-1	Etb 6-21-5	100	0	21	13	65	21
ND092329AB	ND060389B-5Russ	AND97279-5Russ	100	0	0	0	100	0
ND092331AB	ND060389B-5Russ	ND059787AB-3Russ	100	0	10	8	82	10
ND092332AB	ND060397AB-20	King Harry	35	0	1	12	21	3

Table A8. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2010 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2010, isolates 693-3, 714, and 481 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of genotypes evaluated	Frequency of Ratings Within Families				
	Female	Male		0	1	2	3	% Resistant
ND092336AB	ND060397AB-20	ND049475ABC-1	59	0	3	1	55	5
ND092337AB	ND060397AB-20	ND060378B-1	100	1	45	31	23	46
ND092341AB	ND060463C-1	Etb 6-21-5	44	0	0	0	40	0
ND092349CB	ND060475C-2Russ	ND060378B-1	100	1	3	13	83	4
ND092351CA	ND060475C-2Russ	ND060389AB-5Russ	66	0	0	0	66	0
ND092352AB	ND060475C-2Russ	ND060474ABC-1Russ	75	0	0	0	75	0
ND092360AB	ND060475C-11Russ	ND059787AB-3Russ	100	0	0	2	98	0

Table A9. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2011. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2011, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of genotypes evaluated	Frequency of Ratings Within Families				
	Female	Male		0	1	2	3	% Resistant
ND102486B	Umatilla Russet	ND039194-1Russ	100	0	1	2	97	1
ND102598B	ND6934b-6	ND039194-1Russ	23	1	6	1	14	32
ND102599B	ND6934b-6	ND049489B-5Russ	100	6	7	8	78	13
ND102600B	ND6934b-6	ND050032-4Russ	100	5	16	19	60	21
ND102601B	ND6934b-6	ND060761B-3Russ	100	0	4	3	93	4
ND102603B	ND6956b-13	Dakota Trailblazer	100	0	9	11	78	9
ND102606CB	ND6957B-25P	ND050306-1R	100	0	0	5	95	0
ND102609AB	ND7384Ab-4	ND039194-1Russ	39	9	7	1	22	41
ND102610B	ND7403B-5	Dakota Trailblazer	100	0	0	1	99	0
ND102611B	ND7403B-5	AND01804-3Russ	100	0	0	0	100	0
ND102612CB	ND7403B-5	ND038589c-1	100	2	0	0	98	2
ND102613B	ND7403B-5	ND060761B-3Russ	100	1	2	1	96	3
ND102614B	ND7403B-5	ND070927-5Russ	100	10	22	8	60	32

Table A9. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2011 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2011, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND102641CB	ND7799c-1	LBR8	100	14	28	9	49	42
ND102645B	Dakota Russet	Stirling	100	1	8	4	87	9
ND102648B	Dakota Russet	ND060607B-4	38	3	14	4	15	47
ND102650CB	ND8277B-5	ND060618CB-3	100	28	10	6	56	38
ND102652B	ND8304-2	ND8277B-5	100	3	26	19	52	29
ND102660B	ND8314-1R	ND070935B-3R	57	0	0	0	57	0
ND102661B	ND8527B-94	Stirling	99	5	14	12	68	19
ND102662AB	ND8527B-94	ND060421Ab-1	100	8	1	0	91	9
ND102663B	Dakota Ruby	Patagonia	100	0	10	17	73	10
ND102664B	Dakota Ruby	RA90213-60	100	7	1	5	87	8
ND102666CB	Dakota Ruby	ND050067CB-1R	100	3	4	6	87	7
ND102668AB	ND028589c-1	Etb 6-5-5	97	3	1	5	88	4
ND102676b	ND028671B-96	M7	100	2	8	2	87	10
ND102677B	ND028671B-96	ND049546b-	100	0	3	7	90	3
ND102679BV	ND028940B-102R	ND060788bV-3RY	99	2	3	2	92	5
ND102688B	ND049223B-3R	Patagonia	100	21	31	11	37	52
ND102689BC	ND049223B-3R	ND049326C-2P	100	0	6	9	85	6
ND102690CB	ND049223B-3R	ND050060CB-4R	100	1	6	12	81	7
ND102691B	ND049223B-3R	ND059734-4R	100	7	2	10	81	9
ND102694CB	ND049326C-2P	Patagonia	92	3	5	5	79	9
ND102695CB	ND049326C-2P	RA90213-60	88	18	28	15	27	52
ND102697CB	ND049326C-2P	ND049223B-3R	100	13	1	0	86	14
ND102700CB	ND049326C-2P	ND050174B-5R	100	9	7	25	59	16
ND102705CB	ND049326C-2P	ND060822CB-2P	100	5	2	5	88	7
ND102709CB	ND049517b-1Russ	ND060618CB-9	100	0	1	1	98	1
ND102710CB	ND049517b-1Russ	Nd060625Cb-	77	0	2	13	61	3
ND102712B	ND049546B-10Russ	Stirling	47	0	1	4	42	2
ND102715B	ND049546B-10Russ	ND060607B-4	96	0	0	0	96	0

