EVALUATION OF WINTER WHEAT GERMPLASM FOR RESISTANCE TO STRIPE RUST AND LEAF RUST

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MASTER OF SCIENCE

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ABSTRACT

Wheat leaf rust, caused by *Puccinia triticina (Pt),* and wheat stripe rust caused by *P. striiformis* f. sp. *tritici* (*Pst*) are important foliar diseases of wheat (*Triticum aestivum* L.) worldwide. Breeding for disease resistance is the preferred strategy of managing both diseases. The continued emergence of new races of *Pt* and *Pst* requires a constant search for new sources of resistance. Winter wheat accessions were evaluated at seedling stage in the greenhouse with races of *Pt* and *Pst* that are predominant in the North Central US. Association mapping approach was performed on landrace accessions to identify new or underutilized sources of resistance to *Pt* and *Pst*. The majority of the accessions were susceptible to all the five races of *Pt* and one race of *Pst*. Association mapping studies identified 29 and two SNP markers associated with seedling resistance to leaf rust and stripe rust, respectively.

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DEDICATION

To my beloved parents John Okaba and Justine Birungi who passed away before witnessing this great achievement. And to my wife Brandie Kertho who always believes in me.

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CHAPTER ONE. LITERATURE REVIEW

General Introduction

Wheat (*Triticum aestivum* L.) is among the top three important crops of the world. Twenty percent of the food calories consumed by the world population comes from wheat (CYMMYT, 2013; Plains Grains Inc., 2012). The United States exports about half of its entire wheat crop; the majority of which is winter wheat (Plains Grains Inc., 2012; USDA-ERS, 2014). Rusts are the most destructive diseases of wheat. Occurrence of leaf rust, caused by *Puccinia triticina* is more common and widely distributed than stripe rust (caused by *Puccinia striiformis* f. sp *tritici)* or stem rust (caused by *Puccinia graminis* f. sp. *tritici)* (Bolton et al., 2008). Rusts of wheat historically have been a major yield limiting factor in wheat production in the northern Great Plains of the United States.

Managing rusts by fungicides is effective but costly, highly dependent on application timing, damaging to the environment and it doesn't provide a long term solution. The cultivation of resistant varieties has proven to be the most economical and environmentally friendly strategy of managing rusts of wheat (Roelfs and Bushnell, 1985). However, the constant evolution of new virulent races of the rust pathogens has rendered many varieties susceptible to the diseases. Therefore there is need to improve current wheat varieties by deploying longer-lasting resistance gene combinations. This can be achieved by incorporating new sources of resistance from landraces and wild relatives; a strategy that has worked previously for rust diseases and other pathogens of wheat (Cox et al., 1994).

Wheat. Evolution, Domestication and Dissemination

Evolution of wheat

Wheat is a member of grass family (Poaceae) which includes other important food crops such as sorghum, maize, rice, and barley (Kellogg, 2001). These major cereals are further organized into three subfamilies. Wheat, barley, oat, and brome are grouped in the sub-family of Pooideae. The other two sub-families are Panicoideae (represented by sorghum and maize) and Ehrhartoideae (represented by rice) (Kellogg, 2001). All the grass species are believed to have evolved from a common ancestor 50-70 million years ago through genome duplication and chromosome fusion (Bolot et al., 2009). The phylogenetic analysis based on the RFLP genome mapping of the species revealed that wheat has the closest relationship with barley. Brome is relatively more related to wheat compared to oats and rice. Sorghum and maize has been shown to be have a distant relationship to wheat (Kellogg, 2001; Gaut, 2002).

All wheat species belong to the genus *Triticum*. This genus contains six species: *Triticum monococcum* L. (2n=14, AA genomes), *Triticum urartu Tumanian ex Gandilyan* (2n=14, AA genomes), *Triticum turgidum* L. (2n=28, AABB genomes), *Triticum timopheevii (Zhuk.) Zhuk.* (2n=28, AAGG genomes), *Triticum aestivum* L. (2n=42, AABBDD genomes), and *Triticum Zhukovskyi Menabde & Ericz* (2n=42, AAAAGG genomes). These species can further be grouped into three gene pools: *Monococcon*, containing diploid species; *Dicoccoidea*, containing tetraploid species; and *Triticum*, containing hexaploid species. Among these six species, *Triticum urartu Tumanian ex Gandilyan* was found only in the wild, and the two hexaploid species, *Triticum aestivum L.* and *Triticum Zhukovskyi* Menabde & Ericz were found only in the cultivated form. The other three species can be found in both wild and domesticated forms (reviewed in Matsuoka, 2011).

Wheat is generally believed to have evolved by allopolyploidization through hybridization with species in the genus *Aegilops* (Tsunewaki, 2009). Hexaploid *T. aestivum* is believed to have arisen through natural hybridization between tetraploid *T. turgidum* and diploid *A. taushii Coss* (D genome donor) (Kihara, 1944; McFadden and Sears, 1944). The evolution and divergence of *T. turgidum, A. taushii Coss* and other species that contributed to the evolution of bread wheat were reviewed by Matsuoka (2011) and were also discussed by Kihara (1966); Dvorak et al. (1993); Slageren and Areas (1994). It is believed that *A. tauschii* is the donor of D genome, *T. urartu* is the donor of A genome, and *A. speltoides* is the donor of B genome (Petersen et al., 2006).

