Identification and mapping of a resistance gene to barley leaf rust (*Puccinia hordei* G. Otth)

by

John C. Zwonitzer

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

CROP AND SOIL ENVIRONMENTAL SCIENCES

Dr. Carl A. Griffey (co-chair)	Dr. M.A. Saghai Maroof (co-chair)
Dr. Glenn Buss	Dr. Erik L. Stromberg

December 1999

Blacksburg, VA

Identification and mapping of a resistance gene to barley leaf rust (*Puccinia hordei* G. Otth)

by

John C. Zwonitzer

Carl A. Griffey and M. A. Saghai Maroof, Co-major professors

Crop and Soil Environmental Sciences

(Abstract)

Barley leaf rust (*Puccinia hordei* G. Otth) has been the cause of numerous and often devastating disease epidemics since the beginning of agriculture. Leaf rust is one of the most important diseases that affect barley (*Hordeum vulgare* L.) throughout the world. The pathogen *Puccinia hordei* is an obligate parasite. Symptoms of barley leaf rust may range from small chlorotic flecks to large pustules containing spores. Leaf rust epidemics reduce yields and grain quality.

Deployment of resistant cultivars is one of the most effective and economical means of controlling barley leaf rust. Identification and incorporation of new and effective sources of resistance are crucial to the success of barley breeding programs. Two types of resistance have been identified. They are race-specific resistance and partial resistance. A hypersensitive reaction by the host to infection of *P. hordei* isolates lacking corresponding virulence genes is indicative of race-specific resistance that is controlled by major genes. Sixteen race-specific genes (*Rph*1 to *Rph*16) have been identified. Partial resistance is generally polygenic and is often more durable that race-specific resistance.

The purpose of this research is to determine the inheritance of resistance to leaf rust in the barley experimental line VA 92-42-46, to identify the gene(s) conferring

resistance, identify putative resistance related markers, and to map the gene(s) to one or more barley chromosomes using molecular markers. The Virginia barley line 92-42-46 was selected for this research project because it possesses resistance to *P. hordei* race 30, which has overcome resistance conferred by *Rph*7. Crosses were made between VA 92-42-46 and Moore, a susceptible cultivar to leaf rust. Inheritance studies were performed by screening F_2 progeny and $F_{2:3}$ families against race 8 and race 30 to determine the number of leaf rust resistance genes in VA 92-42-46. Allelism tests were performed to determine gene identity. A single dominant gene at the *Rph*5 locus or a tightly linked gene confers the resistance to P. hordei in VA 92-42-46.

Two populations, 'Moore' X VA 92-42-46 and 'Bowman' X 'Magnif', were used in this study for mapping molecular markers to provide comparison and confirmation of results. 'Magnif' possesses the resistance gene *Rph*5. Bulked segregant analysis was used to identify polymorphic RFLP and SSR markers that were used for mapping in each population. Linkage analysis revealed that the *Rph*5 gene maps to barley chromosome 3 (3H) above the centromeric region in the 'Moore' X VA 92-42-46 population. These findings agree with previous research that identified linkage between *Rph*5 and *Rph*7 on chromosome 3. The results obtained in this study do not support previous research that reported the resistance gene *Rph*5 was located on barley chromosome 7 (5H). Further research should be conducted to verify the results of this study using the 'Bowman' X 'Magnif' population. The markers screened in the region above the centromere region of barley chromosome 3 were monomorphic for the 'Bowman' X 'Magnif' population except for the marker MWG561. Therefore, additional markers above the centromere of barley chromosome 3 should be screened.

Acknowledgements

In the course of completing my Masters degree I have had the pleasure of interacting and learning from numerous talented and skillful individuals, without whom completion of this work would not have been possible. I am especially grateful to my two co-major advisors, Dr. C. A. Griffey and Dr. M. A. Saghai Maroof, whose ambition and success have been truly inspiring. I am also indebted to my committee members, Dr. Glenn Buss and Dr. Erik Stromberg for their expertise both in guiding my graduate study, and in preparation of this thesis. I am grateful for the support of Dr. Brian Steffenson who provided F_2 and $F_{2:3}$ disease data and F_3 seed for the 'Bowman' X 'Magnif' mapping population.

There are numerous colleagues who have contributed directly and indirectly to this work and whose efforts are greatly appreciated. They include Dr. Ruslan Biyashev, Dr. Jack Hall, Sixin Liu, and Tom Pridgen. I am also grateful to have been able to work with such a large group of both gifted and amicable scientists who are too numerous to list. I would like to make mention of Wynse Brooks and Wendy Rohrer, who have been both very close friends and a tremendous amount of help throughout this entire work.

I owe a tremendous debt of gratitude to my supportive and loving family. They include my parents, Jim and Sharon; my father-in-law, Lynn; my sister and brother-in-law, Jr. and Jeanne, my brother- and sister-in-law, Mark and Becky; and my niece and nephew, Nicole and Spencer. I love you all and thank you very much for sticking beside me. I also want to thank my BCF family for all of their prayers and support over the past year. You have all made it a joy to be in Blacksburg. I love you all. And to my wife, Martha and daughter, Shannon, thank you for all of your help and patience over the past year and a half. Having you here with me has made all of my work worthwhile. Finally, I thank the Heavenly Father for all of the blessings He has bestowed on me throughout my entire life and for the abilities He has given me.

Table of Contents

List of Tables

CHAPTER II

List of Figures

CHAPTER II.

List of Appendices

CHAPTER II.

CHAPTER I.

INTRODUCTION

Barley Importance and Uses

Barley (*Hordeum vulgare* L.) is a cereal crop that is grown throughout the world and is ranked fifth in world crop production. There are several hypotheses concerning the origin of cultivated barley. The first was postulated by Aberg (1940) who thought that six-rowed wild barley (*Hordeum vulgare* ssp. *spontaneum*) found in Tibet was the progenitor of cultivated barley. Second, Harlan (1976) proposed that barley was first domesticated in Southwest Asia from two-rowed wild barley. Freisleben (1940) postulated a diphyletic explanation that six-rowed cultivated barley in the Orient was derived from six-rowed barley from Tibet, while two-rowed cultivated barley in Southwest Asia originated from two-rowed wild barley. Finally, it was thought that its center of origin was found in the region known as the Fertile Crescent, with its importance dating back to 8000 B.C. (Nevo, 1986; Nevo, 1992). The land area in barley production and its importance have greatly increased since its domestication. Barley has three primary uses: feed for livestock, human consumption, and malting barley for beer production (Jones and Clifford, 1983; Nevo, 1992).

In the United States (U.S.), there are 27 states that produce barley (Virginia Agricultural Statistics Service Bulletin Number 66, 1995; Bulletin Number 71, 1999). From 1989 to 1998 Virginia ranked approximately 12th in total area of barley planted and harvested, and also in total grain production. Virginia barley production has fluctuated over the past 10 years. The highest production year was 1992, with 1989 through 1991 and 1993 through 1998 being considerably lower. In 1992, the total number of hectares of barley harvested was 36 thousand [90 thousand acres (ac)] with a yield of 4.25 metric

tons/hectare (79 bushels/acre). Total barley production for 1992 was, therefore, 155 thousand metric tons (7.11 million bushels). The years 1989 through 1991 and 1993 through 1998 had lower total hectares in barley grain production. From 1989 through 1991, the average area of barley grain harvested was 32,390 hectares (80,000 acres) and from 1993 through 1998 the average harvested area was 30,850 hectares (76,200 acres). The average yield and total production numbers were also lower for 1989-1991 and 1993-1998 with average yield being 3.6 metric tons/ha (67 bushels/ac) and 3.9 metric tons/ha (72.5 bushels/acre), respectively. The average total production for these years was 115 thousand metric tons (5.3 million bushels) and 120 thousand metric tons (5.5 million bushels), respectively.

Barley can be grown in many different climatic regions due to its adaptability to diverse conditions. These climatic conditions include variable growing seasons, temperatures, and precipitation rates (Pomeranz, 1987). In the U.S., the majority of the barley is produced in the Northern Plains and Pacific Northwest. The climate in these regions is favorable for malting barley. Barley produced in North Dakota and Minnesota is almost all for malting. In addition, barley is grown because other grains that are more profitable such as corn, sorghum, and soybeans are not well adapted to the climate in certain geographic regions.

Over the period 1992 through 1998 there has been a general trend of decreasing acres of barley planted, harvested, and total production in the U.S. (http://usda.mannlib.cornell.edu/reports/nassr/field/pcp-bbs/small_grains_summary_09.30.98; Agricultural Statistics Board Statistical Bulletin

SB947a, 1998). Grain yield levels were fairly constant from 1992 through 1997 with a range of 3.08-3.36 metric tons/hectare (57.2-62.5 bushels/acre). In 1992, the grain yields were the highest and in 1995, the average yields were the lowest. From 1992 to 1998 total barley grain area harvested ranged from 2.4-2.9 million hectares (6.0-6.73 million acres). The highest number of hectares harvested occurred in 1992 and the lowest number was in 1998. U.S. barley production ranged from 7.8-9.9 million metric tons (358-455 million bushels).

From 1992 through 1997 North Dakota ranked first in total barley production followed second by Idaho and Montana (USDA Statistical Bulletin SB947a, 1998). Minnesota and Washington were fourth and fifth in total U.S. barley production. More than 75% of the 1998 barley crop was produced in these five states.

The wide adaptability of barley makes it an important cereal crop worldwide. World barley production has tripled since 1960. This increase in production is primarily due to increased production in Europe. Two-thirds of the world barley production is grown in Europe and Russia (Pomeranz, 1987). The world area in barley production for the 1995-96 crop year was 69 million hectares (171 million acres), for 1996-97 and 1997-98 crop year 66 million hectares (164 million acres), and 61 million hectares (150 million acres) for the 1998-99 crop year (http://151.121.3.140/WAP/circular/1999/99-08/tables.html; http://www.usda.gov/nass/pubs/agr99/99_ch1.pdf). World barley production was 143 million metric tons (6555 million bushels) in 1995-96, 154 million metric tons (7059 million bushels) in 1996-97, 155 million metric tons (7100 million bushels) in 1997-98, and 137 million metric tons (6290 million bushels) in 1998-99.

World barley production area decreased over the periods 1995-96 and 1998-99. The world barley production increased in the periods of 1995-96 and 1997-98 crop years with a decline in production for 1996-97 and 1998-99.

Barley is extremely nutritious and can contain a protein content as high as 18%. In addition, barley contains considerably less oil than maize (*Zea mays* L.), thus making it more appealing for low-fat diets. Due to the high fiber content in the hull, there is decreased digestibility in monogastric animals. Over one-half of the world's barley production is used in feed stuffs for livestock, predominantly for ruminants, rather than for human consumption (Jones and Clifford, 1983; Nevo, 1992).

Human foods made with barley use pot barley or pearl barley. These barley products are made by gradually removing the hull and outer layers of the barley kernels through an abrasive action. One hundred pounds of barley grain yields 65 pounds of pot barley and 35 pounds of pearl barley after the abrasive action (Pomeranz, 1987). Barley can be used as a thickener, stabilizer, binder, or protein source in baby foods. It also is used in soups and dressings, pet foods, prepared meats, and malt beverages (Pomeranz, 1987).

High quality grain is required for the malting process. The grain should be mature, plump, have a protein level between 9-13%, have a high starch content, high malt extract concentration, high diastatic power, and high level of alpha-amylase (Hockett et al., 1993; Lu and Ding, 1991; Pomeranz, 1987). Different countries have special standards for malting barley. The Australian protein level for Malt 1 grade barley is less than 11 percent and the Western Canadian malting barley maximum protein requirement

for two-row and six-row barley types is 13.5 percent (http://www.cwb.ca/markets/gfwc/cwmb.html; http://www.grdc.com.au/pub/gc12/gc12_5.html). Another important constituent of malting barley is starch. The starch content must be high since the malting process requires sufficient enzymes to be available to complete the fermentation process of converting starch into sugars (Jones and Clifford, 1983).

Another use of barley is for winter pasture and hay. In addition, barley can be produced as a companion crop with clover (*Trifolium* sp.) and grasses because of its early maturity. A producer can harvest the barley crop first, and then clover and grasses, inter-seed with the barley, will provide forage later in the season.

In Virginia and other temperate climatic regions, barley is often grown in a double-crop system with soybeans. This rotation works effectively and has an advantage over wheat, because barley is usually harvested one to two weeks earlier than wheat. Research performed in Arkansas over a number of years did not indicate significant differences in soybean yields when planted between April 25 and June 15 (Caviness and Thomas, 1979). Arkansas research also showed that soybeans planted within five days of July 15 showed a significant reduction in yield of approximately 0.27 metric tons per hectare (5 bushels/acre). It was also shown that when supplemental moisture was not applied during drought stress, soybeans that had a later planting date had greater reductions in yield (Caviness and Thomas, 1979; Thurlow, 1986). Additional research performed in Alabama at Auburn University showed that soybeans planted after May 25 also had yield reductions (Thurlow, 1986). Therefore soybeans yield more due to a longer available growing season. In addition, soybeans grown after wheat show

phytotoxic effects, such as reduced plant growth and decreased soybean yields. (Caviness et al., 1986). No research was found that showed allopathic effects from barley residues on soybean production.

Barley Leaf Rust

Cereal rust epidemics have been reported to cause significant damage in cereal grains since the beginning of agriculture. The cereal rust diseases gained much attention from plant pathologists in the late 1800s and early 1900s. Much of the research conducted by early pathologists concentrated on leaf rust (*Puccinia recondita* f. sp. *tritici*) of wheat (*Triticum aestivum* L.). These findings proved useful when research expanded to include barley leaf rust (Schafer et al., 1984). This research was valuable because many findings observed with wheat leaf rust could be directly applied in barley research.

There are three rust diseases that affect barley: leaf rust, stem rust (*Puccinia graminis* f. sp. *tritici*), and stripe rust (*Puccinia striiformis* sp. *hordei*). Leaf rust is one of the most important diseases that affects barley throughout the world (Ivandic et al., 1998; Jin et al, 1995). Leaf rust is a disease caused by host-specific fungi in the genus *Puccinia*, which can infect many different plants. *Puccinia hordei* is the pathogen responsible for leaf rust in barley. Most rust fungi are obligate parasites that grow and survive only on green plants.

Barley leaf rust is of particular importance in climatic regions where the crop matures late in the growing season. This occurs to a great extent in both the winter and

spring production regions of the Eastern and Midwestern United States, North Africa, New Zealand, Europe, Australia, and parts of Asia (Mathre, 1982). The symptoms of barley leaf rust may range from small chlorotic flecks to large pustules containing spores. This disease causes an increase in plant transpiration and respiration, which reduces plant efficiency and also causes a decrease in the rate of photosynthesis in diseased plants. Therefore, barley leaf rust affects plant and root growth and ultimately grain yield and seed quality (Mathre, 1982; Wiese, 1987). Yield losses are greatest when leaf rust develops early in the season or when the crop is planted late (Mathre, 1982; Melville et al., 1976; Wiese, 1987). Late season epidemics reduce yield by decreasing kernel weight (Mathre, 1982; Wiese, 1987).

Two stages of the leaf rust cycle occur on cultivated and wild barley. These are the uredial and telial stages (Clifford, 1985). During inter-cropping periods, the fungus survives as urediospores or dormant mycelium on wild, volunteer, or over-wintering barley crops (Gair et al., 1987; Murray et al., 1998). The urediospores are then disseminated by the wind from south to north in the spring, and north to south in the autumn and can mix with water vapor in the clouds and fall back to the earth with rain (Mathre, 1982; Reinhold and Sharp, 1982; Wiese, 1987). Upon contact with a receptive host, germination and penetration of the urediospores is completed in six to eight hours when free moisture is available and optimum temperatures are between 15 and 25 degrees Celsius. Secondary urediospores are then produced in seven to ten days (Mathre, 1982; Murray, et al., 1998; Wiese, 1987). After infection, rust pustules form predominantly on the upper side of the leaf blades and leaf sheaths (Wiese, 1987;

Zillinsky, 1983). Barley leaf rust is macrocyclic since many cycles of urediospores can form in one season (Clifford, 1985; Schafer et al., 1984). In addition, *Puccinia hordei* is a heteroecious rust as it requires more than one host to complete its life cycle. The telial stages form on barley toward the end of the growing season. This occurs in areas that have been infested by the pathogen for the longest portion of the growing season (Murray et al., 1998).

The alternate host of *Puccinia hordei* is the arable weed (a weed that is found in tilled cropland) *Ornithogalum umbellatum* L., commonly known as the Star-of-Bethlehem. The pathogen goes from barley to the alternate host by teliospores and then basidiospores germinate on the alternate host (Schafer et al., 1984). After penetration of the host epidermal cells, pycnia and aecia are formed. Pycniospores are sexual fertilizing bodies that fertilize aecia. The aeciospores formed through the sexual stage produce germ tubes that enter the barley host through the stomata to form uredia (Clifford, 1985; Schafer et al., 1984). This alternate host can be a source of sexual aeciospores (dikaryotic spores) which provides another method of dissemination and increased pathogenic diversity in some areas of Israel and Greece, and possibly in some parts of Southern Australia (Murray et al., 1998). In the U.S., the alternate host has not proven to be a factor in the spread or genetic diversity of leaf rusts (Murray et al., 1998; Reinhold and Sharp, 1982; Zillinsky, 1983). Since the alternate host is not a factor in the disease cycle of barley leaf rust in the U.S., mutations are the primary source of new virulence genes in the pathogen.

Numerous researchers have concluded that leaf rust populations consist of many different races (Anikster, 1984; Mathre, 1982; Parlevliet, 1983; Reinhold and Sharp, 1982). The number of races is much less important than the frequency of specific races (Mathre, 1982). The frequency of leaf rust races is in a constant state of fluctuation, and new races are continually developing and increasing in frequency in response to selection pressure exerted by resistance genes in widely grown cultivars (Mathre, 1982). Levine and Cherewick (1952) performed research on 357 leaf rust isolates. They grouped the isolates into 26 consolidated races. The races found in the highest frequency were races 4, 37, and 47 with the number of isolates that were grouped into these designations being 106, 57, and 54, respectively. The infection types of the selected host differentials 'Bolivia' (CI 1257), 'Egypt 4' (CI 6481), 'Gold' (CI 1145), 'Lechtaler' (CI 6488), 'Oderbrucker' (CI 940), 'Quinn' (CI 1024), 'Reka 1' (CI 5051), 'Speciale' (CI 7536), and 'Sudan' (CI 6489) to race 4 were $1^{=}$, 4^{+}, 1, 1^{-}, 1, $1^{=}$, 1, and 1^{-}, respectively. The infection types of the differentials to race 37 were 0;, 2±, 0;, 2-, 0;, 0;, 1=, 0;, and 1±, respectively. Finally, race 47 infection types to the differentials were 0;, 4+, 1, 1, 2, 0;, 1-, 4=, and 1++, respectively. During the period from 1956 to 1964, leaf rust race 4 was the most prevalent race and was present in 67% of all collections (Andres et al., 1983). From 1979 to 1982, Andres et al. (1983) performed 93 field collections from 16 states and Mexico. They identified six races (race 4, 8, 13, 19, 40, and 42) from the isolates collected with race 8 being most predominant followed by race 4.