Table A9. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2011 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2011, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND102721B	ND049589B-5Russ	Dakota Trailblazer	100	0	4	15	81	4
ND102722AB	ND049589B-5Russ	ND7443Ab-72Russ	100	25	32	10	33	57
ND102723B	ND049589B-5Russ	ND039194-1Russ	100	0	1	12	87	1
ND102724B	ND050005-1P	ND8277B-5	100	13	22	9	56	35
ND102731CB	ND050060CB-4R	Dakota Jewel	40	0	0	2	38	0
ND102732CB	ND050060CB-4R	Patagonia	100	0	0	8	92	0
ND102733CB	ND050060CB-4R	ND060822CB-2P	100	0	0	3	97	0
ND102734CB	ND050060CB-4R	ND071007B-3R	100	0	0	12	88	0
ND102736CB	ND050067CB-1R	ND028940B-102R	100	0	0	0	100	0
ND102738CB	ND050067CB-1R	ND050174B-5R	100	0	1	25	74	1
ND102741CB	ND050067CB-1R	ND060822CB-2P	100	0	0	2	98	0
ND102742CB	ND050067CB-1R	ND071007B-3R	100	10	2	1	87	12
ND102743CB	ND050132C-6R	Patagonia	100	0	2	19	79	2
ND102744CB	ND050132C-6R	ND028940B-102R	69	0	0	0	69	0
ND102759CB	ND050306-1R	ND060822CB-2P	100	0	0	0	100	0
ND102764B	ND050306-1R	ND070935B-3R	100	0	0	4	96	0
ND102768AB	ND059769AB-1Russ	ND060761B-3Russ	21	0	0	0	21	0
ND102771CB	ND059809C-1P	Patagonia	100	0	0	3	97	0
ND102777CB	ND059818C-5	Stirling	100	0	7	16	77	7
ND102778CA	ND059818C-5	Etb 6-5-5	100	0	0	3	97	0
ND102779CA	ND059818C-5	ND7384Ab-4	100	0	0	17	83	0
ND102781CA	ND059818C-5	ND060380Ab-5	100	0	1	8	91	1
ND102782CB	ND059818C-5	ND060607B-4	100	0	1	8	91	1
ND102785AB	ND059823-4R	ND071007B-3R	35	0	0	0	35	0
ND102787B	ND059825-4R	ND071007B-3R	100	0	0	0	100	0
ND102795AB	ND059999C-4	ND060392AB-4	100	0	0	14	86	0
ND102796CB	ND059999C-4	ND060618CB-3	100	0	0	4	96	0
ND102810AB	ND060421Ab-1	ND028984B-1	100	0	0	16	84	0

Table A9. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2011 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2011, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND102813CA	ND060476CAb-6	Dakota Trailblazer	100	0	0	11	89	0
ND102815CA	ND060476CAb-6	Stirling	100	0	0	2	98	0
ND102821CA	ND060476CAb-15	Dakota Trailblazer	100	0	0	10	90	0
ND102824CA	ND060476CAb-15	AH66-4	100	0	0	5	95	0
ND102825CA	ND060476CAb-15	M7	68	0	0	0	68	0
ND102826CA	ND060476CAb-15	ND049546b-	100	0	0	0	100	0
ND102836CB	ND060564C-3Russ	Stirling	100	0	13	10	77	13
ND102849AB	ND060607B-4	ND060384Ab-5	100	0	0	0	100	0
ND102851B	ND060607B-4	ND060753-8	72	0	0	5	67	0
ND102853CB	ND060618CB-3	ND049553B-50	100	0	0	0	100	0
ND102854AB	ND060618CB-3	ND060380Ab-5	100	0	2	0	98	2
ND102855CA	ND060618CB-3	ND060421Ab-1	100	0	0	0	100	0
ND102859CB	ND060618CB-9	Dakota Trailblazer	50	0	0	2	47	0
ND102860CB	ND060618CB-9	Ranger Russet	100	0	0	0	98	0
ND102863CB	ND060618CB-9	ND049546b-	100	0	0	1	99	0
ND102865CB	ND060625cB-1Russ	Dakota Trailblazer	100	0	0	15	85	0

Table A10. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2012. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2012, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND113028CB	Dakota Diamond	Stirling	100	5	10	9	76	15
ND113033B	Dakota Jewel	LBR8	100	2	5	11	82	7
ND113035B	Dakota Pearl	LBR8	100	6	2	3	89	8
ND113038B	Dakota Trailblazer	Russet Norkotah	100	12	13	16	59	25
ND113039B	Dakota Trailblazer	AH66-4	100	0	0	9	91	0

Table A10. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2012 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2012, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND113040B	Dakota Trailblazer	M7	100	0	0	5	95	0
ND113041B	Dakota Trailblazer	ND070927-2Russ	100	2	9	9	80	11
ND113042	Gala	Dakota Jewel	100	1	4	0	95	5
ND113043	Gala	ND4659-5R	100	0	0	0	100	0
ND113044	Gala	ND7192-1	100	0	3	7	90	3
ND113054	Ivory Crisp	Gala	100	5	5	11	79	10
ND113074CB	Patagonia	ND050067cb-1R	100	0	0	8	92	0
ND113075CB	Patagonia	ND050167C-3R	100	0	0	9	91	0
ND113077CB	Patagonia	ND059809C-1P	55	0	0	8	47	0
ND113078CB	Patagonia	ND060822CB-2p	100	0	0	11	89	0
ND113084AB	Remehue 7	ND860-2	100	0	2	12	86	2
ND113085B	Remehue 7	ND7519-1	100	1	6	28	65	7
ND113092CB	Romanze	ND050060cb-1R	38	0	0	1	37	0
ND113094CB	Romanze	ND050167C-3R	100	0	0	1	99	0
ND113095CB	Romanze	ND060822CB-2p	100	2	2	0	96	4
ND113109	Yagana	AND01804-3Russ	100	0	0	13	87	0
ND113113B	Yagana	ND028742b-12REY	100	0	1	1	1	33
ND113114CA	Yagana	ND039104CAB-5	100	13	8	18	61	21
ND113115B	Yagana	ND049289B-1Russ	100	0	3	7	90	3
ND113116CB	Yagana	ND059809C-1P	100	0	3	12	85	3
ND113322CB	ND8331Cb-3	Stirling	100	4	2	10	84	6
ND113323CB	ND8331Cb-3	M3	100	1	0	5	94	1
ND113325CB	ND8331Cb-3	ND6620-14	99	1	0	2	96	1
ND113326CB	ND8331Cb-3	ND7519-1	100	1	9	10	80	10
ND113328AB	ND8331Cb-3	ND060873Ab-7	85	0	1	4	80	1
ND113350B	ND028671B-96Y	AND01804-3Russ	100	0	0	7	93	0
ND113353CB	ND028671B-96Y	ND060566CB-	100	2	5	24	69	7
ND113354B	ND028671B-96Y	ND070927-2Russ	100	0	0	0	100	0