Economic Importance of Leaf Rust and Yield Losses

Historically, rust diseases have had a major impact on barley crop yields. For this reason, the study of leaf rust resistance has become one of major economic importance. The occurrence of leaf rust epidemics has increased in the past 15-20 years, especially in Europe (Clifford, 1985). In Australia, there were few reports of leaf rust epidemics prior to 1978 (Cotterill et al., 1992a). However since that time, there have been epidemics during five years from 1978 to 1992 (Cotterill et al., 1992b).

In the United Kingdom (U.K.), Melville et al. (1976) reported yield losses of 17-31% in non-treated field trials versus those treated with broad-spectrum fungicides. The majority of yield loss from leaf rust epidemics was due to reduced kernel weight (King and Polley, 1976; Melville et al., 1976). From 1981 through 1991, surveys were conducted in the U.K. on leaf rust infection of winter barley. Two major epidemics were reported, with the first being in 1989 and the second in 1990 (Murray et al., 1998). The estimated annual losses due to barley leaf rust averaged 1.2% of the U.K.'s national yield throughout the survey period.

In the eastern U.S., Griffey et al. (1994) reported that average barley yield losses caused by leaf rust in Virginia were between 6 and 16% in 1991 and 1992 at two Virginia locations (Painter and Warsaw). Yield losses as high as 32% in susceptible cultivars were reported by Griffey et al. (1994), based on estimates obtained from regression analysis of disease severity versus grain yield. It has been estimated that an average yield loss of 0.42% occurs for each 1% increment of leaf rust on the upper two leaves at the early dough stage of development (Griffey, et al., 1994; Murray, et al., 1998). Melville et

al. (1976) determined that an increase of 10 percent in leaf rust that was observed on the first leaf below the penultimate leaf at growth stage 75 resulted in a yield reduction of 7.7 percent. King and Polley (1976) reported that percent yield loss can be calculated by multiplying the percent of leaf rust on leaf 1 (not clearly defined in source) by 0.6.

Leaf Rust Resistance

Deployment of resistant cultivars is one of the most effective and economical means of controlling leaf rust (Mathre, 1982; Zillinsky, 1983); therefore, research has focused largely on identification and incorporation of new sources of resistance into breeding programs. There are two types of resistance: race-specific and partial.

Race-specific resistance is controlled by major genes that confer a hypersensitive reaction in response to infection by *P. hordei* isolates lacking corresponding virulence genes (Parlevliet, 1976a, 1983; Parlevliet and Kuiper, 1977; Roane, 1962). Sixteen race-specific genes (*Rph*1 to *Rph*16) have been identified (Franckowiak et al., 1997; Ivandic et al., 1998). Franckowiak et al. (1997) reported on the allelic associations and found 12 alleles at the *Rph*2 locus, three alleles at the *Rph*3 locus, two alleles at the *Rph*7 locus, two alleles at the *Rph*9 locus, while the other *Rph* genes have only one identified allele at the given locus. Jin and Steffenson (1994) have reported races of P. hordei possessing virulence to resistance genes Rph1-12. Subsequently, *Rph* genes 13 to 16 were identified since 1996 and confer resistance to all known races of leaf rust (Chicaiza et al., 1996; Ivandic et al., 1998; Jin et al., 1996). New sources of effective leaf rust resistance have rarely been found in the cultivated barley gene pool. Jin et al. (1995) evaluated 1,997 *H.*

vulgare accessions from the United States Department of Agriculture (USDA) National Small Grains Collection (NSGC) for reaction to 4 isolates of *P. hordei*, but only two accession, PI 531901 and PI 531941, were resistant to all the isolates used in their study. New sources of effective resistance must be identified and transferred from wild barley since resistance is fairly common in these species (Jin et al., 1995).

Hypersensitive resistance has been used extensively in breeding programs because it usually is governed by single dominant genes that are inherited in a simple Mendelian fashion and, therefore, is easily recognized and incorporated into adapted germplasm (Niks, 1982; Russell, 1978). However, the deployment of race-specific *Rph* genes has not provided durable resistance since new leaf rust races generally overcome these genes in a short period of time (Niks, 1982; Steffenson et al., 1993). Breeding strategies that may provide more durable resistance in cultivars include combining (pyramiding) several *Rph* genes in each genotype, developing isogenic multilines, and incorporating effective *Rph* genes into lines possessing partial resistance (Dreiseitl and Steffenson, 1999; Jin et al., 1995). Major gene (*Rph* genes) resistance has been used more frequently than partial resistance and has resulted in a *Vertifolia effect* or erosion of partial resistance genes in the host in favor of major genes (Parlevliet, 1983; Parlevliet and Kupier, 1977; Russell, 1978; Van Der Plank, 1968).

The second type of resistance is partial resistance. This resistance is generally polygenic, controlled by several to many genes, and is more durable than race-specific resistance (Parlevliet, 1976b; Parlevliet, 1978). Partial resistance results in a reduced rate of disease in genotypes that otherwise produce a susceptible reaction type (Parlevliet,

1983; Parlevliet and van Ommeren, 1985). Partial resistance, therefore, is conferred by low infection efficiency, long latent period, and low sporulation rate (Niks, 1982; Parlevliet, 1976b).

As previously mentioned, breeding programs tend to use single gene resistance at the expense of partial resistance. Programs that commonly select for and deploy major *Rph* genes over large regions have greatly reduce the level of partial resistance in modern cultivars (Parlevliet, 1983). Relying on major gene resistance has replaced an effective and more durable form of resistance that is offered by partial resistance (Parlevliet, 1983). No interaction between the presence or absence of *Rph* genes and partial resistance was found by Parlevliet (1983); therefore, these types of resistance could be used simultaneously.

Breeding Schemes

The use of resistant cultivars has been shown to be the most effective, economically feasible, and environmentally sound method to control barley leaf rust (Mathre, 1982; Zillinsky, 1983). In the past, traditional plant breeding was the only effective means to provide resistant cultivars. More recently, the use of biotechnology has allowed researchers to identify, tag and clone genes and to manipulate plants at the DNA level. In addition, utilizing a combination of traditional plant breeding methods and biotechnology tools has resulted in the identification and release of cultivars with superior disease resistance and other traits of interest in a shorter time frame (Tanksley et

al., 1989). Traditional breeding methods often require 12 to 15 years to release a new cultivar (Lawes, 1988).

Plant breeders improving barley are interested in developing short, early maturing cultivars with good straw strength, plumper kernels, increased yields, and superior resistance to diseases. In order to achieve the desired goals and objectives set forth in a plant breeding program, breeders must understand the morphology, physiology, pathology, and environment of the plant (Poehlman and Sleper, 1995). Therefore, development of cultivars that are resistant to rust and other diseases requires that plant breeders continually search for new resistance sources and make crosses to incorporate new resistance genes into superior cultivars that are adapted to the region of interest. This is particularly important to plant breeders because of the ephemeral nature of resistance genes and the rapidly evolving dynamics of the pathogen population (Ivandic et al., 1998).

Molecular markers have recently become an important tool for plant breeding (Tanksley et al., 1989). Through the use of molecular markers, traits of interest can be identified, selected, and even cloned directly into the cultivar using map-based cloning (Tanksley et al., 1989). Tight linkages between molecular markers and a gene of interest allow for the efficient and effective use of marker-based selection in plant breeding programs. The use of molecular markers can reduce the amount of time required to develop a cultivar. In addition, molecular markers could be used to develop cultivars that possess desirable combinations of genes or traits that otherwise would have been too

difficult, labor intensive, and time-consuming to develop using traditional breeding methods.

One important use of molecular markers in barley is to transfer traits or genes of interest, such as leaf rust resistance, from distant relatives and wild barley (*Hordeum vulgare* ssp. *spontaneum*) into adapted cultivars (Brown, 1992). Barley cultivars selected by today's plant breeders contain much less genetic diversity than landraces. Molecular markers could be used to identify and aid in introduction of genetic diversity, thus reducing genetic vulnerability. Barley is known to possess linkage disequilibrium; if an individual has a distinctive allele at one locus, it also will have a higher than random likelihood of possessing a distinctive allele at another locus. On a broader scope, when comparing populations, a population that results in a polymorphism at one locus is more likely to be polymorphic at another locus than a population lacking polymorphism for the first locus (Brown, 1992).

Barley Molecular Maps

Barley is a model crop for genetics research and has been extensively studied because it is a diploid self-pollinating crop, that can be successfully grown under many different environmental conditions and barley has a relatively short life cycle (Forster et al., 1997; Klienhofs and Kilian, 1994). Unfortunately, the large genome size of barley is one of the major limitations that has slowed progress in developing molecular maps and molecular analyses (Moore et al., 1993; Qi et al., 1996). The barley genome contains 5.3 X 10^9 base pairs (bp) (C= 5.5pg, where 'constant' (C) is the amount of DNA

characteristic of a particular genotype) per haploid nucleus (Bennett and Smith, 1976; 1991). Recent research performed by Bennett and Leitch (1997) reported that the 'C' value for the barley genome was 3.6pg. In contrast, the rice (*Oryza sativa* L.) genome contains 4.3 X 10^8 bp (C=0.6pg), which is relatively small compared to barley, wheat, and rye genomes (Bennett and Smith, 1976; 1991; Kurata et al., 1994). The barley genome consists of seven chromosomes that are highly homoeologous to wheat analogs. Barley chromosomes 1 to 7 are analogous to wheat chromosomes 7H, 2H, 3H, 4H, 1H, 6H, and 5H, respectively (Heun et al., 1991; Kleinhofs and Kilian, 1994).

There are a number of barley molecular genome maps that have been published (Becker et al., 1995; Grain Genes web site; Graner et al., 1991; Heun et al., 1991; Kasha et al., 1995; Kleinhofs et al., 1993). The updated versions of these maps can be found on the Grain Genes web site. Most comprehensive molecular genetic maps of barley came from the North American Barley Genome Mapping Project (NABGMP). Two principal double haploid populations derived from crosses between 'Steptoe' X 'Morex' (SM) and 'Harrington' X 'TR306' (HT) were used to construct barley molecular maps in this project. The number of markers in these two maps of SM and HT are 453 and 222 respectively (Grain Genes web site; Kasha et al., 1995; Kleinhofs et al., 1993; Liu et al., 1996. Qi et al. (1996) used marker data from four mapping populations that were previously published to compare and develop an integrated map consisting of 898 markers. The map covered 1060 cM (centimorgans), and markers were placed in many of the large gaps existing in the individual maps.

Chromosome Location of Known Leaf Rust Resistance Genes in Barley

Genes at 16 different loci governing leaf rust resistance have been identified and named (Franckowiak et al., 1997; Ivandic et al., 1998). These genes are designated *Rph*1-*Rph*16. *Rph*1 was the first resistance gene identified in the cultivar Oderbrucker Waterhouse (1948) and *Rph*16 was most recently identified by Ivandic et al. (1998) in the *H. spontaneum* accessions 078-2-0-002 9490 1831 and 084-2-0-016 6180 2002. Tuleen and McDaniel (1971) located *Rph*1 (*Pa*1) on chromosome 2 using six primary trisomics, which included all of the barley chromosomes except chromosome 1. Borovkova et al. (1997) reported that gene *Rph*Q, identified in the Australian barley line Q21861, is allelic or closely linked to the *Rph*2.b (*Pa*2) allele found in 'Peruvian'. *Rph*Q was mapped to the centromeric region of chromosome 7. The *Rph*3.c (*Pa*3) allele in 'Estate' barley is located on the long arm of chromosome 1 (Jin et al., 1993). McDaniel and Hathcock (1969) mapped the *Rph*4.d (*Pa*4) allele in the barley cultivar Gold to chromosome 5 using the *Reg*1 (Ml-a) locus as a genetic marker. Tan (1978) confirmed that the *Rph*4.d allele in 'Gold' was located on chromosome 5 using trisomic analyses.

There are discrepancies in published data regarding the chromosome location of *Rph*5 (Pa5). Research performed by Jin et al. (1996) and Borovkova et al. (1997) indicated linkage between *Rph*2.b and the *Rph*5.e allele of 'Magnif' on barley chromosome 7. However, Tan (1978) previously reported that *Rph*5.e was located on barley chromosome 3. Parlevliet (1976a) concluded that *Rph*5 and *Rph*7 are tightly linked and independent of *Rph*2. The *Rph*7.g allele in 'Cebada Capa' was located on barley chromosome 3 by Tuleen and McDaniel (1971). Based on trisomic analyses, Tan

(1978) confirmed that *Rph*7.g was on chromosome 3. *Rph*7.g in 'Cebada Capa' was not linked to morphological markers on chromosomes 1 to 7 utilized in the research of Jin et al. (1993). The resistance allele in 'Triumph' barley was recently re-designated as *Rph*9.z (previously designated as *Rph*12.z) by Borovkova et al. (1998), and was located on the long arm of barley chromosome 7. Recent research performed by Borovkova et al. (1998) determined that the leaf rust resistance alleles *Rph*9.i of Hor2596 and *Rph*9.z of 'Triumph' were actually alleles at a single locus and not independent genes at different loci as previously reported by Jin et al. (1993). The *Rph*10.o allele in 'Clipper BC8' was located on the long arm of barley chromosome 3 by Feuerstein et al. (1990). The *Rph*11.p allele in 'Clipper BC67' was identified on chromosome 6 by Feuerstein et al. (1990). Research performed by Borovkova et al. (1997) and Jin et al. (1996) indicated linkage between *Rph*9.i and *Rph*13.x, the allele identified in PI 531849. Since, *Rph*9.i was located on the long arm of barley chromosome 7; *Rph*13.x also should be located on chromosome 7. *Rph*16 was identified on the short arm of chromosome 2 by Ivandic et al., (1998).

No work has been published on the chromosomal location of *Rph*6 and *Rph*8. Research performed by Jin et al. (1996) identified a resistance gene in barley accession PI 584760 that is different than *Rph*1 to *Rph*13. Therefore, the gene was designated as *Rph*14. The allele conferring resistance to barley leaf rust in the barley accession PI 584760 was classified as *Rph*14.ab (Franckowiak et al., 1997). The association of *Rph*14.ab to any particular chromosome has not been determined. Chicaiza et al. (1996) identified a resistance gene in PI 355447 that is different than *Rph*1 to *Rph*14. Therefore,

the gene was designated *Rph*15. The allele conferring resistance to barley leaf rust in the *Hordeum spontaneum* accession PI 355447 was classified as *Rph*15.ad (Franckowiak et al., 1997). The chromosomal location of *Rph*15.ad has not been determined.

Molecular mapping research performed by Borovkova et al. (1997), Borovkova et al. (1998), and Ivandic et al. (1998) indicated that *Rph*2, *Rph*9, and *Rph*16 are located on barley chromosomes 7, 7, and 2, respectively. Qi et al. (1998) reported that *Rph*1, *Rph*2, *Rph*3, *Rph*4, *Rph*7, *Rph*9, *Rph*10, *Rph*11, and *Rph*12 were linked to molecular markers on chromosomes 2, 7, 1, 5, 3, 7, 3, 6, and 7, respectively.

Borovkova et al. (1997) indicated that *Rph*Q (an allele of *Rph*2 or a closely linked locus) is located near the centromeric region of barley chromosome 7. The *Rph*Q locus was mapped 3.5 cM below marker CDO749. A sequence tagged site (STS) marker ITS1 was mapped 1.6 cM below the *Rph*Q locus. Another marker less than 5.0 cM from the *Rph*Q locus was the RAPD marker OPI-04 that was mapped 3.4 cM below the *Rph*Q locus.

Borovkova et al. (1998) mapped the *Rph*9.i locus to chromosome 7 using a mapping population derived from a cross between 'Bowman' and 'Hor 2596'. A SSR marker was mapped 10.2 cM from the *Rph*9.i locus. Esterase isozymes were analyzed and Est 9 was found linked to the *Rph*9.i locus at a distance of 9.3 cM. The second mapping population was derived from a cross between 'Triumph' and a near-isogenic line I91-533-*va*, which has the chromosome 7 morphological trait locus va (controlling variegated leaf color). The *Rph*9.z locus (previously designated as *Rph*12) was placed between two molecular markers (OPA19 and ABC155).

The leaf rust resistance gene *Rph*16 was mapped to barley chromosome 2 (Ivandic et al., 1998). RFLP markers MWG874 and MWG2133 were converted to STS markers and linked to the *Rph*16 locus at a distance of 1.0 cM above the locus. RFLP marker MWG950 was linked at a distance of 5.2 cM below the *Rph*16 locus.

Research Objectives

The major goals of this research are to determine the inheritance of resistance to leaf rust in the barley experimental line VA 92-42-46, to identify the gene(s) conferring resistance to leaf rust, and to use molecular markers to map the gene to a barley chromosome. Interest in VA 92-42-46 arose as a result of its resistance to leaf rust during epidemics in 1990 that virtually overcame resistance in all other cultivars and experimental lines that were previously resistant. These genotypes primarily possessed the *Rph*7 gene for which *P. hordei* race 30 possesses virulence. This race quickly became the predominant race in regions relying on resistance conferred by *Rph*7. Resistance conferred by *Rph*7 was effective for approximately 30 years (Griffey et al, 1994; Steffenson et al, 1993). Virulence to *Rph*7 exacerbated the need to identify new sources of resistance to barley leaf rust. Because VA92-42-46 was the most accessible source of resistance, it was deemed necessary to determine the number of resistance genes present in the line and identify the gene(s) conferring resistance. Molecular markers then could be utilized to map the resistance gene to a specific barley chromosome. After linkage between the gene of interest and molecular markers is

identified, marker-assisted selection can be used to identify other lines in the breeding program that may possess the same gene, and to pyramid resistance genes.

The specific objectives of this research are:

1) To conduct inheritance studies and allelism tests to identify the number of leaf rust resistance genes present in VA 92-42-46 and to determine the gene(s) identity.
2) To identify putative resistance related markers [markers closely linked to the resistance gene(s) of interest].
3) To use molecular markers to localize the gene(s) on one or more barley chromosomes.

Literature Cited

Aberg, E. 1940. The taxonomy and phylogeny of *Hordeum L.* Sect. Cerealia Ands., with special reference to Tibetan barleys. Symboine Botan. Upsalieanses 4:1-156.

Agricultural Statistics Board. 1998. Field Crops Final Estimates 1992-1997. NASS,USDA Statistical Bulletin Number SB947a. U.S. Government Printing Office, Washington D.C.. pp. 22-25.