Table A10. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2012 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2012, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND113355B	ND028671B-96Y	ND071302B-1Russ	100	6	5	7	82	11
ND113362AB	ND028799C-3	ND060873Ab-7	100	19	12	18	51	31
ND113364B	ND028984B-1	Ivory Crisp	100	1	1	5	93	2
ND113365CB	ND028984B-1	ND059804C-13	100	0	5	8	87	5
ND113366CA	ND039104CAB-3	Gala	100	0	0	0	100	0
ND113368CA	ND039104CAB-3	Puren	100	0	0	10	90	0
ND113370CA	ND039104CAB-3	Stirling	100	1	2	14	83	3
ND113371CA	ND039104CAB-3	LBR8	99	0	0	9	90	0
ND113372CA	ND039104CAB-3	ND860-2	100	0	0	3	97	0
ND113373CA	ND039104CAB-3	ND6620-14	100	0	1	13	86	1
ND113374CA	ND039104CAB-3	ND7519-1	100	0	1	9	90	1
ND113376CA	ND039104CAB-3	ND091829-1	100	0	0	11	89	0
ND113379AB	ND039194AB-1Russ	AND01804-3Russ	100	0	0	7	93	0
ND113380AB	ND039194AB-1Russ	AOND95292-3Russ	100	0	0	5	95	0
ND113381AB	ND039194AB-1Russ	Dakota Russet	100	0	0	2	98	0
ND113382AB	ND039194AB-1Russ	ND049546b-15Russ	93	0	1	7	85	1
ND113383AB	ND039194AB-1Russ	ND050032-4Russ	100	1	3	5	91	4
ND113384AB	ND039194AB-1Russ	ND060761B-3Russ	100	0	0	4	96	0
ND113385AB	ND039194AB-1Russ	ND070927-2Russ	100	0	1	8	91	1
ND113386AB	ND049219AB-5	Dakota Pearl	90	0	3	4	83	3
ND113387AB	ND049219AB-5	Yagana	100	0	0	5	95	0
ND113388AB	ND049219AB-5	ND860-2	100	0	1	0	99	1
ND113389B	ND049219AB-5	Ranger Russet	100	0	0	6	94	0
ND113390B	ND049289B-1Russ	AH66-4	100	0	1	8	91	1
ND113391B	ND049289B-1Russ	ND049546b-15Russ	100	0	0	5	95	0
ND113393AB	ND049289B-1Russ	ND060796AB-	85	0	0	0	85	0
ND113394CA	ND049321CAB-3	Dakota Pearl	100	0	0	5	95	0
ND113398CB	ND049322C-5	Stirling	100	0	1	5	94	1