Andres, M.W., R.D. Wilcoxson, A.P. Roelfs, and Z.A. Pretorius. 1983. Races of *Puccinia hordei* in the United States from 1979 through 1982. Plant Disease 67:1336-1337.

Anikster, Y. 1984. Parasitic specialization of *Puccinia hordei* in Israel. Phytopathology 74:1061-1064.

Becker, J., P. Vos, M. Kuiper, F. Salamini, M. Heun. 1995. Combined mapping of AFLP and RFLP markers in barley. Molecular General Genetics 249:65-73.

Bennett, M.D. and I.J. Leitch. 1997. Nuclear DNA amounts in angiosperms-583 new estimates. Annals of Botany 80:169-196.

Bennett, M.D. and J.B. Smith. 1976. Nuclear DNA amounts in angiosperms. Philosophical Transactions of the Royal Society of London. B Biol. Sci. 274:227-274.

Bennett, M.D., and J.B. Smith. 1991. Nuclear DNA amount in angiosperms. Philosophical Transactions of the Royal Society of London. B Biol. Sci. 334:309-345.

Borovkova, I.G., Y. Jin, and B.J. Steffenson. 1998. Chromosomal location and genetic relationship of the leaf rust resistance genes *Rph*9 and *Rph*12 in barley. Phytopathology 88:76-80.

Borovkova, I.G., Y. Jin, B.J. Steffenson, A. Kilian, T.K. Blake, and A. Kleinhofs. 1997. Identification and mapping of a leaf rust resistance gene in barley line Q21861. Genome 40(2):236-241.

Brown, A.H.D. 1992. Genetic variation and resources in cultivated barley and wild *Hordeum*. pp. 669-682. *In* Munck, L. (ed) Barley genetics 6, vol 2. Proc 6th Barley Genetics Symposium, Mnksgaard Interational Publishers Ltd, Helsingborg, Sweden.

Caviness, C.E., F.C. Collins, and M. Sullivan. 1986. Effect of wheat on early growth of soybean. Arkansas Farm Research 35:8.

Caviness, C.E., and J.D. Thomas. 1979. Influence of planting date on three soybean varieties. Arkansas Farm Research 28(2):8.

Chicaiza, O., J.D. Franckowiak, and B.J. Steffenson. 1996. New sources of resistance to leaf rust in barley. pp. 706-708. *In* Slinkard, A.E., G.J. Scoles, and B.G. Rossnagel (eds.) Proc. Fifth International Oat Conference & Seventh International Barley Genetics Symposium, Saskatoon. University of Saskatchewan, Saskatoon.

Clifford, B.C. 1985. Barley leaf rust. pp. 173-205. *In* Roelfs, A.P. and W.R. Bushnell (eds.) The Cereal Rust. Vol. II. Diseases, Distribution, Epidemiology, and Control. Academic Press, New York.

Cotterill, P.J., R.G. Rees, G.J. Platz, and R. Dill-Macky. 1992a. Effects of leaf rust on selected Australian barleys. Australian Journal of Experimental Agriculture 32:747-751.

Cotterill, P.J., R.G. Rees, and W.A. Vertigan. 1992b. Detection of *Puccinia hordei* virulent on the *Pa9* and Triumph resistance genes in barley in Australia. Australasian Plant Pathology 21:32-34.

Dreiseitl, A. and B.J. Steffenson. 1999. Population of leaf rust resistance genes in Czech and Slovak barley cultivars and breeding lines. Plant Breeding: (In press).

Feuerstein, U., A.H.D. Brown, and J.J. Burdon. 1990. Linkage of rust resistance genes from wild barley (*Hordeum spontaneum*) with isozyme markers. Plant Breeding 104:318-324.

Forster, B.P., J.R. Russell, R.P. Ellis, L.L. Handley, D. Robinson, C.A. Hackett, E. Nevo, R. Waugh, D.C. Gordon, R. Keith, and W. Powell. 1997. Locating genotypes and genes for abiotic stress tolerance in barley: a strategy using maps, markers and the wild species. New-Phytologist 137 (1) 141-147.

Franckowiak, J.D., Y. Jin, and B.J. Steffenson. 1997. Recommended allele symbols for leaf rust resistance genes in barley. Barley Genetics Newsletter 27:36-44.

Freisleben, R. 1940. Die phylogenetische Bedeutung asiatisher Gersten. Zuchter, 12:257:272.

Gair, R., J.E.E. Jenkins, and E. Lester. 1987. Cereal Pests and Diseases. 4th ed. Farming Press Limited, Wharfedale Road, Ipswich, Suffolk. pp. 179-181.

Graner, A., A. Johoor, J. Schondelmaier, H. Siedler, K. Pillen, G. Fischbeck, G. Wenzel, and R.G. Herrmann. 1991. Construction of an RFLP map of barley. Theoretical and Applied Genetics 83:250-256.

Griffey, C.A., M.K. Das, R.E. Baldwin, and C.M. Waldenmaier. 1994. Yield losses in winter barley resulting from a new race of *Puccinia hordei* in North America. Plant Disease 78:256-260.

Harlan J.R. 1976. Barley. pp. 93-98. *In* Simmonds, N.W. (ed.) Evolution of crop plants. Longman, London and New York.

Heun, M., A.E. Kennedy, J.A. Anderson, N.L.V. Lapitan, M.E. Sorrells, and S.D. Tanksley. 1991. Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437-447.

Hockett, E.A., A.F. Cook, M.A. Khan, J.M. Martin, and B.L. Jones. 1993. Hybrid performance and combining ability for yield and malt quality in a diallel cross of barley. Crop Science 33:1239-1244.

Ivandic, V., U. Walther, and A. Graner. 1998. Molecular mapping of a new gene in wild barley conferring complete resistance to leaf rust (*Puccinia hordei* Otth). Theoretical and Applied Genetics. 97:1235-1239.

Jin, Y., and B.J. Steffenson. 1994. Inheritance of resistance to *Puccinia hordei* in cultivated and wild barley. Journal of Heredity. 85:451-454.

Jin, Y., G.H. Cui, B.J. Steffenson, and J.D. Franckowiak. 1996. New leaf rust resistance genes in barley and their allelic and linkage relationships with other *Rph* genes. Phytopathology 86:887-890.

Jin, Y., B.J. Steffenson, and H.E. Bockelman. 1995. Evaluation of cultivated and wild barley for resistance to pathotypes of *Puccinia hordei* with wide virulence. Genetic Resources and Crop Evolution. 42:1-6.

Jin, Y., G.D. Statler, J.D. Franckowiak, and B.J. Steffenson. 1993. Linkage between leaf rust resistance genes and morphological markers in barley. Phytopathology. 83(2):230-233.

Jones, D.G. and B.C. Clifford. 1983. Cereal diseases: their pathology and control. 2nd ed. John Wiley and Sons Limited, New York, NY. pp. 7-51, 230-231.

Kasha, K.J., A. Kleinhofs, A. Kilian, M.A. Saghai Maroof, R.M. Biyashev, G. Scoles, P.M. Hayes, D. Hoffman, Dahleen, T.K. Blake, B. Rossnagel, B.J. Steffenson, Pl.L. Thomas, D.E. Falk, A. Laroche, W. Kim, S. Molnar, D. Mather. 1995. The North American barley genome map on the cross 'Harrington' X 'TR306' and its comparison to the map on cross 'Steptoe' X 'Morex'. pp. 73-88. *In* Tsunewaki, K. (ed) Plant genome and plastome: their structure and evolution. Kodansha Publishers Ltd, Tokyo, Japan.

King, J.E. and R.W. Polley. 1976. Observations on the epidemiology and effect on grain yield of brown rust in spring barley. Plant Pathology 25:63-73.

Kleinhofs, A. and A. Kilian. 1994. RFLP maps of barley. pp. 163-198. *In* Philips, R.L. and I.K. Basil (ed) DNA-based markers in plants. Edited by. Kluwer Academic Publishers, Dordrecht.

Kleinhofs, A., A. Kilian, M.A. Saghai Maroof, R.M. Biyashev, P. Hayes, F.Q. Chen, N. Lapitan, A. Fenwick, T.K. Blake, V. Kanazin, E. Ananiev, L. Dahleen, D. Kudrna, J. Bollinger, S.J. Knapp, B. Liu, M. Sorrells, M. Heun, J.D. Franckowiak, D. Hoffman, R. Skadsen, B.J. Steffenson. 1993. A molecular, isozyme and morphological map of barley (*Hordeum vulgare*) genome. Theoretical and Applied Genetics 86:705-712.

Kurata, N., Y. Nagamura, K. Yamamoto, Y. Harushima, N. Sue, J. Wu, B.A. Antonio, A. Shomura, T. Shimizu, S.Y. Lin, T. Inoue, A. Fukuda, T. Shimano, Y. Kuboki, T. Toyama, Y. Miyamoto, T. Kirihara, K. Hayasaka, A. Miyao, L. Monna, H.S. Zhong, Y. Tamura, Z-X. Wang, T. Momma, Y. Umehara, M. Yano, T. Sasaki and Y. Minobe. 1994. A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nature Genetics 8:365-372.

Lawes, D.A. 1988. The costs of providing disease-resistant cultivars. pp. 213-219. *In* Clifford, B.C., and E. Lester (eds.) Control of Plant Diseases: Costs and Benefits. Blackwell Scientific Publications, Oxford.

Levine, M. N., and W. J. Cherewick. 1952. Studies on dwarf leaf rust of barley. U.S. Department of Agriculture Technical Bulletin. No. 1056, Washington D.C.

Liu, Z.–W., R.M. Biyashev, and M.A. Saghai Maroof. 1996. Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theoretical and Applied Genetics 93:869-876.

Lu, M. and S. Ding. 1991. Genetic analysis of some malting quality traits in two-rowed barley. *In* Munck, L. (ed) Barley Genetics VI, Volume 1. Munksgaard International Publishers Ltd, Copenhagen, pp. 481-483.

Mathre, D.E. 1982. Compendium of barley diseases. The American Phytopathological Society, St. Paul, MN. pp. 32-41.

McDaniel, M.N. and B.R. Hathcock. 1969. Linkage of the Pa-4 and Mla loci in barley. Crop Science 9:822-823.

Melville, S.C., G.W. Griffin, and J.L. Jemmett. 1976. Effects of fungicide spraying on brown rust and yield in spring barley. Plant Pathology 25:99-107.

Moore, G., M.D. Gale, N. Kurata, R.B. Flavell. 1993. Molecular analysis of small grain cereal genomes: current status and prospects. Bio/Technology 11:584-589.

Murray, T.D., D.W. Parry, and N.D. Cattlin. 1998. A Color Handbook of Diseases of Small Grain Cereal Crops. Iowa State University Press, Ames, Iowa. pp. 49-51.

Nevo, E. 1986. Genetic resources of wild cereals and crop improvement: Israel, a natural laboratory. Israel Journal of Botany 35:255-278.

Nevo, E. 1992. Origin, evolution, population genetics and resources for breeding of wild barley *Hordeum spontaneum* in the fertile crescent. pp. 19-43. *In* Shewry P.R. (ed) Barley: genetics, biochemistry, molecular biology and biotechnology. CAB International Oxon, UK.

Niks, R.E. 1982. Early abortion of colonies of leaf rust, *Puccinia hordei*, in partially resistant barley seedlings. Canadian Journal of Botany 60:714-723.

Parlevliet, J.E. 1976a. The genetics of seedling resistance to leaf rust, *Puccinia hordei* Otth. in some spring barley cultivars. Euphytica 25:249-254.

Parlevliet, J.E. 1976b. Partial resistance of barley to leaf rust, *Puccinia hordei*. III The inheritance of the host plant effect on latent period in four cultivars. Euphytica 25:241-248.

Parlevliet, J.E. 1978. Further evidence of polygenic inheritance of partial resistance in barley to leaf rust, *Puccinia hordei*. Euphytica. 27:369-379.

Parlevliet, J.E. 1983. Race-specific resistance and cultivar-specific virulence in the barley-leaf-rust pathosystem and their consequences for the breeding of leaf rust resistant barley. Euphtica 32:367-375.

Parlevliet, J.E. and H.J. Kupier. 1977. Resistance of some barley cultivars to leaf rust, *Puccinia hordei*; polygenic, partial resistance hidden by monogenic hypersensitivity. Netherlands Journal of Plant Pathology 83:85-89.

Parlevliet, J.E. and A. van Ommeren. 1985. Race-specific effects in major genic and polygenic resistance of barley to barley leaf rust in the field: identification and distinction. Euphytica 34:689-695.

Poehlman, J.M. and D.A. Sleper. 1995. Breeding Field Crops. 4th ed. Iowa State University Press, Ames, Iowa.

Pomeranz, Y. 1987. Modern Cereal Science and Technology. VCH Publishers, Inc., New York, NY. pp. 8, 15, 21-22, 419-422.

Qi, X., R.E. Niks, P. Stam, and P. Lindhout. 1998. Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley. Theoretical and Applied Genetics 96:1205-1215.

Qi, X., P. Stam, and P. Lindhout. 1996. Comparison and integration of four barley genetic maps. Genome 39:379-394.

Reinhold, M. and E.L. Sharp. 1982. Virulence types of *Puccinia hordei* from North America, North Africa, and the Middle East. Plant Disease 66:1009-1011.

Roane, C.W. 1962. Inheritance of reaction to *Puccinia hordei* in barley. I. Genes for resistance among North America race differentiating varieties. Phytopathology 52:1288-1295.

Russell, G.E. 1978. General conclusions on breeding for resistance. pp. 431-432. *In* D.J.A. Cole, W. Haresign, J.P. Hudson, G.E. Russell, D.E. Tribe (eds.) Plant Breeding for Pest and Disease Resistance. Butterworths, Boston, MA.

Schafer, J.F., A.P. Roelfs, and W.R. Bushnell. 1984. Contributions of early scientists to knowledge of cereal rusts. pp. 3-38. *In* Bushnell, W.R. and A.P. Roelfs (eds.) The Cereal Rusts. Vol. I. Origins, Specificity, Structure, and Physiology. Academic Press, Inc., Orlando, Florida.

Steffenson, B. J., Y. Jin, and C. A. Griffey. 1993. Pathotypes of *Puccinia hordei* with virulence for the barley leaf rust resistance gene *Rph*7 in the United States. Plant Diseases 77:867-869

Tan, B.H. 1978. Verifying the genetic relationships between three leaf rust resistance genes in barley. Euphytica 27:317-323.

Tanksley, S.D., N.D. Young, A.H. Paterson, and M.W. Bonierbale. 1989. RFLP mapping in plant breeding: new tools for an old science. Bio/Technology 7:257-264.

Thurlow, D.L. 1986. Planting date effect on soybean growth and yield. Research Report Series Alabama Agricultural Experiment Station Auburn University 4:17-19.

Tuleen, N.A., and M.E. McDaniel. 1971. Location of *Pa* and *Pa5*. Barley Newsletter 15:106-107.

Van Der Plank, J.E. 1968. The loss of horizontal resistance. pp. 153-155. Disease Resistance in Plants. Academic Press, New York, NY.

Virginia Agricultural Statistics Service. 1995. 1994 Virginia Agricultural Statistics Bulletin. USDA Bulletin Number 66. Richmond, Virginia. pp. 9.

Virginia Agricultural Statistics Serivce. 1999. 1998 Virginia Agricultural Statistics Bulletin. National Agricultural Statistics Service, USDA Bulletin Number 71. Richmond, Virginia. pp. 9.

Waterhouse, W.L. 1948. Studies in the inheritance of resistance to rust of barley. II. J. Proc. Royal Society. New South Wales 81:198-205.

Wiese, M.V. 1987. Compendium of wheat diseases. 2nd ed. The American Phytopathological Society, St. Paul, MN. pp. 37-41.

Zillinsky, F.J. 1983. Common diseases of small grain cereals: a guide to identification. The International Maize and Wheat Improvement Center (CIMMYT). Mexico. pp. 11-16.

CHAPTER II.

INHERITANCE OF LEAF RUST RESISTANCE IN VA 92-42-46 AND MOLECULAR MAPPING OF THE *Rph*5 RESISTANCE GENE

Abstract

In 1990, the Virginia breeding program reported leaf rust outbreaks on cultivars that possessed the *Rph*7 gene. *Rph*7 was introduced into the Virginia breeding program in the early 1950s from Cebada Capa, a spring barley cultivar. Most of the Virginia cultivars released from 1968 to 1990 contained the leaf rust resistance gene *Rph*7 for which *P. hordei* race 30 possesses virulence. Therefore, a need for resistance to *Puccinia hordei* race 30 arose. The purpose of this research is to determine the inheritance of resistance to leaf rust in the barley experimental line VA 92-42-46, to identify the gene(s) conferring resistance, identify putative resistance related markers, and to map the gene(s) to one or more barley chromosomes using molecular markers. The Virginia barley line 92-42-46 was selected for this research project because it possesses resistance to *P. hordei* race 30, which has overcome resistance conferred by *Rph*7. Crosses were made between VA 92-42-46 and Moore, a susceptible cultivar to leaf rust. Crosses were also made between VA 92-42-46 and selected host-differentials. Inheritance studies were performed by screening F_2 progeny and $F_{2:3}$ families against race 8 and race 30 to determine the number of leaf rust resistance genes in VA 92-42-46. Allelism tests were performed to determine gene identity. A single dominant gene at the *Rph*5 locus or a tightly linked gene confers the resistance to P. hordei in VA 92-42-46. Two populations, 'Moore' X VA 92-42-46 and 'Bowman' X 'Magnif', were used in this study for mapping molecular markers, to provide comparison and confirmation of results. 'Magnif' possesses the resistance gene *Rph*5. Bulked segregant analysis was used to identify polymorphic RFLP and SSR markers that were used for mapping in each population.

Linkage analysis revealed that the *Rph*5 gene maps to barley chromosome 3 (3H) above the centromeric region in the 'Moore' X VA 92-42-46 population. These findings agree with previous research that identified linkage between *Rph*7 and *Rph*5 on chromosome 3. The research performed in this study does not support the research that had reported linkage between *Rph*5 and *Rph*2 on barley chromosome 7 (5H). Further research should be conducted to verify the results of this study using the 'Bowman' X 'Magnif' population. The markers screened above the centromere region of barley chromosome 3 were monomorphic for the 'Bowman' X 'Magnif' population except for the RFLP marker MWG561. Therefore, additional markers above the centromere of barley chromosome 3 should be screened.

Introduction

Barley (*Hordeum vulgare* L.) is an important crop throughout the world and is ranked fifth in world crop production (Nevo, 1992). Foliar diseases are one of the major biotic stresses affecting cereal crops (Zillinsky, 1983). Barley leaf rust (*Puccinia hordei* G. Otth), a fungal pathogen, is the most important foliar disease in barley (Zillinsky, 1983).