Table A10. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2012 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2012, isolates 693-3 and 714 of *P. infestans* strain US-8 were used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND113399C	ND049322C-5	Yagana	100	0	0	0	100	0
ND113400CB	ND049322C-5	ND7381B-17	100	0	1	12	87	1
ND113401B	ND049517b-1Russ	AH66-4	100	0	1	6	93	1
ND113402B	ND049517b-1Russ	M7	100	0	0	0	100	0
ND113404B	ND049517b-1Russ	ND070927-2Russ	100	0	0	10	90	0
ND113405B	ND049546b-15Russ	AH66-4	100	0	1	7	92	1
ND113406B	ND049546b-15Russ	M7	100	0	1	2	97	1
ND113407B	ND049546b-15Russ	ND070927-2Russ	100	0	0	9	91	0
ND113408B	ND049546b-27Russ	AND01804-3Russ	100	0	0	2	98	0
ND113409B	ND049546b-27Russ	M7	100	0	0	1	99	0
ND113410AB	ND049546b-27Russ	ND039194AB-	100	0	0	1	99	0
ND113411B	ND049546b-27Russ	ND070927-2Russ	100	0	9	9	82	9
ND113412B	ND049589B-5Russ	Dakota Trailblazer	100	0	7	0	93	7
ND113414B	ND049589B-5Russ	M7	100	1	13	7	79	14
ND113415AB	ND049589B-5Russ	ND039194AB-	100	0	2	7	91	2
ND113416B	ND050032-4Russ	Stirling	100	1	13	5	81	14
ND113417CB	ND050060Cb-4R	Gala	100	1	23	6	70	24
ND113419CB	ND050060Cb-4R	Patagonia	100	0	3	6	91	3
ND113421CB	ND050060Cb-4R	95043.11	100	0	25	12	63	25
ND113422CB	ND050067cb-1R	Gala	100	4	22	16	58	26
ND113423CB	ND050067cb-1R	Patagonia	100	1	25	11	63	26
ND113424CB	ND050067cb-1R	Romanze	85	1	19	11	54	24
ND113425CB	ND050067cb-1R	AND00272-1R	100	0	24	22	54	24
ND113427CB	ND050067cb-1R	ND4659-5R	100	2	24	8	66	26
ND113428CB	ND050067cb-1R	ND060822CB-2p	100	0	24	12	64	24
ND113429CB	ND050132C-6R	Patagonia	100	0	8	2	90	8
ND113440CB	ND050167C-3R	Patagonia	100	0	1	4	95	1
ND113443CB	ND059624C-4	LBR8	100	3	35	16	46	38
ND113448CB	ND059809C-1P	Patagonia	100	2	21	23	54	23

Table A11. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2013. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2013, isolate 1044 of *P. infestans* strain US-24 was used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND123CB	Dakota Diamond	ND7379B-6	100	0	1	3	96	1
ND125B	Dakota Pearl	J138-A12	100	0	0	0	100	0
ND126B	Dakota Trailblazer	Ranger Russet	100	0	0	0	100	0
ND127B	Dakota Trailblazer	M7	100	0	0	0	100	0
ND128B	Dakota Trailblazer	ND028672-1Russ	63	0	0	0	63	0
ND129AB	Dakota Trailblazer	ND039194AB-1Russ	100	0	0	2	98	0
ND1227B	Ranger Russet	Dakota Trailblazer	100	0	0	2	98	0
ND1228CB	Ranger Russet	ND039194AB-1Russ	100	0	0	0	100	0
ND1229CB	Ranger Russet	ND081555CB-2Russ	100	0	1	0	99	1
ND1230B	Romanze	AND00272-1R	100	0	0	0	100	0
ND1231B	Romanze	ATND98459-1RY	100	0	0	3	97	0
ND1232B	Romanze	Dakota Ruby	100	0	0	1	99	0
ND1235B	Umatilla Russet	Dakota Trailblazer	100	1	1	1	97	2
ND1236AB	Umatilla Russet	ND039194AB-1Russ	100	0	0	1	99	0
ND1237CB	Umatilla Russet	ND081555CB-2Russ	100	0	0	0	100	0
ND1239B	AND00272-1R	Romanze	100	0	0	0	100	0
ND1255B	AND99362B-1Russ	Dakota Trailblazer	100	0	0	0	100	0
ND1257AB	AND99362B-1Russ	ND039194AB-1Russ	110	0	0	0	110	0
ND1258B	AND99362B-1Russ	ND049251B-9Russ	100	0	0	1	99	0
ND1259B	AND99362B-1Russ	ND049546b-15Russ	100	0	0	0	100	0
ND1261B	AND99362B-1Russ	ND060340-8Russ	100	0	0	3	97	0
ND1262CB	AND99362B-1Russ	ND081555CB-2Russ	100	0	1	1	98	1
ND1271B	J103-K7	Dakota Trailblazer	100	0	1	1	86	1
ND1273CB	J103-K7	ND7799c-1	100	0	1	4	95	1
ND1276CB	J103-K7	ND081555CB-2Russ	100	0	1	6	93	1
ND1278B	J138-A12	ND049251B-9Russ	100	3	5	1	82	8
ND1280B	LBR8	ND049546b-27Russ	100	0	4	8	88	4
ND1281B	LBR8	ND081555CB-2Russ	100	0	0	3	97	0