The spring barley cultivar Cebada Capa was used as a source of leaf rust resistance in the early 1950s in the Virginia breeding program. 'Cebada Capa' possesses the resistance gene *Rph*7. The first cultivar released from the Virginia breeding program that possessed *Rph*7 was 'Hanover' and it was released in 1968 (Starling et al., 1970). Most of the barley cultivars developed in the Virginia breeding program between 1968 and 1990 had the *Rph*7 gene. In 1990, leaf rust outbreaks were reported on cultivars that possessed the *Rph*7 gene in Virginia (Steffenson et al., 1993). Leaf rust isolates that were virulent to *Rph*7 were also identified in Pennsylvania and California in 1990 (Steffenson et al., 1993). These are the earliest reports of *Puccinia hordei* isolates in North America with virulence to *Rph*7.

The resistance provided by 'Cebada Capa' remained effective and durable for approximately 30 years, which is extremely rare for a single dominant gene (Steffenson et al., 1993). Under selection pressure exerted by a resistant host, pathogens usually mutate giving rise to new races possessing virulence to widely deployed resistance genes. Resistance conferred by a single dominant gene will usually be overcome much more quickly than occurred with *Rph*7. It is not uncommon for plant breeders to develop a

resistant cultivar and have the pathogen become virulent to the cultivar by the time of release or shortly thereafter. *Puccinia hordei* race 30 is virulent to *Rph*7 and has become one of the most prevalent races in Virginia and the surrounding region (Steffenson et al., 1993; Griffey et al., 1994). Because the primary resistance gene, *Rph*7, found in most Virginia cultivars, was overcome by leaf rust race 30 new sources of leaf rust resistance needed to be identified quickly and transferred into desirable agronomic backgrounds. During ensuing years of leaf rust epidemics, one Virginia experimental line (VA 92-42-46) was identified as having a high degree of resistance when most other lines and cultivars were severely infected with leaf rust. Therefore, research was needed to identify the gene(s) conferring resistance against the new race (race 30) and to transfer the resistance into other cultivars with good agronomic backgrounds.

Preliminary genetics research was conducted prior to this study on the experimental line VA 92-42-46 from crosses with the susceptible cultivar Moore. Crosses were also made between VA 92-42-46 and barley differential lines possessing genes *Rph*3, 5, and 9 that confer resistance to race 30. Four F_2 populations from a cross between 'Moore' X VA 92-42-46 were selected for disease screening in the preliminary study. One of these populations designated as Population 1 was used in the current study for additional genetics and mapping research. Results from the preliminary study indicated that VA 92-42-46 likely possesses *Rph*5 (Appendix B). The cultivar Magnif was shown to possess the single dominant resistance gene *Rph*5 to barley leaf rust (Franckowiak et al., 1997; Jin et al., 1996; Yahyaoui and Sharp, 1987). Research performed by Jin et al. (1996) and Borovkova et al. (1997) reported linkage between

*Rph*2 and *Rph*5 on barley chromosome 7 (5H).

Dr. Brian Steffenson of North Dakota State University made a cross between 'Bowman', a leaf rust-susceptible spring cultivar, and 'Magnif', the donor of *Rph*5 (Steffenson, 1997, personal communication). Steffenson obtained leaf rust infection type data of F_2 and $F_{2:3}$ populations for race 8. Since resistance in 'Magnif' is conferred by the single dominant gene *Rph*5, the 'Bowman' X 'Magnif' population was included to provide comparison and confirmation of results obtained for the 'Moore' by VA 92-42-46 population in mapping studies.

The purpose of this research is to determine the inheritance of resistance to leaf rust in the barley experimental line VA 92-42-46, to identify the gene(s) conferring resistance, identify putative resistance related markers, and to map the gene(s) to one or more barley chromosomes using molecular markers.

Materials and Methods

Evaluation of leaf rust resistance in the 'Moore' X VA 92-42-46 barley populations

The Virginia experimental barley line 92-42-46 was selected for this research primarily because it possesses resistance to *P. hordei* race 30, which had overcome resistance conferred by *Rph*7. The pedigree of VA 92-42-46 is unknown. Crosses between 'Moore', a universal susceptible cultivar, and VA 92-42-46 were made. One population that was evaluated in the preliminary study was also used for further evaluation in this study that population was designated as Population 1 (Table 1). The other crosses with 'Moore' X VA 92-42-46 that were used for disease screening are

identified as Population 2 and 3 (Table 1, Appendix E). F_1 plants from this cross were grown in the greenhouse to produce F_2 progeny. Each F_2 population was derived from a single F_1 plant. Three F_1 plants that produced a minimum of 100 F_2 seed were selected for disease screening as shown by the non-pooled data in Appendix E.

The parents, 'Moore' and VA 92-42-46, their F_2 progeny, and a set of host-differential lines (Table 2) were screened using *P. hordei* race 30 to determine their infection type. Race 30 is virulent to *Rph*1, *Rph*2, *Rph*4, *Rph*2+6, *Rph*7, *Rph*8, *Rph*11, and *Rph*13 (Griffey et al., 1994; T. Fetch, 1999, personal communication). Race 30 is avirulent to *Rph*3, *Rph*5, *Rph*9, *Rph*10, *Rph*14, and *Rph*15 (Griffey et al., 1994; T. Fetch, 1999, personal communication). Race 30 used in disease screening in this study originated in Virginia and was purified as a single pustule isolate to obtain a pure race. Isolate 90-34 was collected on volunteer barley on November 2, 1990 in Blacksburg, VA (Steffenson et al., 1993). Isolate 90-34 from NDSU has the same virulence pattern as race 30 (Steffenson et al., 1993; T. Fetch, 1999, personal communication). *Rph*16, a newly named gene, has not been tested against leaf rust race 30 or isolate 90-34.

Infection type was determined on five seedlings of each parent, five to seven seedlings of the host-differential lines, and 100 seedlings from each of the three F_2 populations. Seedlings were grown in small plastic pots (75 mm in diameter and 65 mm in depth). Pots were filled to within 12 mm of the top with a soil and peat mixture (3:1 peat/soil), and the pots were placed in wooden flats (35 pots per flat and 5 seeds/pot). Seeds were space planted in each pot in a systematic way and greenhouse stakes were used to identify the seedlings for each population. Flats were placed on a greenhouse

bench where the temperature was maintained at 22 $\pm$ 3^{o}C. Seedlings were inoculated at the two-leaf stage, which was 10 to 14 days after planting. Seedlings were inoculated with a mixture of *P. hordei* (race 30) urediospores and talcum powder (1g of spores per 5g talc) using a vacuum pressure pump model # DDA-P104D-AA (Thomas Scientific, Swedesboro,NJ) to evenly cover the seedlings with the spore/talc mixture. A canvas tent was used to cover the inoculated seedlings and to maintain free-moisture provided by an intermittent mist from a humidifier for 16 hours. The humidifier was controlled by a timer and was set on a cycle to run for two hours and then shut off for two hours. The sides of the canvas tent were raised after the 16-hr dew period, and the seedlings were allowed to dry slowly. Artificial lighting was provided by 300-watt incandescent Phillips model #21F bulbs (Phillips lighting Co., Somerset, NJ) in the greenhouse and was set to maintain 16 hr days. The bulbs are rated at 0.566 photons per square meter.

Infection types were scored using the 0-4 scale of Levine and Cherewick (1952). Readings were performed 10 to 14 days after inoculation. Plants with infection types of 0, 1, and 2 were considered resistant, and plants with infection types 3 and 4 were considered susceptible. Infection types of the F_2 progeny were compared with infection types of the parents and barley host-differentials to assure proper classification and assignment into resistant and susceptible classes. A Chi-square test was utilized to determine goodness-of-fit of observed segregation patterns versus expected genetic ratios. Chi-square tests were performed for all genetic data. Subsequent to disease scoring, plant tissue from individual F_2 progeny was cut, wrapped in cheesecloth, labeled,

and stored in a –70°C freezer until used for DNA extraction from 'Moore' X VA 92-42-46 Population 1.

In addition, $F_{2:3}$ families of Population 1 were screened for reaction to *P. hordei* race 30. Ninety-six $F_{2:3}$ families were screened against race 30 using 50 seeds per family. The disease screening procedures used were the same as previously described.

$F_{2:3}$ families of Population 1 were screened for reaction to race 8 (ND8702) (Steffenson et al., 1993). Ninety-one $F_{2:3}$ families were screened against race 8 using 50 seeds per family. The disease screening procedures used were the same as previously described. The initial race 8 leaf rust inoculum was provided by Dr. Brian Steffenson of North Dakota State University. Race 8 is virulent to *Rph*1, *Rph*4, *Rph*8, *Rph*10, and *Rph*11 (Griffey et al., 1994; T. Fetch, 1999, personal communication). Race 8 is avirulent to *Rph*2, *Rph*3, *Rph*5, *Rph*2+6, *Rph*7, *Rph*9, *Rph*13, *Rph*14, and *Rph*15 (Griffey et al., 1994; T. Fetch, 1999, personal communication). Rph16 has not been tested against race 8.

Inheritance of resistance to *P. hordei* race 8 for Populations 2 and 3

Two additional crosses between 'Moore' and VA 92-42-46 were made in order to produce genetic populations for evaluation with *P. hordei* race 8. These crosses are identified as Populations 2 and 3 in Table 2 and Appendix E. F_1 plants from these crosses were grown in the greenhouse to produce F_2 progeny. Each F_2 population was derived from a single F_1 plant. Seed from each cross were planted and three F_1 plants that produced from 64 to 100 F_2 seed were selected for disease screening for both Population

2 and 3. F_2 progeny were screened for reaction to *P. hordei* race 8 (ND8702), using the same procedures described previously. Infection types of the F_2 progeny were compared with infection types of the parents and barley host-differentials to assure proper classification and assignment into resistant and susceptible classes. Data from F_2 plants derived from separate F_1 plants were pooled when the variance was homogenous using the Test for Homogeneity of Ratio (Gomez and Gomez, 1984).

Allelism Study

An allelism study was performed via the evaluation of segregation patterns among F_2 progeny derived from crosses between VA92-42-46 and four host-differential lines ['Estate' (*Rph*3.c), 'Aim' (*Rph*3), 'Magnif' (*Rph*5.e), and 'Triumph' (*Rph*9.z)], that are resistant to race 30. Three F_1 plants from each cross that produced approximately 100 F_2 seed were selected for disease screening. Disease screening was performed on the F_2 progeny using the procedures previously described, gene number and action were determined in populations where segregation occurred. Data from F_2 plants derived from individual F_1 plants were pooled when the variance was homogenous using the Test for Homogeneity of Ratio (Gomez and Gomez, 1984). Chi-square tests were performed to test goodness-of-fit of observed segregation patterns to expected genetic ratios. Appearance of leaf rust susceptible plants among F_2 progeny derived from a cross between VA 92-42-46 and a selected host-differential would strongly indicate that the gene present in VA 92-42-46 is different from that of the differential. Lack of segregation among such F_2 progeny would provide strong evidence that the host

differential and VA 92-42-46 have alleles at the same locus or tightly linked genes.

'Bowman' X 'Magnif' Population

Dr. Brian Steffenson at North Dakota State University (NSDU) made crosses between 'Bowman', a leaf rust susceptible cultivar, and 'Magnif', a resistant cultivar that possesses the *Rph*5 gene. Parental infection types to race 8 are presented in Table 1. F_2 and $F_{2:3}$ progeny were screened for reaction to race 8 (ND8702) by Dr. Steffenson at NDSU using procedures described by Jin et al. (1996). Segregation data and $F_{2:3}$ seed were sent to Virginia Polytechnic and State University for use in mapping studies. $F_{2:3}$ plants were grown in the greenhouse, and tissue was collected and stored at -70^{o}C prior to DNA extraction.

Molecular mapping of the *Rph*5 resistance gene

DNA was isolated from barley leaf tissue, previously collected and stored at –70^{o}C, by freeze-drying and grinding 0.75 g (dry weight) into a fine powder with a mechanical mill. After powdered, 15 ml of extraction buffer [50 mM Tris =, pH 8.0/0.7 M NaCl/10 mM EDTA / 1% hexadecyltrimethylammonium bromide/0.1% 2-mercaptoethanol (CTAB)] was added to the samples as described by Saghai Maroof et al. (1984). DNA concentration was measured using a DNA TKO 100 Fluorometer (Hoefer Scientific, San Francisco, CA). DNA integrity was determined by running undigested DNA in 0.8% agarose gel followed by staining in ethidium bromide (EtBr) and visualized under ultraviolet (UV) light.

Bulked segregant analysis (BSA) was used to identify the target region with all available polymorphic markers (Michelmore et al., 1991). In this study, BSA was performed using the $F_{2:3}$ disease data to identify six genotypes that were homozygous resistant and six genotypes that were homozygous susceptible to *P. hordei*. Respective genomic DNAs were pooled to form bulk resistant and bulk susceptible samples. These blots were used for initial screening of RFLP probes for polymorphism.

RFLP analysis was performed with slight modifications as described by Biyashev et al. (1997). Diagnostic blots were prepared by digesting 8 µg of parental DNA and 8 µg of DNA from the bulk samples with each of six restriction enzymes; BamHI, DraI, EcoRI, HindIII, SstI, and XbaI; according to manufacturer's protocols. Only four restriction enzymes DraI, EcoRI, HindIII, and XbaI that detected the highest level of polymorphism were used to digest DNA from F_2 genotypes and DNA from $F_{2:3}$ families to make population blots. After digestion, DNA was electrophoresed on 0.8% agarose gels, followed by standard Southern transfer to nylon membranes. Blots were hybridized overnight with randomly primered ^{32}P-dCTP labeled probes. Following hybridization, blots were washed three times, twice for 5 min. at room temperature (22^{o}C) with 1X SSC and 0.1% SDS and once for 15 min. at 65^{o}C with 0.5X SSC and 0.1% SDS. After washing, blots were covered with plastic wrap and the blots were exposed to X-ray film for 5-7 days.

In addition to screening for RFLPs, a set of microsatellite or simple sequence repeat (SSR) markers were used in this study. Parents and bulks from both the 'Moore' X VA 92-42-46 and 'Bowman' X 'Magnif' populations were screened with 31 SSR

primers pairs. Depending on primers used, amplification of the SSRs was performed using one of the two PCR profiles: 1) one cycle of 94^{o}C for 3 min., 47^{o}C for 2 min., and 72^{o}C for 1.5 min., followed by 30 cycles at 94^{o}C for 1 min., 47^{o}C for 2 min and 72^{o}C for 1.5; 2) one cycle of 94^{o}C for 3 min., 55^{o}C for 2 min., and 72^{o}C for 1.5 min., followed by 30 cycles at 94^{o}C for 1 min., 55^{o}C for 2 min and 72^{o}C for 1.5 min min (Liu et al., 1996). PCR was performed in a thermal cycler (Perkin-Elmer Cetus 480, Norwalk, CT). The PCR reaction preparation and polyacrylamide gel electrophoresis were conducted as previously described by Saghai Maroof et al. (1994).

Genetic mapping and linkage analysis were performed using the computer program MAPMAKER version 3.0b (Lander et al., 1987). Analysis was conducted using threshold LOD scores of 3.0 and 2.0 (the default setting is 3.0), with a maximum Haldane distance of 50 cM. When there was no difference in the number of markers or map distance between markers a LOD of 3.0 was used. When additional markers were included in a linkage group using a LOD of 2.0 versus 3.0, an LOD of 2.0 was used.

Results and Discussion

Infection types (IT) of 'Moore', VA 92-42-46, 'Bowman', 'Magnif', and host-differential lines to *P. hordei* races 8 and 30 are presented in Table 2. The host-differential lines 'Estate' (*Rph*3.c) and 'Aim' (*Rph*3) exhibited the lowest infection type (0; and 0;N, respectively) to race 30. VA 92-42-46, 'Magnif' (*Rph*5.e), and 'Estate' (*Rph*3.c) gave the lowest infection type (0;) to race 8. Leaf rust race 8 was included in

this research because it gave infection types that were more definitive for grouping into resistant and susceptible classes than race 30.

Genetic analysis of leaf rust resistance in 'Moore' X VA 92-42-46 and 'Bowman' X 'Magnif' populations

Segregation patterns, genetic analyses and proposed inheritance of leaf rust resistance in all genetic populations are presented in Table 1. F_2 progeny from Population 1 (cross between 'Moore' and VA 92-42-46) fit a 3 resistant: 1 susceptible genetic ratio when screened with race 30 as verified by the $F_{2:3}$ generation (Appendix A). The original F_2 data from the preliminary study fit a 7 resistant:9 susceptible genetic ratio (Appendix B). The number of F_2 plants in the susceptible class from the preliminary study was larger than the number indicated by the disease screening results from the $F_{2:3}$ families. The F_2 plants that were misclassified in the F_2 generation were likely misclassified because the greenhouse temperature was too high to provide accurate infection types. The 'Moore' X VA 92-42-46 $F_{2:3}$ families from Population 1 segregated to fit a 1 homozygous resistant: 2 segregating: 1 homozygous susceptible genotypic ratio for a single dominant gene when tested against both race 8 and 30. Genetic analyses of the 'Moore' X VA 92-42-46 populations tested against race 8 and race 30 corroborate dominant monogenic inheritance of leaf rust resistance in VA 92-42-46. The pooled F_2 segregation data of two independent crosses between 'Moore' and VA 92-42-46 (Population 2 and Population 3), screened against race 8 of *P. hordei*, fit a 3 resistant: 1

susceptible genetic ratio, and indicated that a single dominant gene governs resistance to leaf rust in VA 92-42-46.

The 'Bowman' X 'Magnif' F_2 segregation data presented in Table 1 fit a 3 resistant: 1 susceptible genetic ratio for reaction to race 8. The $F_{2:3}$ families of this cross segregated to fit a 1 homozygous resistant: 2 segregating: 1 homozygous susceptible genotypic ratio in response to race 8. Data from the F_2 and $F_{2:3}$ generations support previous reports by Franckowiak et al. (1997), Jin et al., (1996) and Yahyaoui and Sharp (1987) that leaf rust resistance in 'Magnif' is governed by the single dominant gene *Rph*5.

Analysis of crosses between VA 92-42-46 and four host-differential lines

Results of preliminary allelism tests for crosses between VA 92-42-46 and selected host-differentials screened against race 30 of *P. hordei* are presented in Appendix B. There was one susceptible F_2 plant derived from a cross between VA 92-42-46 and 'Magnif' (*Rph*5.e). Another cross between VA 92-42-46 and 'Magnif' resulted in all resistant F_2 progeny. Since one susceptible plant was observed in crosses between VA 92-42-46 and 'Magnif' that possesses the *Rph*5 gene, additional crosses were made between VA 92-42-46 and 'Magnif'. Segregation for susceptible F_2 progeny was observed in crosses of VA 92-42-46 with 'Estate' (*Rph*3.c), 'Aim' (*Rph*3), 'Hor2596' (*Rph*9.i), and 'Triumph' (*Rph*9.z) and indicated that resistance in VA 92-42-46 is not governed by alleles at the *Rph*3 or *Rph*9 loci. Preliminary results indicated that VA 92-42-46 possesses two genes for resistance to barley leaf rust.