Table A11. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2013 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2013, isolate 1044 of *P. infestans* strain US-24 was used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND1286CB	M7	ND081555CB-2Russ	100	0	0	3	97	0
ND1287B	ND060544-4	LBR8	100	1	8	9	82	9
ND1289B	ND060544-4	ND049289B-1Russ	100	0	1	3	96	1
ND1290B	WND8624-2Russ	ND049546b-15Russ	100	0	2	0	98	2
ND12107CB	ND5873-21	ND7379B-6	100	0	1	2	97	1
ND12108CAB	ND5873-21	ND039194AB-1Russ	100	0	1	0	99	1
ND12109CB	ND5873-21	ND081761b-9Russ	100	0	1	0	99	1
ND12111CB	ND5873-53	LBR8	100	0	7	3	90	7
ND12112CB	ND5873-53	ND7379B-6	100	0	0	5	95	0
ND12114CB	ND5873-53	ND049251B-9Russ	100	0	0	2	98	0
ND12115CB	ND5873-53	ND049546b-15Russ	100	0	0	2	98	0
ND12117CB	ND5873-55	ND081555CB-1Russ	100	0	0	0	100	0
ND12118CB	ND5873-55	ND081555CB-2Russ	100	0	0	1	99	0
ND12119CB	ND6400C-1Russ	ND049546b-15Russ	100	0	0	1	99	0
ND12120CB	ND6400C-1Russ	ND060761b-3Russ	42	0	0	0	42	0

Table A12. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2014. A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2014, isolate 1044 of *P. infestans* strain US-24 was used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND12212B	ND070927-2Russ	Dakota Trailblazer	100	38	17	0	45	55
ND12214CB	ND071127-1Russ	ND081555CB-2Russ	100	28	17	1	41	45
ND12215B	ND071127-1Russ	ND081761b-9Russ	100	14	27	1	43	41
ND12216CB	ND071239b-1Russ	ND081555CB-2Russ	100	26	40	7	27	66
ND12218CB	ND071416-1Russ	ND081555CB-2Russ	100	0	0	0	100	0
ND12219B	ND081476B-8Russ	ND071127-1Russ	100	23	9	7	61	32

Table A12. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2014 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2014, isolate 1044 of *P. infestans* strain US-24 was used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND12220CB	ND081555CB-2Russ	Ranger Russet	100	0	0	0	100	0
ND12221CB	ND081555CB-2Russ	Umatilla Russet	100	0	2	1	97	2
ND12222CB	ND081555CB-2Russ	AND97279-5Russ	85	0	0	0	85	0
ND12223CA	ND081555CB-2Russ	ND039194AB-1Russ	100	0	0	0	100	0
ND12224B	ND081555CB-2Russ	ND059846C-4Russ	100	0	1	4	95	1
ND12225CB	ND081555CB-2Russ	ND060770B-5Russ	100	0	0	1	99	0
ND12226CB	ND081555CB-2Russ	ND071127-1Russ	100	6	7	1	76	13
ND12227B	ND081626B-48Russ	ND049251B-9Russ	100	6	21	2	51	27
ND12228AB	ND081701B-48Russ	ND039194AB-1Russ	80	0	1	3	76	1
ND12229B	ND081701B-48Russ	ND071416-1Russ	100	0	1	2	97	1
ND12230B	ND081752B-6R	Inka Dawn	54	0	0	0	54	0
ND12231B	ND081752B-6R	Romanze	100	0	1	2	97	1
ND12232B	ND081752B-6R	ND071176-5R	72	0	2	2	68	3
ND12233CAB	ND092233CAB-	Inka Dawn	100	0	0	1	99	0
ND12234B	ND113089B-1	ND5858	100	15	14	1	57	29
ND12235B	ND113089B-1	ND081752B-6R	100	26	28	1	36	54
ND12241YB	90245.1	Dakota Trailblazer	100	22	25	7	46	47
ND12245YCB	90245.1	ND081555CB-2Russ	99	0	0	2	97	0
ND12247YB	93057.1	ND049546b-27Russ	100	0	4	9	87	4
ND135B	5441	ND071410B-3R	90	1	6	3	80	8
ND137YB	79101.3	Dakota Trailblazer	100	0	5	3	92	5
ND1312TB	87HM12-16	Dakota Trailblazer	82	0	0	3	79	0
ND1315TB	87HM12-16	ND081626B-48Russ	40	0	11	7	22	28
ND1317YB	90245.1	Dakota Trailblazer	100	0	0	2	98	0
ND1450CAB	Dakota Diamond	ND102800ABC-1	100	0	0	0	100	0
ND1462ABC	Dakota Pearl	ND102800ABC-1	100	0	0	0	100	0
ND1463AB	Dakota Pearl	ND102809AB-2	100	0	0	0	100	0
ND14311CAB	ND092095C-1	ND102809AB-2	100	0	0	0	100	0