Results of allelism tests for crosses between VA 92-42-46 and selected host-differentials screened against race 8 of *P. hordei* in the current study are presented in Table 1. All of the 299 F_2 plants derived from the cross between VA 92-42-46 and 'Magnif' (*Rph*5.e) were resistant to leaf rust. The lack of segregation for susceptible progeny provides strong evidence that resistance in VA 92-42-46 is conferred by an allele at the *Rph*5 locus or a tightly linked gene. Segregation for susceptible F_2 progeny was observed in crosses between VA 92-42-46 with 'Estate' (*Rph*3.c), 'Aim' (*Rph*3), and 'Triumph' (*Rph*9.z) and the observed segregation patterns fit 15 resistant: 1 susceptible ratios expected for two independent dominant genes. Therefore, the single dominant gene in VA 92-42-46 is not allelic or linked to genes *Rph*3 or *Rph*9.

RFLP markers

A total of 133 RFLP markers were used to determine polymorphism between parental lines and bulks in the 'Moore' X VA 92-42-46 and 'Bowman' X 'Magnif' mapping populations. Six restriction enzymes (BamHI, DraI, EcoRI, HindIII, SstI, and XbaI) were used in an initial screening for polymorphism. Of 133 markers, 83 were genomic clones including 73 from barley (ABG and MWG) and 10 from wheat (KSU and WG) (Graner et al., 1991; Heun et al., 1991; Kleinhofs et al., 1993; and Sherman et al., 1995). Besides genomic clones, 47 cDNA and three functional gene clones were screened. The cDNA clones included nine from oat (CDO) and 35 from barley (ABC, BCD, and PSR) (Graner et al., 1991; Heun et al., 1991; Kleinhofs et al., 1993; and Sherman et al., 1995).

Out of a total of 133 clones, 85 revealed polymorphism between parental lines (Table 3). The overall level of polymorphism was 63.9%. There were 61 polymorphic clones for the 'Moore' X VA 92-42-46 mapping population and 62 polymorphic clones for the 'Bowman' X 'Magnif' mapping population. The level of polymorphism was about 46% for each population. Forty-one out of a total of 133 clones (30.8%) were polymorphic for both populations. Comparison between restriction enzymes in terms of level of polymorphism each can generate showed that parental DNA digested with DraI and XbaI displayed the highest level of polymorphism in combination with 43 (32.3%) and 41 (30.8%) clones, respectively. The level of polymorphism observed with HindIII was 32 out of 133 or 24.1%, and EcoRI resulted in the lowest level of polymorphism with 18.8%.

Microsatellite or Simple Sequence Repeat (SSR) markers

Since the level of polymorphism revealed by RFLP markers was not high enough for mapping purposes, a set of microsatellite markers was also used in this study. SSR markers were chosen based on their map location in the barley genome in an attempt to facilitate the search of linkage with *Rph*5 and to gain better coverage of barley chromosomes with molecular markers. Thirty-one SSR primer pairs were screened for polymorphism. There were 16 polymorphic SSRs (Table 3). The overall level of polymorphism was 51.6%. Each population had 11 polymorphic SSRs out of 31. There were seven SSRs that were polymorphic for both populations.

Molecular mapping of *Rph*5

Linkage analysis of segregation data allowed for construction of genetic maps for the 'Moore' X VA 92-42-46 and 'Bowman' X 'Magnif' mapping populations. The barley chromosome maps (Figures 1-7) were constructed using MAPMAKER and the map distance in cM is indicated between adjacent markers for the 'Moore' X VA 92-42-46 and 'Bowman' X 'Magnif' mapping populations. The estimated location of previously mapped *Rph* genes (qualitative resistant genes) and *Rphq* genes (quantitative resistant genes) are presented in Figures 1-7 (Borovkova et al., 1997; Ivandic et al., 1998; Qi et al., 1998).

The distribution of RFLP and SSR markers among the seven barley chromosomes and the level of polymorphism for each chromosome are presented in Table 4 for the 'Moore' X VA 92-42-46 mapping population and Table 5 for the 'Bowman' X 'Magnif' mapping population. Barley chromosome 7 had the highest number of RFLP markers screened and the most polymorphic RFLP markers compared to the other chromosomes for both mapping populations. Markers first screened in this research were located on barley chromosome 7 as reported on maps constructed by Kasha et al. (1995), Kleinhofs et al. (1993), and Liu et al. (1996).

Two linkage groups were identified using MAPMAKER on chromosome 7 for the 'Moore' X VA 92-42-46 mapping population (Figure 7). The gap between the two linkage groups is estimated at 80 cM as compared to the Kleinhofs et al. (1993) map that was based on a more extensive set of markers. Linkage between the markers mapped on chromosome 7 and *Rph*5 was not detected.

Two linkage groups were identified on chromosome 7 for the 'Bowman' X 'Magnif' population (Figure 7). The marker furthest above the centromere on the plus arm in the 'Bowman' X 'Magnif' population was ABG497 (Linde-Laursen and Jensen, 1992). The order of ABG497 and ABG705 is inverted when compared to the 'Steptoe' X 'Morex' map (Kleinhofs et al., 1993). The gap between the two linkage groups is approximately 30 cM as compared to the Kleinhofs et al. (1993) map. However, for the specific cross of 'Bowman' X 'Magnif' the map distance between the two markers ABC390 and MWG716a is likely greater than 50 cM, otherwise MAPMAKER should have combined these two linkage groups into one linkage group.

While linked markers on both sides of the centromere of chromosome 7 were identified for both mapping populations, no linkage between any of these markers and the leaf rust resistance gene *Rph*5 was found. Since several of the markers used in this research cover the region identified by Borovkova et al. (1997) containing *Rph*2, the markers should have shown linkage with *Rph*5 if it is linked to *Rph*2 as reported by Jin et al. (1996) and Borovkova et al. (1997). Since linkage was not observed in the current study, it is proposed that the resistance gene *Rph*5 is not located in the region covered by markers on chromosome 7 (Figure 7).

Tan (1978) reported linkage between *Rph*7 and *Rph*5 on barley chromosome 3. Therefore, after screening approximately 35 markers on chromosome 7 and not finding linkage between the markers and *Rph*5 it was logical to screen markers on chromosome 3 in an attempt to identify markers linked to the *Rph*5 resistance gene. A MAPMAKER LOD setting of 3.0 was used to identify a linkage group consisting of KSUA3b and the

*Rph*5 resistance gene in the 'Moore' X VA 92-42-46 mapping population. Because KSUA3 is a multi-locus marker this linkage group could not be identified to a specific chromosome without additional markers of known chromosome location linked together with KSUA3b and *Rph*5. Using an LOD score of 2.0, six markers and *Rph*5 were shown to form a linkage group on chromosome 3 for the 'Moore' X VA 92-42-46 population (Figure 3). The findings of the current research agree with the report by Tan (1978) that identified *Rph*5 on barley chromosome 3. The results of this research do not support research by Borovkova et al. (1997) and Jin et al. (1996) that showed *Rph*5 on barley chromosome 7.

The linkage group on chromosome 3 for the 'Bowman' X Magnif population is presented in Figure 3. The following markers located above MWG561 were monomorphic: BCD828, ABG462, ABG460, and ABC171 when screened in this population. Therefore, markers in the region where KSUA3b and *Rph*5 were mapped in the 'Moore' X VA 92-42-46 population were not mapped in the 'Bowman' X 'Magnif' population. Linkage between markers and *Rph*5 was not observed on chromosome 3 for the 'Bowman' X 'Magnif' population.

Markers from barley chromosomes 1, 2, 4, 5, and 6 were also screened and the linkage maps for these chromosomes for both mapping populations are presented in Figures 1, 2, 4, 5, and 6. Linkage between markers and *Rph*5 was not identified for either mapping population on barley chromosomes 1, 2, 4, 5, 6, or 7.

The number of genomic, cDNA clones and SSR primers placed on the barley chromosomes are presented in Table 6 for the 'Moore' X VA 92-42-46 mapping

population. The number of markers mapped includes 57 RFLP and 3 SSR markers. The RFLP markers include 42 genomic clones and 15 cDNA clones. Their distribution among the seven barley chromosomes is reported in Table 6. The 60 polymorphic markers that were mapped for the 'Moore' X VA 92-42-46 population cover a total distance of 833 cM with an average distance 14 cM covered by each marker (Table 6 and Figures 1-7). The map distance covered for each chromosome in the 'Moore' X VA 92-42-46 population varied from 95 cM to 146 cM for the seven chromosomes.

The number of genomic, cDNA clones and SSR primers placed on the barley chromosomes are reported in Table 7 for the 'Bowman' X 'Magnif' population. This map includes 61 RFLP and 2 SSR markers. The RFLP markers include 40 genomic probes and 21 cDNA probes. Their distribution among the seven barley chromosomes is presented in Table 7. The 63 polymorphic markers that were mapped for the 'Bowman' X 'Magnif' population cover a total distance of 931 cM with an average distance 15 cM covered by each marker (Table 7 and Figures 1-7). The distance covered for each chromosome in the 'Bowman' X 'Magnif' mapping population varied from 58 cM to 196 cM for the seven chromosomes.

Further research should be conducted with markers above the centromeric region of chromosome 3 in an attempt to identify linkage between markers and the *Rph*5 disease data in the 'Bowman' X 'Magnif' population. Additional research is warranted to identify markers which are within 5 to 10 cM of the *Rph*5 gene that could prove useful in marker-assisted selection for *Rph*5 in the Virginia breeding program and other programs

that are interested in quickly transferring the *Rph*5 resistance gene into barley cultivars with good agronomic traits.

The leaf rust resistance gene in VA 92-42-46 was identified as *Rph*5. Virulence to *Rph*5 has been reported to exist in Europe (Clifford, 1974). Brody and Rivadeneira (1996) identified 55 isolates from Ecuador and 72 isolates from Israel with virulence to 'Magnif' (*Rph*5.e formerly known as Pa 5) among 100 isolates tested from each country. Virulence to *Rph*5 has not been identified in the United States. This is likely due to the lack of deployment of *Rph*5 in the U.S. Combining the resistance gene *Rph*5 with other genes such as *Rph*14 or *Rph*15 should provide effective and more durable resistance than deployment of these genes individually. In the U.S., *Rph*5 could also be combined with genes such as *Rph*3 and *Rph*9, which have not been deployed, nor has virulence been widely reported. Using *Rph*5 in conjunction with adult-plant (quantitative) resistance as identified by Parlevliet and Kuiper (1977) could also prove effective in providing durable resistance to barley leaf rust. Marker-assisted selection would also facilitate the pyramiding of *Rph*5 with other leaf rust resistance genes.

Literature Cited

Biyashev, R.M., R.A. Ragab, P.J. Maughan, and M.A. Saghai Maroof. 1997. Molecular mapping, chromosomal assignment, and genetic diversity analysis of phytochrome loci in barley (*Hordeum vulgare*). Journal of Heredity 88:21-26.

Borovkova, I.G., Y. Jin, B.J.Steffenson, A. Kilian, T.K. Blake, and A. Kleinhofs. 1997. Identification and mapping of a leaf rust resistance gene in barley line Q21861. Genome 40(2):236-241.

Brodny, U. and M. Rivadeneira. 1966. Physiologic specialization of *Puccinia hordei* in Israel and Ecuador, 1992-1994. Canadian Journal of Plant Pathology 18:375-378.

Clifford, B.C. 1974. The choice of barley genotypes to differentiate races of *Puccinia hordei* Otth. Cereal Rusts Bulletin 2(1):5-6.

Franckowiak, J.D., Y. Jin, and B.J. Steffenson. 1997. Recommended allele symbols for leaf rust resistance genes in barley. Barley Genetics Newsletter 27:36-44.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley and Sons, New York, NY. pp. 464-467.

Graner, A., A. Jahoor, J. Schondelmaier, H. Siedler, K. Pillen, G. Fischbeck, G. Wenzel, and R.G. Herrmann. 1991. Construction of an RFLP map of barley. Theoretical and Applied Genetics 83:250-256.

Griffey, C.A., M.K. Das, and C.M. Waldenmaier. 1994. Yield losses in winter barley resulting from a new race of *Puccinia hordei* in North America. Plant Disease 78:256-260.

Heun, M., A.E. Kennedy, and J.A. Anderson. 1991. Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare)*. Genome 34:437-447.

Ivandic, V., U. Walther, and A. Graner. 1998. Molecular mapping of a new gene in wild barley conferring complete resistance to leaf rust (*Puccinia hordei* Otth). Theoretical and Applied Genetics. 97:1235-1239.

Jin, Y., G.H. Cui, B.J. Steffenson, and J.D. Franckowiak. 1996. New leaf rust resistance genes in barley and their allelic and linkage relationships with other *Rph* genes. Phytopathology 86:887-890.

Kasha, K.J., A. Kleinhofs, A. Kilian, M.A. Saghai Maroof, R.M. Biyashev, G. Scoles, P.M. Hayes, D. Hoffman, Dahleen, T.K. Blake, B. Rossnagel, B.J. Steffenson, P.L. Thomas, D.E. Falk, A. Laroche, W. Kim, S. Molnar, D. Mather. 1995. The North American barley genome map on the cross 'Harrington' X 'TR306' and its comparison to the map on cross 'Steptoe' X 'Morex'. pp. 73-88. *In* Tsunewaki, K. (ed) Plant genome and plastome: their structure and evolution. Kodansha Publishers Ltd, Tokyo, Japan.

Kleinhofs, A., A. Kilian, M.A. Saghai Maroof, R.M. Biyashev, P. Hayes, F.Q. Chen, N. Lapitan, A. Fenwick, T.K. Blake, V. Kanazin, E. Ananiev, L. Dahleen, D. Kudrna, J. Bollinger, S.J. Knapp, B. Liu, M. Sorrells, M. Heun, J.D. Franckowiak, D. Hoffman, R. Skadsen, and B.J. Steffenson. 1993. A molecular, isozyme and morphological map of barley (*Hordeum vulgare*) genome. Theoretical and Applied Genetics 86:705-712.

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, J.M. Daly, S.E. Lincoln, and L. Newberg. 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181.

Levine, M.N., and W.J. Cherewick. 1952. Studies on dwarf leaf rust of barley. U.S. Department of Agriculture Technical Bulletin. No. 1056, Washington D.C.

Linde-Laursen, I. and J. Jensen. 1992. Proposal for new designations of chromosome arms and positions of chromosomal markers. *In* Munck L. (ed) Barley geneticsVI, Vol 1. Munksgaard International Publishers Limited, Copenhagen, Denmark. pp. 287-289.

Liu, Z.W., R.M. Biyashev, and M.A. Saghai Maroof. 1996. Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theoretical and Applied Genetics 93:869-876.

Michelmore, R.W., I. Paran, and R.V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proceedings of the National Academy of Sciences, USA 88:9828-9832.

Nevo, E. 1992. Origin, evolution, population genetics and resources for breeding of wild barley *Hordeum spontaneum* in the fertile crescent. pp. 19-43. *In* Shewry P.R. (ed) Barley: genetics, biochemistry, molecular biology and biotechnology. CAB International Oxon, UK.

Parlevliet, J.E., and H.J. Kuiper. 1977. Resistance of some barley cultivars to leaf rust, *Puccinia hordei*; polygenic, partial resistance hidden by monogenic hypersensitivity. Netherlands Journal of Plant Pathology 83:85-89.

Qi, X., R.E. Niks, P. Stam, and P. Lindhout. 1998. Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley. Theoretical and Applied Genetics 96:1205-1215.

Saghai Maroof, M.A., R.M. Biyashev, G.P. Yang, Q. Zhang, and R.W. Allard. 1994. Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. Proceedings of the National Academy of Sciences, USA 91: 5466-5470.

Saghai Maroof, M.A., K.M. Soliman, R.A. Jorgensen, and R.W. Allard. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences, USA 81:8014-8018.

Sherman, J.D., A.L. Fenwick, D.M. Namuth, and N.L.V. Lapitan. 1995. A barley RFLP map: alignment of three barley maps and comparisons to Gramineae species. Theoretical and Applied Genetics 91:681-690.

Starling, T.M., C.W. Roane, and H.M. Camper. 1970. Registration of Hanover barley. Crop Science 10:456.

Steffenson, B.J., Y. Jin, and C.A. Griffey. 1993. Pathotypes of *Puccinia hordei* with virulence for the barley leaf rust resistance gene *Rph*7 in the United States. Plant Disease 77:867-869.

Tan, B.H. 1978. Verifying the genetic relationships between three leaf rust resistance genes in barley. Euphytica 27:317-323.

Yahyaoui, A.H. and E.L. Sharp. 1987. Virulence spectrum of *Puccinia hordei* in North Africa and the Middle East. Plant Disease 71:597-598.

Zillinsky, F.J. 1983. The International Maize and Wheat Improvement Center (CIMMYT). Common diseases of small grain cereals: a guide to identification. Mexico. pp. 11-16.

Table 1. Observed versus expected segregation of progeny from 'Moore' X VA 92-42-46, VA 92-42-46 X selected host-differentials, and 'Bowman' X 'Magnif' populations.

Cross	Generation Studied	Leaf Rust Race	Reaction Class				Ratio Tested	Chi-square Probability
			Res.	Seg.	Susc.	Total		
Moore (CI 7251) X VA 92-42-46								
Population 1	F_2	Race 30	42	0	49	91	7:9	0.66[a]
Population 1	F_2	Race 30	71	0	20	91	3:1	0.51[b]
Population 1	$F_{2:3}$	Race 30	22	49	20	91	1:2:1	0.73
Population 1	$F_{2:3}$	Race 8	21	49	18	88	1:2:1	0.51
Population 2	F_2	Race 8	166	0	41	207	3:1	0.14
Population 3	F_2	Race 8	215	0	67	282	3:1	0.65
VA 92-42-46								
x Estate (*Rph* 3.c)[c]	F_2	Race 8	287	0	12	299	15:1	0.12
x Aim (*Rph* 3)[c]	F_2	Race 8	237	0	21	258	15:1	0.21
x Triumph (*Rph* 9.z)[c]	F_2	Race 8	216	0	19	235	15:1	0.025
x Magnif (*Rph* 5.c)[c]	F_2	Race 8	299	0	0	299	No seg.	
Bowman (PI 483237)								
x Magnif (CI 13860)[d]	F_2	Race 8	70		23	93	3:1	0.77

[a] The p-value was based on infection types as scored in the preliminary study.

[b] The p-value was determined for the F_2 generation as verified by the $F_{2:3}$ generation in Appendix A.

[c] Non-pooled data is presented in Appendix F.

[d] Disease data for the 'Bowman' X 'Magnif' population was collected and provided by Dr. Brian Steffenson at North Dakota State University.

Table 2. Infection types of barley parents and host-differential lines to *Puccinia hordei* race 30 and race 8.

Line	Accession number	Resistance gene(s)	Infection type	
			Race 30[a]	Race 8[a]
Parents				
Moore[b]	CI 7251	none	34	4
VA92-42-46[b]		*Rph*5	;1^{-}N	0;
Bowman[c]	PI 483237	none	33^{-}	33^{-}
Magnif[c]	CIho13806	*Rph*5.e[d]	0;N	0;
Host-Differential lines				
Sudan	CI 6489	*Rph*1	34	4
Peruvian	CIho 935	*Rph*2.b	3^{+}	;1^{-}N
Aim	CIho 3737	*Rph*3	0;N	0;N
Estate	PI 57700	*Rph*3.c	0;	0;
Gold	CIho 1145	*Rph*4.d	34	33^{+}
Bolivia	PI 36360	*Rph*6.f + *Rph*2.r	3^{+}	;1^{-}N
Cebada Capa	PI 53911	*Rph*7.g	3	;1^{-}
Egypt 4	CIho 6481	*Rph*8.h	4	23C
Hor 2596	CIho 1243	*Rph*9.i	;12C	1^{+}N
Triumph	PI 268180	*Rph*9.z	;1CN	;N
Clipper BC8	T39-3	Rph10.o	2^{+}	3
Clipper BC67	T38-26	*Rph*11.p	3	;12^{-}
Berac*3/HS2986	PI 531849	*Rph*13.x	3	0;
PI 531901-1	PI 584760	*Rph*14.ab	2^{+}C	;1^{+}CN
Bowman*4/PI 3555447	PI 355447	*Rph*15.ad	0;N	0;N

[a] Infection type as described by Levine and Cherewick (1952) where 0, 1 and 2 are resistant and 3-4 are susceptible. The symbols + and – denote more or less sporolation, respectively, N=necrosis, and C=chlorosis.

[b] Parents used to develop the Virginia mapping population.

[c] Parents used to develop the mapping population from NDSU.

[d] Allele symbols proposed by Franckowiak et al., 1997.

Table 3. Summary of polymorphic RFLP clones and SSR primer pairs out of 133 RFLP clones and 31 SSR primer pairs screened for the 'Moore' X VA 92-42-46 and 'Bowman' X 'Magnif' populations.

	RFLPs	Percent of Total RFLPs	SSRs	Percent of Total SSRs
Polymorphic	85	63.9	16	51.6
Moore X VA 92-42-46	61	45.9	11	35.5
Bowman X Magnif	62	46.6	11	35.5
In both populations	41	30.8	7	22.6

Table 4. Number of RFLP clones and SSR primer pairs screened and level of polymorphism for each barley chromosome for the 'Moore' X VA 92-42-46 mapping population.

Chromosome	Number of RFLPs	Number of Polymorphic RFLPs	Number of SSRs	Number of Polymorphic SSRs
1(7H)	19	11	6	3
2(2H)	21	6	10	2
3(3H)	21	9	4	2
4(4H)	19	9	8	2
5(1H)	16	9	2	1
6(6H)	20	8	1	1
7(5H)	33	18	3	1

Table 5. Number of RFLP clones and SSR primer pairs screened and level of polymorphism for each barley chromosome for the 'Bowman' X 'Magnif' mapping population.

Chromosome	Number of RFLPs	Number of Polymorphic RFLPs	Number of SSRs	Number of Polymorphic SSRs
1(7H)	19	9	6	1
2(2H)	21	12	10	5
3(3H)	21	9	4	0
4(4H)	19	7	8	1
5(1H)	16	8	2	2
6(6H)	20	8	1	1
7(5H)	33	17	3	2

Table 6. Distribution of markers and map distance covered on the seven barley chromosomes in the 'Moore' X VA 92-42-46 mapping population.

Chromosome	cM	Markers			
		Genomic	cDNA	SSRs	Total
1(7H)	123	3	2	1	6
2(2H)	127	5	1	0	6
3(3H)	114	4	1	1	6
4(4H)	95	7	2	0	9
5(1H)	146	7	3	0	10
6(6H)	113	7	0	0	7
7(5H)	115	7	6	1	14
Total	833	40	15	3	58

Table 7. Distribution of markers and map distance in cM covered on the seven barley chromosomes in the 'Bowman' X 'Magnif' mapping population.

Chromosome	cM	Markers			
		Genomic	cDNA	SSRs	Total
1(7H)	142	5	3	0	8
2(2H)	138	12	3	0	15
3(3H)	110	4	1	0	5
4(4H)	196	5	2	0	7
5(1H)	93	5	2	0	7
6(6H)	58	4	3	0	7
7(5H)	194	5	7	2	14
Total	931	40	21	2	63

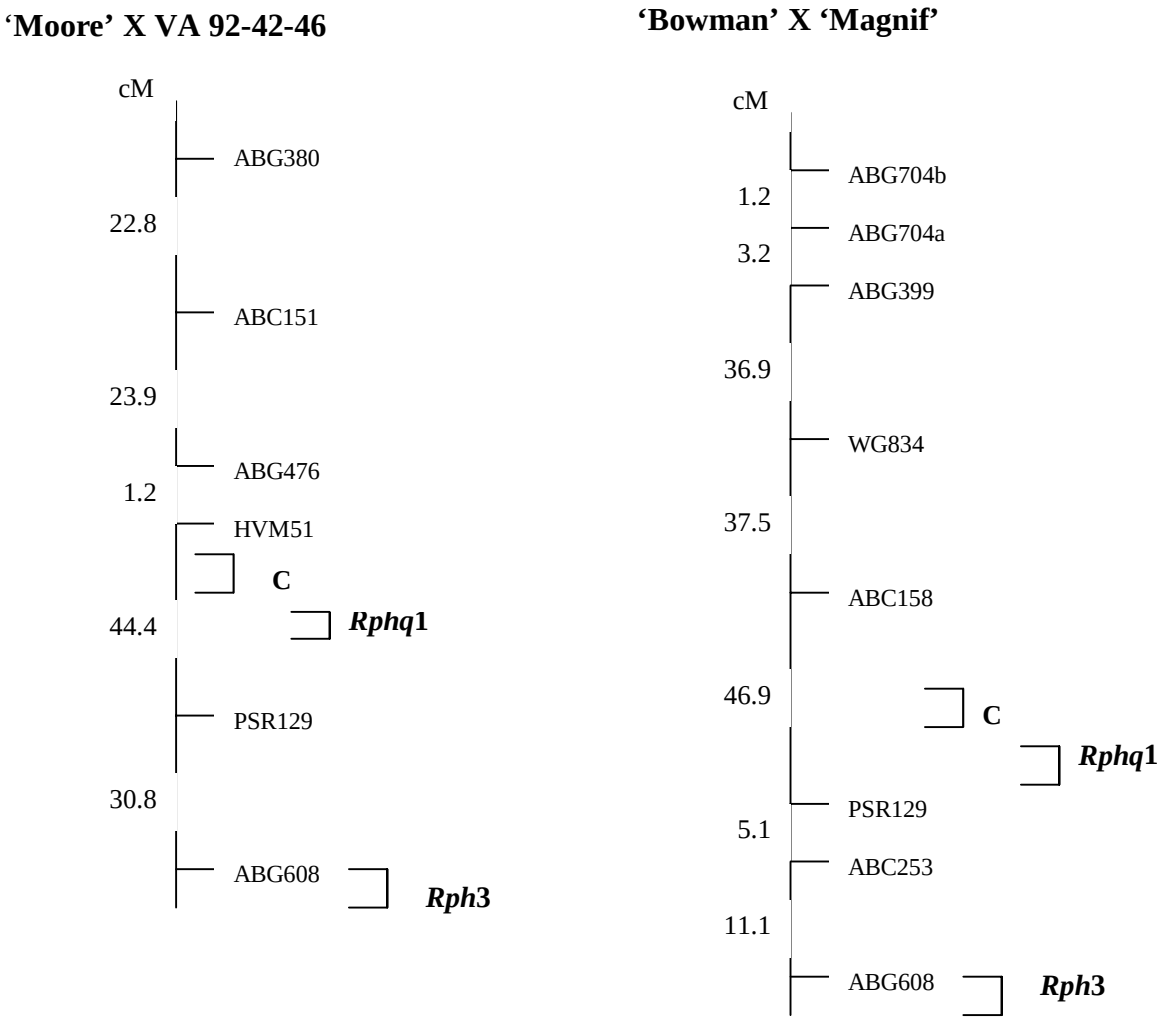

Figure 1. Chromosome 1 (7H) linkage maps of two barley mapping populations. The approximate location of *Rph*3 and *Rphq*1 are shown as reported by Qi et al. (1998).

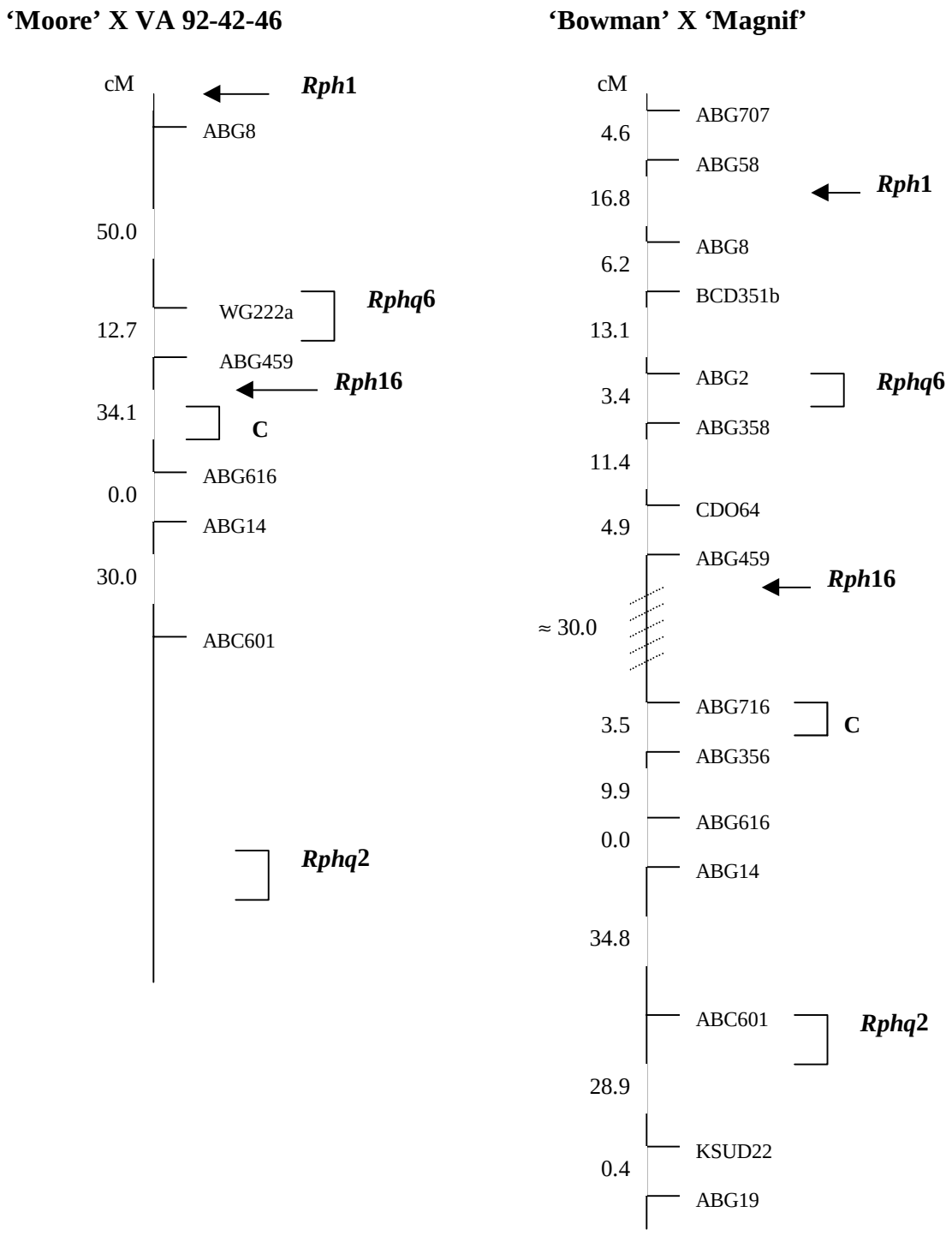

Figure 2. Chromosome 2 (2H) linkage maps of two barley mapping populations. The dashed lines represent a gap between the two linkage groups in the 'Bowman' X 'Magnif' population. The estimated distance between the two linkage groups was obtained by comparing maps by Kasha et al. (1995) and Kleinhofs et al. (1993).

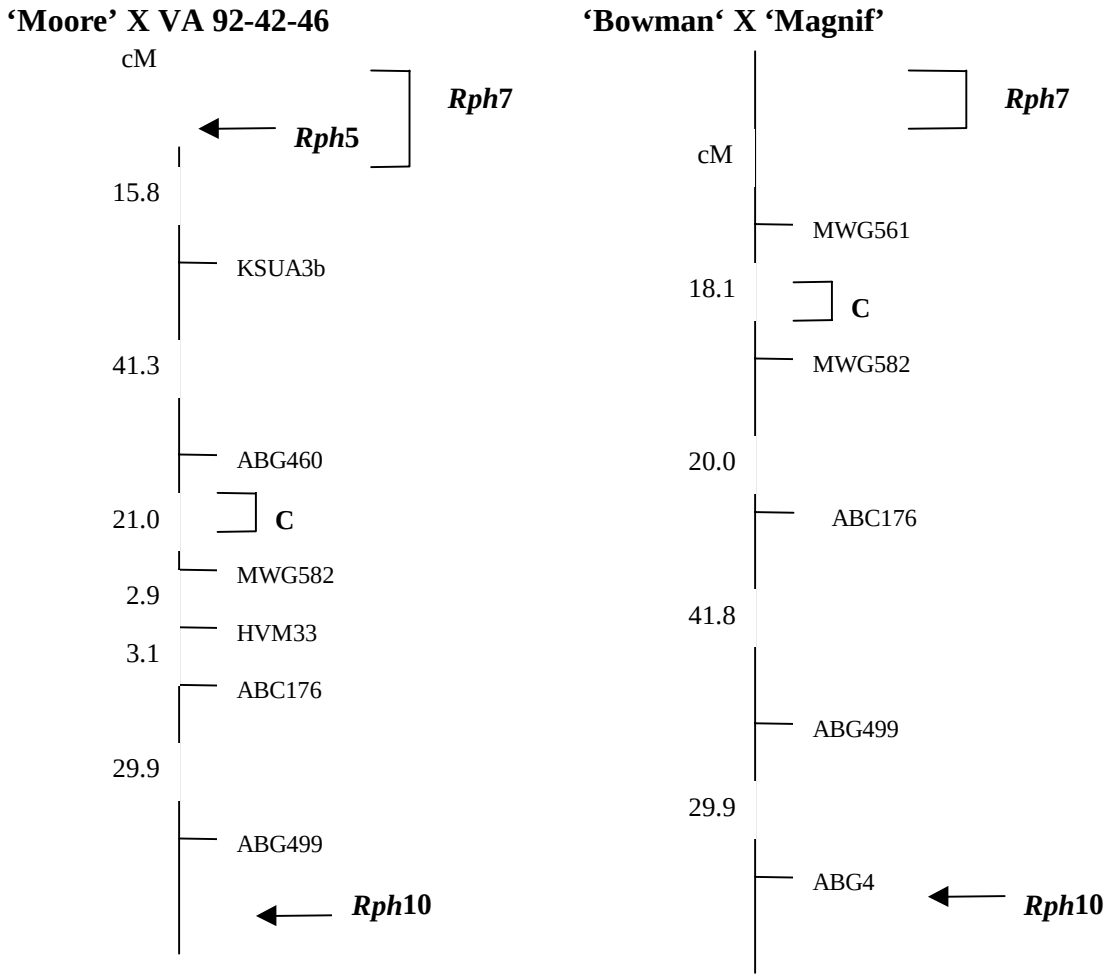

Figure 3. Chromosome 3 (3H) linkage maps of two barley mapping populations. *Rph*5 was mapped above the centromeric region in the 'Moore' X VA 92-42-46 mapping population.

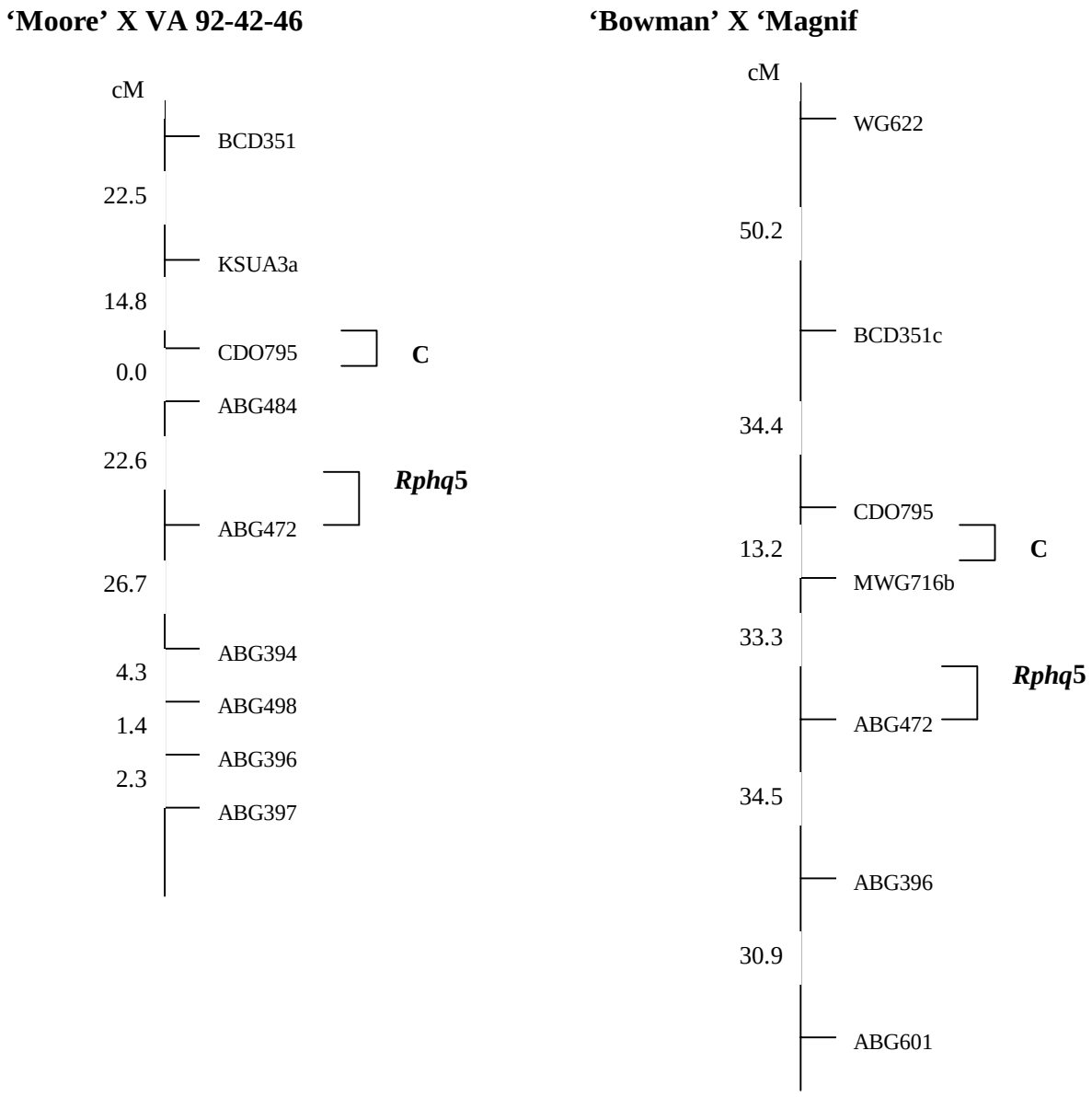

Figure 4. Chromosome 4 (4H) linkage maps of two barley mapping populations. The approximate location of *Rphq*5 is shown as reported by Qi et al. (1998).