Table A12. Families evaluated using a detached leaf assay, with their respective parentages and evaluated late blight ratings of progeny, conducted in 2014 (continued). A 0 and a 1 rating were considered resistant with no reaction and a hypersensitive reaction, respectively. A rating of 2 indicated a lesion less than 1 cm in diameter, and a rating of 3 had a lesion more than 1 cm in diameter with sporulation. In 2014, isolate 1044 of *P. infestans* strain US-24 was used.

Family	Parentage		Number of	Frequency of Ratings Within Families				
	Female	Male	genotypes evaluated	0	1	2	3	% Resistant
ND14312CB	ND092095C-1	ND102857CB-1	48	0	0	0	48	0
ND14352ABY	ND102809AB-2	Eva	100	0	2	1	97	2
ND14353AB	ND102809AB-2	Ivory Crisp	100	0	1	2	97	1
ND14355ABY	ND102809AB-2	793101.3	79	0	1	4	74	1
ND14356ABY	ND102809AB-2	90245.1	100	2	0	3	95	2
ND14357AB	ND102809AB-2	J103-K7	100	1	1	6	92	2
ND14358AB	ND102809AB-2	ND860-2	100	0	1	7	92	1
ND14360ABC	ND102809AB-2	ND028799C-3	100	0	3	1	85	3
ND14362AB	ND102809AB-2	ND028984B-1	100	2	1	3	94	3
ND14363AB	ND102809AB-2	ND059624c-4	100	0	2	0	98	2
ND14364ABC	ND102809AB-2	ND060839C-7	100	0	0	2	98	0
ND14365ABC	ND102809AB-2	ND091831CB-8	100	0	0	5	95	0
ND14366ABC	ND102809AB-2	ND092095C-1	100	1	0	1	88	1
ND14367ABC	ND102809AB-2	ND102800ABC-1	100	0	4	1	95	4
ND14368AB	ND102809AB-2	ND113172-1	100	0	0	1	99	0
ND14369ABC	ND102809AB-2	ND113306C-2	99	0	1	5	93	1
ND14370AB	ND102809ABC-2	ND113307-2	100	0	0	9	91	0
ND14371AB	ND102809ABC-2	ND113317-5	100	0	0	0	100	0
ND14407AB	ND113060-2	ND102809AB-2	100	0	0	0	100	0
ND14463CAB	ND113306C-1	ND102809AB-2	100	0	0	0	100	0
ND14474AB	ND113317-5	ND102809AB-2	64	0	0	0	64	0
ND14484B	ND113356B-2PEY	ND4659-5R	67	0	1	0	66	1