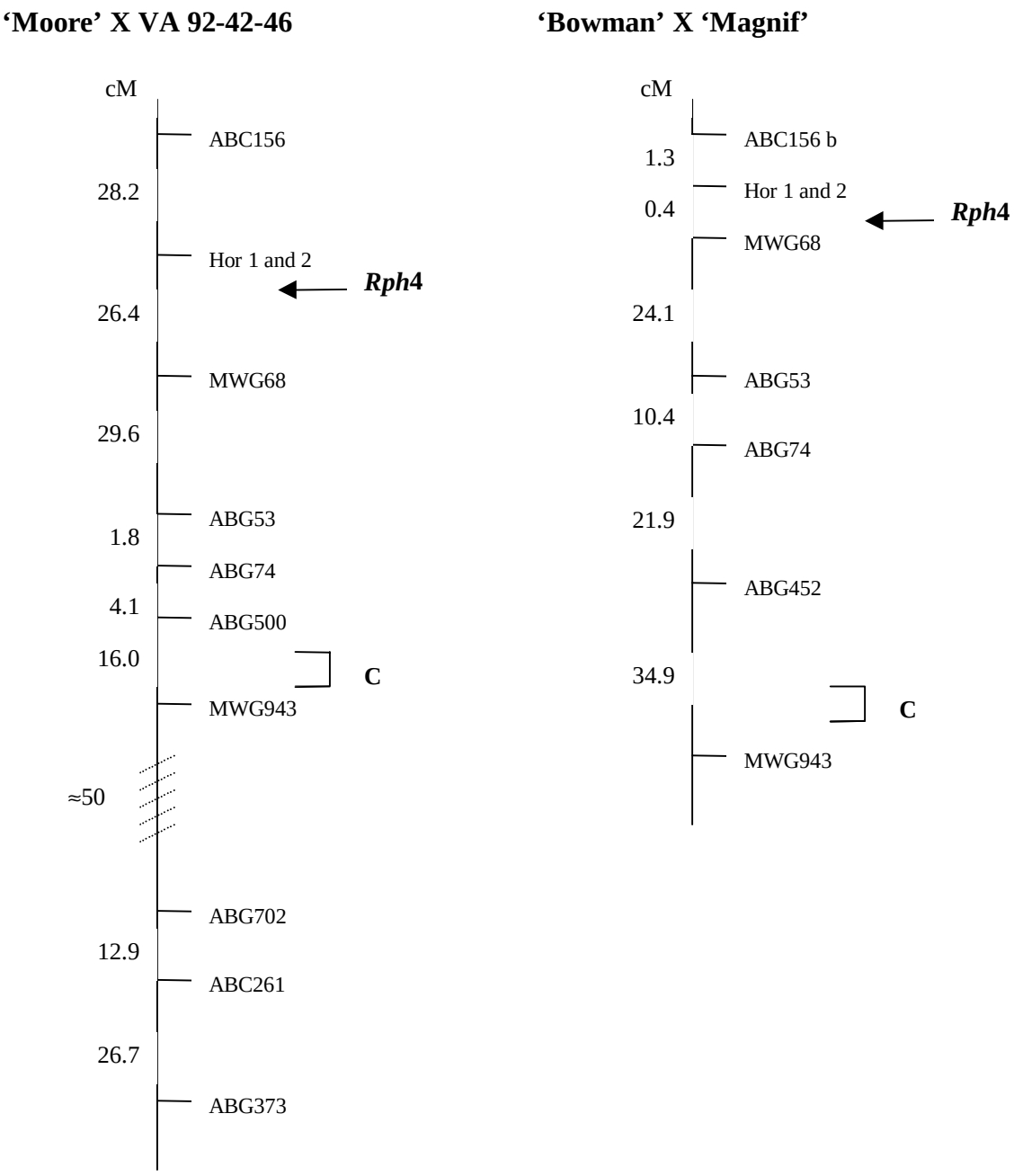

Figure 5. Chromosome 5 (1H) linkage maps of two barley mapping populations. The dashed lines represent a gap between the two linkage groups in the 'Moore' X VA 92-42-46 mapping population. The estimated distance was obtained by comparing published maps by Kasha et al. (1995) and Kleinhofs et al. (1993).

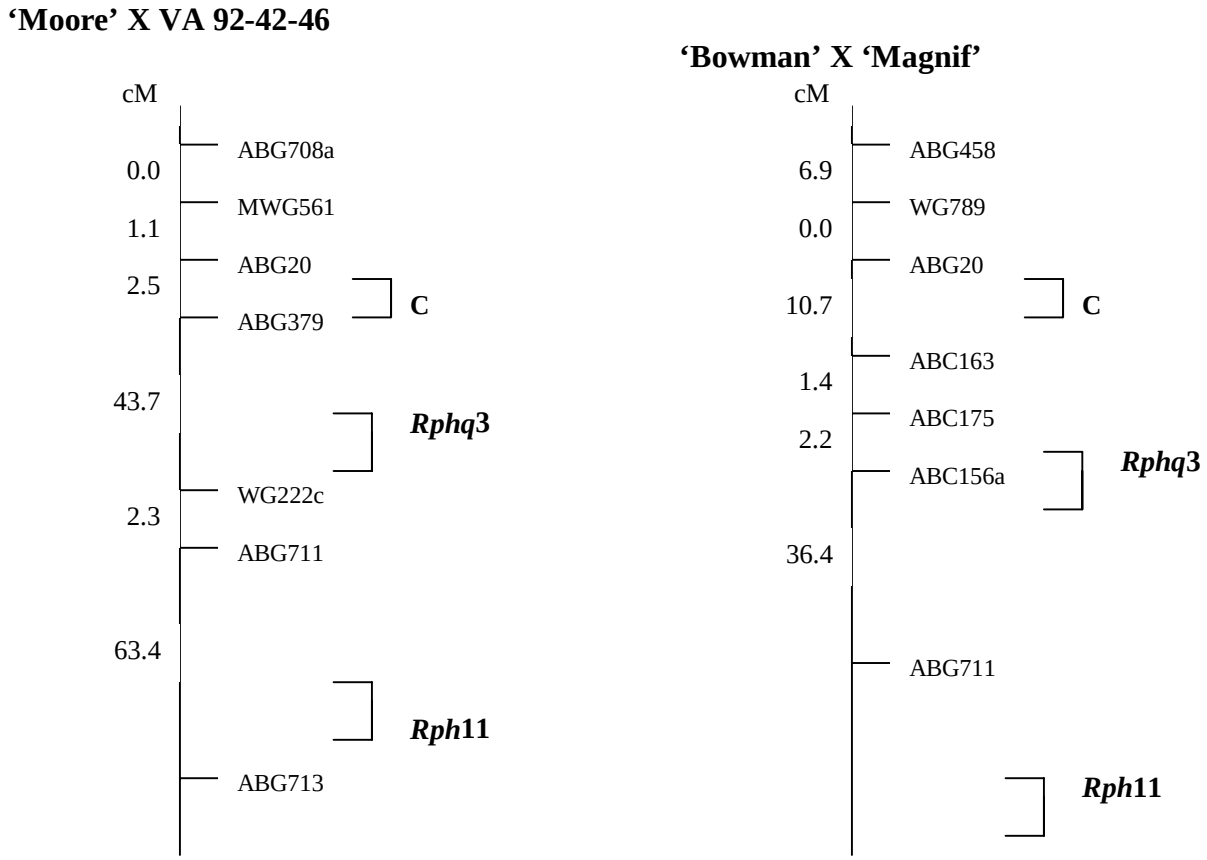

Figure 6. Chromosome 6 (6H) linkage maps of two barley mapping populations. The approximate locations of *Rphq*3 and *Rph*11 are shown by Qi et al. (1998).

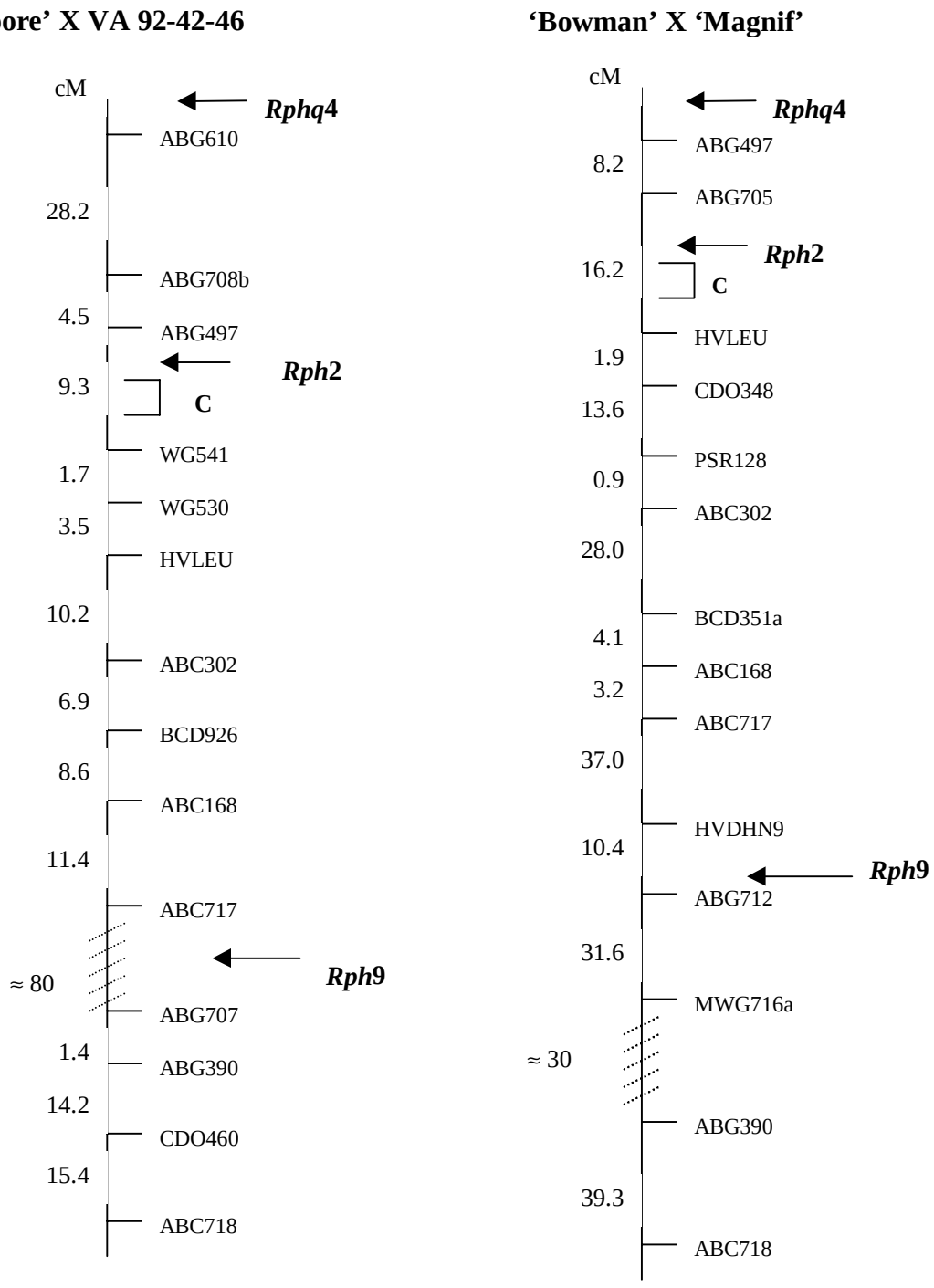

Figure 7. Chromosome 7 (5H) linkage maps of two barley mapping populations. *Rph*2 was mapped to the centromeric region and *Rph*9 was mapped to the minus arm by Borovkova et al. (1997). The dashed lines represent a gap between linkage groups. The estimated distance between the linkage group was obtained by comparing maps by Kasha et al. (1995) and Kleinhofs et al. (1993). *Rphq*4 is mapped to the teliomeric region of the plus arm as reported by Qi et al. (1998).

Appendix A. 'Moore' X VA 92-42-46 barley mapping population (Population 1) F_2 reaction to leaf rust race 30 and $F_{2:3}$ reaction to race 30 and race 8 of *Puccinia hordei*.

ID Number	Race 30 F_2 IT[a]	Race 30 F_2 Rxn type[b]	Race 30 $F_{2:3}$ Rxn type[c]	Race 8 $F_{2:3}$ Rxn type[c]
326-1	3	S	HS	HS
326-2[d]	3C	S	Seg	Seg
326-3	;1=N	R	HR	HR
326-5	3	S	HS	HS
326-6	12C	R	Seg	Seg
326-7	;N	R	HR	HR
326-9	23C	R	Seg[e]	Seg
326-10	;N	R	HR	HR
326-11	;N	R	HR	HR
326-12	;N	R	HR	HR
326-13[d]	3	S	Seg	Seg
326-14	;N	R	HR	HR
326-15	23C	R	Seg	Seg
326-16	3	S	HS	HS
326-17	23C	R	Seg	Seg
326-18	3	S	HS	HS
326-19	3	S	HS	No seed[f]
326-20	;N	R	HR	No seed[f]
326-21	3	S	HS	No seed[f]
326-24	;N	R	HR	HR
326-25[d]	3	S	Seg	Seg
326-26[d]	3	S	Seg	Seg
326-27[d]	3	S	Seg	Seg
326-28	12C	R	Seg	Seg
326-29	2C	R	Seg	Seg
326-30	12C	R	Seg[e]	Seg
326-31	2C	R	Seg	Seg
326-32	3	S	HS	HS

ID Number	Race 30 F_2 IT[a]	Race 30 F_2 Rxn type[b]	Race 30 $F_{2:3}$ Rxn type[c]	Race 8 $F_{2:3}$ Rxn type[c]
326-33	23C	R	Seg	Seg
326-34[d]	3C	S	Seg	Seg
326-36	3	S	HS	HS
326-37	;N	R	HR	HR
326-38	23C	R	Seg	Seg
326-39	3	S	HS	HS
326-40	;N	R	HR	HR
326-41[d]	3C	S	Seg	Seg
326-42	23C	R	Seg	Seg
326-43	;N	R	HR	HR
326-44	23C	R	Seg	Seg
326-45	23C	R	Seg[e]	Seg
326-46	3	S	HS	HS
326-47	12C	R	HR	HR
326-48	23C	R	Seg	Seg
326-49[d]	3C	S	Seg[e]	Seg
326-50	;N	R	HR	HR
326-51	;N	R	HR	HR
326-52	3	S	HS	HS
326-53[d]	3C	S	Seg	Seg
326-54	3	S	HS	HS
326-55[d]	3C	S	Seg	Seg
326-56[d]	3	S	Seg[e]	Seg
326-57	2C	R	Seg	Seg
326-58[d]	3C	S	Seg[e]	Seg
326-59[d]	3C	S	Seg	Seg[e]
326-60[d]	3C	S	Seg[e]	Seg
326-62[d]	3C	S	Seg	Seg
326-63	3	S	HS	HS
326-64	;N	R	HR	HR

ID Number	Race 30 F_2 IT[a]	Race 30 F_2 Rxn type[b]	Race 30 $F_{2:3}$ Rxn type[c]	Race 8 $F_{2:3}$ Rxn type[c]
326-66[d]	3C	S	Seg	Seg
326-67	3	S	HS	HS
326-68[d]	3C	S	Seg	Seg
326-69[d]	3C	S	Seg	Seg
326-70	23C	R	Seg	Seg
326-72	23C	R	Seg	Seg
326-73	;N	R	HR	HR
326-74	;N	R	HR	HR
326-75	;N	R	HR	HR
326-76[d]	3C	S	Seg	Seg
326-77	;N	R	HR	HR
326-78[d]	3C	S	Seg	Seg
326-79	3	S	HS	HS
326-80[d]	3C	S	Seg	Seg
326-81	3	S	HS	HS
326-82[d]	3C	S	Seg	Seg
326-83	3	S	HS	HS
326-84	3	S	HS	HS
326-85[d]	3C	S	Seg	Seg
326-87	;N	R	HR	HR
326-88	3	S	HS	HS
326-89	2C	R	Seg[e]	Seg
326-90[d]	3	S	Seg[e]	Seg
326-91	23C	R	Seg	Seg
326-92	;N	R	HR	HR
326-93[d]	3C	S	Seg	Seg
326-94[d]	3	S	Seg	Seg
326-95[d]	3	S	Seg	Seg
326-96	;N	R	HR	HR
326-97[d]	3C	S	Seg	Seg

ID Number	Race 30 F_2 IT[a]	Race 30 F_2 Rxn type[b]	Race 30 $F_{2:3}$ Rxn type[c]	Race 8 $F_{2:3}$ Rxn type[c]
326-98	2C	R	Seg	Seg
326-99[d]	3	S	Seg	Seg
326-100	3	S	HS	HS

[a] F_2 infection type (IT) as described by Levine and Cherewick (1952) where 0, 1, and 2 are resistant and 3-4 are susceptible. The symbols + and – denote more or less sporolation, respectively, N=necrosis, and C=chlorosis.

[b] F_2 reaction type where S=susceptible and R=resistant.

[c] $F_{2:3}$ reaction type where HR=homozygous resistant, Seg=segregating resistant and susceptible plants, and HS=homozygous susceptible.

[d] Reaction type and infection type were misclassified in the F_2 generation as verified in the $F_{2:3}$ generation to leaf rust race 8 and/or race 30.

[e] Families in the $F_{2:3}$ generation that did not fit the expected 3:1 genetic ratio.

[f] No seed was available for disease screening.

Appendix B. Chi-square analyses of F_2 segregation patterns from a preliminary genetics study of resistance to leaf rust race 30 in VA 92-42-46 barley[a].

F_2 Population	Resistant		Susceptible	Ratio Tested	Chi-square	p-value
	0;N	23c	3-c / 3			
'Moore' X VA 92-42-46	**166**		**232**	**7:9**	**0.67**	**0.3-0.5**
	61	68	169	3:4:9	1.05	0.3-0.5
'Estate' X VA 92-42-46 (*Rph* 3)	142		17	55:9	1.50	0.2-0.3
'Aim' X VA 92-42-46	91		9	55:9	2.12	0.1-0.2
(*Rph* 3)	88		8	55:9	2.61	0.1-0.2
'Hor 2596' X VA 92-42-46 (*Rph* 9)	66		8	55:9	1.03	0.3-0.5
'Triumph' X VA 92-42-46 (*Rph* 12)	176		23	55:9	14.12	<0.001
'Magnif' X VA 92-42-46	95		0	55:9		
(*Rph* 5)	99		1			

[a] Research performed by Dr. C. A. Griffey and A. M. Price at VPI & SU.

Appendix C. 'Moore' X VA 92-42-46 (Population 1) barley leaf rust genetics study using race 30.

F3 Family	F2 Rxn	F_3 Reaction Type				Total #	Chi-square 3:1 ratio
		# R	# MR	# MS[a]	# S		
326-1	3				50	50	
326-2	3C	7	5	19	16	47	2.04
326-3	;1⁻N	48				48	
326-4	12C	14	11	12	12	49	0.01
326-5	3			2	46	48	
326-6	12C	10	5	18	16	49	1.53
326-7	;N	50				50	
326-8	23C	13	4	14	12	43	0.19
326-9	23C	11	7	9	20	47	7.72
326-10	;N	49				49	
326-11	;N	49				49	
326-12	;N	50				50	
326-13	3	15	1	22	8	46	1.42
326-14	;N	51				51	
326-15	23C	15		28	7	50	3.23
326-16	3				50	50	
326-17	23C	16	5	17	10	48	0.44
326-18	3			12	37	49	
326-19	3				32	32	
326-20	;N	27				27	
326-21	3			1	37	38	
326-22	3	9	14	16	6	45	3.27
326-23	3					no seed	
326-24	;N	50				50	
326-25	3	8		26	14	48	0.44
326-26	3	12	2	20	13	47	0.18
326-27	3	14	2	16	10	42	0.03
326-28	12C	12	4	21	13	50	0.03
326-29	2C	12	2	25	8	47	1.60
326-30	12C	15	11	17	5	48	5.44
326-31	2C	15	1	23	10	49	0.55
326-32	3				49	49	
326-33	23C	11	7	15	15	48	1.00

F3 Family	F2 Rxn	F_3 Reaction Type				Total #	Chi-square 3:1 ratio
		# R	# MR	# MS[a]	# S		
326-34	3C	10	3	23	14	50	0.24
326-35	3C					no seed	
326-36	3				49	49	
326-37	;N	49				49	
326-38	23C	8	7	21	11	47	0.06
326-39	3				50	50	
326-40	;N	50				50	
326-41	3C	12	1	20	14	47	0.57
326-42	23C	18	1	17	8	44	1.09
326-43	;N	51				51	
326-44	23C	21	5	15	6	47	3.75
326-45	23C	12	25	6	6	49	4.25
326-46	3				49	49	
326-47	12C	43	6			49	
326-48	23C	11	9	19	10	49	0.55
326-49	3C	13	21	13	1	48	13.44
326-50	;N	49				49	
326-51	;N	48				48	
326-52	3			20	27	47	
326-53	3C	11	16	10	12	49	0.01
326-54	3			17	32	49	
326-55	3C	11	15	15	8	49	1.97
326-56	3	5	9	14	19	47	5.96
326-57	2C	15	2	16	17	50	2.16
326-58	3C	5	3	13	22	43	15.69
326-59	3C	8	6	18	16	48	1.78
326-60	3C	7	5	17	19	48	5.44
326-61	NA					no seed	
326-62	3C	12	7	17	13	49	0.061
326-63	3				45	45	
326-64	;N	44				44	
326-65	3				33	33	
326-66	3C	10	5	21	14	50	0.24
326-67	3				50	50	
326-68	3C	13	4	21	10	48	0.44
326-69	3C	16		15	16	47	2.05

F3 Family	F2 Rxn	F_3 Reaction Type				Total #	Chi-square 3:1 ratio
		# R	# MR	# MS[a]	# S		
326-70	23C	12		16	16	44	3.03
326-71	3				51	51	
326-72	23C	12	1	25	9	47	0.86
326-73	;N	48				48	
326-74	;N	49				49	
326-75	;N	50				50	
326-76	3C	18		21	10	49	0.55
326-77	;N	51				51	
326-78	3C	14	2	19	15	50	0.67
326-79	3				50	50	
326-80	3C	11	2	26	9	48	1.00
326-81	3				47	47	
326-82	3C	12	5	17	15	49	0.82
326-83	3				50	50	
326-84	3				49	49	
326-85	3C	9	1	24	17	51	1.89
326-86	NA					no seed	
326-87	;N	46				46	
326-88	3				40	40	
326-89	2C	12	6	12	20	50	6.00
326-90	3	8	1	13	26	48	21.77
326-91	23C	11	1	24	11	47	0.06
326-92	;N	49				49	
326-93	3C	15	4	21	10	50	0.67
326-94	3	13	1	25	9	48	1.00
326-95	3	9		22	16	47	2.05
326-96	;N	46				46	
326-97	3C	11	2	24	13	50	0.03
326-98	2C	12		33	9	54	2.00
326-99	3	9		29	9	47	0.86
326-100	3				50	50	

[a] For chi-square analysis, the resistant class consisted of resistant, moderately resistant, and moderately susceptible reaction types (0-23); the susceptible class consisted of reaction types 3-4.

Appendix D. 'Moore' X VA 92-42-46 (Population 1) barley leaf rust genetics study using race 8.

F3 Family	F2 Rxn R30	F_3 Reaction Type (Race 8)				Total #	Chi-square 3:1 ratio
		# R	# MR	# MS	# S		
326-1	3				50	50	
326-2	3C	39			11	50	0.24
326-3	;1=N	50				50	
326-4	12C	39			10	49	0.55
326-5	3				49	49	
326-6	12C	35			15	50	0.67
326-7	;N	50				50	
326-8	23C					0	
326-9	23C	19			10	29	1.39
326-10	;N	50				50	
326-11	;N	48				48	
326-12	;N	50				50	
326-13	3	39			8	47	1.60
326-14	;N	50				50	
326-15	23C	31			9	40	0.13
326-16	3				49	49	
326-17	23C	33			13	46	0.26
326-18	3				50	50	
326-19	3					0	
326-20	;N					0	
326-21	3					0	
326-22	3	37			12	49	0.01
326-23	3					no seed	
326-24	;N	50				50	
326-25	3	27			6	33	0.82
326-26	3	41			9	50	1.31
326-27	3	36			10	46	0.26
326-28	12C	39			10	49	0.55
326-29	2C	34			15	49	0.82
326-30	12C	36			11	47	0.06
326-31	2C	38			12	50	0.03
326-32	3				48	48	
326-33	23C	36			13	49	0.06

F3 Family	F2 Rxn R30	F_3 Reaction Type (Race 8)				Total #	Chi-square 3:1 ratio
		# R	# MR	# MS	# S		
326-34	3C	32			18	50	3.23
326-35	3C					no seed	
326-36	3				50	50	
326-37	;N	42				42	
326-38	23C	35			15	50	0.67
326-39	3				49	49	
326-40	;N	50				50	
326-41	3C	39			10	49	0.55
326-42	23C	37			13	50	0.03
326-43	;N	50				50	
326-44	23C	42			8	50	2.16
326-45	23C	39			11	50	0.24
326-46	3				50	50	
326-47	12C	43				43	
326-48	23C	35			13	48	0.11
326-49	3C	38			10	48	0.44
326-50	;N	50				50	
326-51	;N	46				46	
326-52	3				50	50	
326-53	3C	39			11	50	0.24
326-54	3				49	49	
326-55	3C	38			12	50	0.03
326-56	3	35			11	46	0.03
326-57	2C	35			14	49	0.17
326-58	3C	35			12	47	0.01
326-59	3C	28			22	50	9.63
326-60	3C	42			7	49	3.00
326-61	NA					no seed	
326-62	3C	37			13	50	0.03
326-63	3				41	41	
326-64	;N	41				41	
326-65	3				1	1	
326-66	3C	37			14	51	0.16
326-67	3				50	50	
326-68	3C	34			15	49	0.82
326-69	3C	33			17	50	2.16

F3 Family	F2 Rxn R30	F_3 Reaction Type (Race 8)				Total #	Chi-square 3:1 ratio
		# R	# MR	# MS	# S		
326-70	23C	37			8	45	1.38
326-71	3				36	36	
326-72	23C	31			18	49	3.60
326-73	;N	15				15	
326-74	;N	46				46	
326-75	;N	50				50	
326-76	3C	15			5	20	0.00
326-77	;N	50				50	
326-78	3C	43			7	50	3.23
326-79	3				49	49	
326-80	3C	33			16	49	1.53
326-81	3				49	49	
326-82	3C	33			15	48	1.00
326-83	3				49	49	
326-84	3				48	48	
326-85	3C	40			10	50	0.67
326-86	NA					no seed	
326-87	;N	46				46	
326-88	3				48	48	
326-89	2C	35			16	51	1.10
326-90	3	35			14	49	0.33
326-91	23C	35			11	46	0.03
326-92	;N	50				50	
326-93	3C	41			10	51	0.79
326-94	3	37			12	49	0.01
326-95	3	35			14	49	0.33
326-96	;N	46				46	
326-97	3C	41			9	50	1.31
326-98	2C	38			12	50	0.03
326-99	3	40			9	49	1.15
326-100	3				44	44	

Appendix E. Barley leaf rust genetics study of 'Moore' X VA 92-42-46 and host-differentials X VA 92-42-46 F_2 populations using race 8.

Diff X VA 92-42-46 & Moore X VA 92-42-46	F_2 Reaction Type[a]			Ratio Tested	Chi-sq.[b]	p-value[b]
	#R	#S	#Total			
Population 2 9621-1 (Moore X VA)	52	12	64	(3:1)	2.30	0.14
Population 2 9621-3 (Moore X VA)	59	16	75	(3:1)		
Population 2 9621-4 (Moore X VA)	55	13	68	(3:1)		
Population 3 9622-2 (Moore X VA)	66	21	87	(3:1)	0.23	0.65
Population 3 9622-3 (Moore X VA)	75	23	98	(3:1)		
Population 3 9622-4 (Moore X VA)	74	23	97	(3:1)		
9327-1 (Estate X VA)	97	3	100	(15:1)	2.55	0.12
9627-2 (Estate X VA)	95	5	100	(15:1)		
9627-3 (Estate X VA)	95	4	99	(15:1)		
9630-1 (Aim X VA)	64	5	69	(15:1)	1.57	0.21
9630-2 (Aim X VA)	86	6	92	(15:1)		
9630-3 (Aim X VA)	87	10	97	(15:1)		
9633-1 (Magnif X VA)	99	0	99	no seg. Rph5		
9633-2 (Magnif X VA)	100	0	100			
9633-3 (Magnif X VA)	100	0	100			
9638-1 (Trph X VA)	78	4	82	(15:1)	1.35	0.25
9638-2 (Trph X VA)	70	8	78	(15:1)		
9638-3 (Trph X VA)	68	7	75	(15:1)		

[a] F_2 infection type (IT) as described by Levine and Cherewick (1952) where 0, 1, and 2 are resistant and 3-4 are susceptible. The symbols + and – denote more or less sporolation, respectively, N=necrosis, and C=chlorosis.

[b] Chi-square and p-values were determined using polled data for each cross.

Appendix F. 'Bowman' X 'Magnif' F_2 and $F_{2:3}$ infection types to *Puccinia hordei* race 8.

Line	F_2 Infection Type (IT)	$F_{2:3}$ Infection Type (IT)
BowMag1	33^+	$3^-3/23^-$
BowMag2	33^+	$3^-3/0$ (Escape)
BowMag3	33^+	33^-
BowMag4	33^+	3^-3
BowMag5	33^+	3^-3
BowMag6	33^+	33^-
BowMag7	33^+	33^-
BowMag8	33^+	3^-3
BowMag9	33^+	33^-
BowMag10	33^+	3^-2
BowMag11	33^+	3^-3
BowMag12	33^+	3^-3
BowMag13	33^+	3^-3
BowMag14	33^+	33^-
BowMag15	33^+	3^-3
BowMag16	33^+	3^-3
BowMag17	33^+	3^-3
BowMag18	33^+	3^-3
BowMag19	33^+	33^-
BowMag20	33^+	3^-3
BowMag21	33^+	33^-
BowMag22	33^+	3^-3
BowMag23	$0;1^-$	0;1N
BowMag24	$0;1^-$	0;
BowMag25	$0;1^-$	0;1N
BowMag26	$0;1^-$	0;N

Line	F_2 Infection Type (IT)	$F_{2:3}$ Infection Type (IT)
BowMag27	0;1^{-}	0;N
BowMag28	0;1^{-}	0;
BowMag29	0;1^{-}	0;1N
BowMag30	0;1^{-}	0;1N
BowMag31	0;1^{-}	0;N
BowMag32	0;1^{-}	0;N
BowMag33	0;1^{-}	0;1
BowMag34	0;1^{-}	0;N
BowMag35	0;1^{-}	0;N
BowMag36	0;1^{-}	0;N
BowMag37	0;1^{-}	0;N
BowMag38	0;1^{-}	0;N
BowMag39	0;1^{-}	0;1N
BowMag40	0;1^{-}	0;N
BowMag41	0;1^{-}	0;N
BowMag42	0;1^{-}	0;N
BowMag43	0;1^{-}	0;N
BowMag44	0;1^{-}	0;N
BowMag45	0;1^{-}	0;N
BowMag46	0;1^{-}	0;1N
BowMag47	0;1^{-}2C	0;/21NC/33^{-}
BowMag48	0;1^{-}2C	0;/21NC/33^{-}
BowMag49	0;1^{-}2C	0;/21NC/33^{-}
BowMag50	0;1^{-}2C	0;/21NC/33^{-}
BowMag51	0;1^{-}2C	0;/21NC/33^{-}
BowMag52	0;1^{-}2C	0;/21NC/33^{-}
BowMag53	0;1^{-}2C	0;/21NC/33^{-}
BowMag54	0;1^{-}2C	0;/21NC/33^{-}
BowMag55	0;1^{-}2C	0;/21NC/33^{-}
BowMag56	0;1^{-}2C	0;/21NC/33^{-}

Line	F_2 Infection Type (IT)	$F_{2:3}$ Infection Type (IT)
BowMag57	0;1-2C	0;/21NC/33-
BowMag58	0;1-2C	0;/21NC/33-
BowMag59	0;1-2C	0;/21NC/33-
BowMag60	0;1-2C	0;/21NC/33-
BowMag61	0;1-2C	0;/21NC/3-3
BowMag62	0;1-2C	0;/21NC/33-
BowMag63	0;1-2C	0;/21NC/33-
BowMag64	0;1-2C	0;/21NC/33-
BowMag65	0;1-2C	0;/21NC/33-
BowMag66	0;1-2C	0;/21NC/33-
BowMag67	0;1-2C	0;/21NC/33-
BowMag68	0;1-2C	0;/21NC/3-3
BowMag69	0;1-2C	0;/21NC/3-3
BowMag70	0;1-2C	0;/21NC/33-
BowMag71	0;1-2C	0;/21NC/3-3
BowMag72	0;1-2C	0;/21NC/3-3
BowMag73	0;1-2C	0;/21NC/33-
BowMag74	0;1-2C	0;/21NC/33-
BowMag75	0;1-2C	0;/21NC/3-3
BowMag76	0;1-2C	0;/21NC/33-
BowMag77	0;1-2C	0;/21NC/33-
BowMag78	0;1-2C	0;/21NC/33-
BowMag79	0;1-2C	0;/21NC/33-
BowMag80	0;1-2C	0;/21NC/33-
BowMag81	0;1-2C	0;/21NC/33-
BowMag82	0;1-2C	0;/21NC/33-
BowMag83	0;1-2C	0;/21NC/3-3
BowMag84	0;1-2C	0;/21NC/33-
BowMag85	0;1-2C	0;/21NC/33-
BowMag86	0;1-2C	0;/21NC/33-

Line	F_2 Infection Type (IT)	$F_{2:3}$ Infection Type (IT)
BowMag87	0;1-2C	0;/21NC/33-
BowMag88	0;1-2C	0;/21NC/33-
BowMag89	0;1-2C	0;/21NC/33-
BowMag90	0;1-2C	0;/21NC/33-
BowMag91	0;1-2C	0;/21NC/33-
BowMag92	0;1-2C	0;/21NC/33-
BowMag93	0;1-2C	0;/21NC/33-

[a] Research performed by B.J. Steffenson at NDSU

Vita

John Zwonitzer was born on May 11, 1973, in Horton, Kansas to Jim and Sharon Zwonitzer. His entire secondary education was undertaken in Atchison County, KS. He graduated from Atchison County Community High School in the spring of 1991. For the next five years he studied at Kansas State University in Manhattan, KS. He completed his undergraduate degree at K-State, obtaining his B.S. in Agronomy with a minor in Animal Science and Industry and a secondary major in Natural Resources and Environmental Sciences. While attending KSU, John participated in Crop and Soil Judging and completed an honors research project entitled "Heavy Metal Availability in Contaminated Soils".

After graduating in the spring of 1996, he began a Master's degree under the guidance of Dr. Carl A. Griffey and Dr. M. A. Saghai Maroof at Virginia Polytechnic Institute and State University. There he learned and practiced the skills of genetic analysis utilizing various molecular techniques. The emphasis of his study in this capacity has been identification and mapping of the barley leaf rust resistance gene, *Rph*5. In addition to his laboratory and greenhouse work, he served as Vice-President and President of the Crop and Soil Environmental Sciences departmental graduate student organization, served as assistant crops judging coach, and served as a teaching assistant for one semester in Soil Taxonomy and Classification.

John was inducted into the Gamma Sigma Delta agricultural honor society at Kansas State University and the Sigma Phi biological honor society while attending Virginia Tech.

In the fall of 1998, he married Martha Nokes formerly of Siloam Springs, Arkansas and in 1999 they had a beautiful daughter, Shannon Leigh.