Molecular characterization of wheat

The whole genome of common bread wheat contains 21 pairs of chromosomes, 7 pairs for each of the genomes A, B and D. The total DNA on all chromosomes amounts to 16 billion bp which equals an average of 810Mb per chromosome (Khan et al., 2005; Gupta et al., 1999). The average wheat chromosome is 25 fold larger than the average rice chromosome. Therefore, three average wheat chromosomes equal the haploid maize genome, and 1.5 wheat chromosomes equal a haploid rice genome. The large genome of wheat may stem from polyploidization and duplication. More than 80% of the total DNA are repetitive sequences. Most of the remaining DNA is arranged as clusters in small chromosome regions (Gupta et al., 1999).

Domestication of wheat

Archeological records and advances in genetic technology have enabled us to trace the origin of wheat domestication. The question of when wheat was first cultivated is open to debate. The general agreement is that wheat cultivation started about 10,000 years ago during the Neolithic period when hunters and gatherers started practicing agriculture (Shewry, 2009).

Einkorn wheat (*Triticum monococcum*) was the first species of wheat to be domesticated and eventually cultivated (Heun et al., 1997). This diploid species of wheat was a very useful crop before the Bronze Age. Einkorn arose from its wild progenitor, *Triticum boeticum*. Salamani *et al.* (2002) and others (Brown et al., 2009; Shewry, 2009) described the morphological differences between wild and domesticated wheat. Just like other wheat species, domesticated einkorn is distinguishable from its progenitor by its seed size and ear structure. The seeds of domesticated wheat are larger than seeds of its wild progenitor. The rachis of wild einkorn is weak, breaking apart easily at maturity thus scattering spikelets. Domesticated einkorn, on the other hand, has tough rachis that keep seeds firmly attached making it easier to handle during harvest and threshing. Last but not least, the glumes that protect the seeds are tightly attached to the seed in wild einkorn, whereas in domesticated einkorn glumes are weakly attached to the seeds and release the seeds at maturity. It's cultivation, however, ceased in the Bronze Age in favor of polyploid wheat that was better suited to varied climates and had better harvesting properties (Salamini et al., 2002).

Emmer (*T. dicoccum*) was the second species of wheat to be domesticated. Emmer was domesticated from its wild progenitor *T. dicoccoides*. The difference in ploidy number gave emmer a cultivation advantage over einkorn. Domesticated emmer is easy to handle during harvest due to its tough rachis that keeps spikelets intact (Salamini et al., 2002). Like einkorn, emmer wheat was of great economic and social importance in the Neolithic age in the Fertile Crescent as well as being the main crop used for making bread in ancient Egypt (Salamini et al., 2002; Peng et al., 2011). Durum wheat is tetraploid and is known to have been domesticated from its ancestor *T. dicoccum.* Durum wheat, like most hexaploid wheat, is free-threshing and is cultivated worldwide for making pasta (Peng et al., 2011).

The final and probably the most important stage of wheat domestication was the discovery of free-threshing wheat of even higher ploidy status. This increase in polyploidy occurred with changes in genes that conferred the free-threshing character. An exception is spelt wheat (*T. spelta*), which is hexaploid but not free-threshing (Shewry 2009). The modern, most cultivated species of wheat is hexaploid bread wheat, *T. aestivum*. Bread wheat resulted from the cross between two different species; the diploid wild grass *A. tauschii* and tetraploid emmer wheat (Shewry, 2009; Dubcovsky and Dvorak, 2007; Charmet, 2011). This hybridization probably happened by accident as the cultivation of emmer wheat expanded.

Center of origin, dissemination and commercialization

The generally accepted place of origin of wheat is a region in the Fertile Crescent. Archeological records show that kernels of *T. boeoticum* have been found to exist at different geographical locations and times in the central Fertile Crescent (Salamini et al., 2002). The remains of domesticated einkorn and emmer wheat have also been recorded in the western Fertile Crescent (Brown et al., 2009). The evidence for the domestication of einkorn in the Fertile Crescent is strongly supported by the work of Heun and his colleagues (1997). They analyzed 288 amplified fragment length polymorphism (AFLP) marker loci in einkorn and its wild ancestor. They found a striking similarity between wild einkorn, *T. boeoticum,* growing in the western foothills of the Karacadag Mountains southeast of Turkey, and domesticated einkorn. This finding suggests that einkorn wheat was first domesticated in Karacadag. Other studies (Dubcovsky and Dvorak, 2007) of genetic relationships have pointed to the same region as the origin of domestication of einkorn and emmer wheat.

The spread of wheat outside the Fertile Crescent into adjacent areas and later to all parts of the world was mainly due the adaptability of hexaploid wheat to diverse environmental

conditions. This adaptability enabled the hexaploid, *T. aestivum*, to spread to hot and humid conditions of Eastern Asia as well as the cold and dry conditions in central and northern Europe (reviewed in Matsuoka, 2011). Feldman (2000) gave a step-by-step account of how wheat cultivation spread from its origin to the entire world. Wheat spread to Europe quite fast; from Greece to the Balkans and then to Italy, France, Spain, the UK and Scandinavia within 5000 years. The spread of wheat via Iran into central Asia and into China was even faster. In Africa, wheat cultivation spread through Egypt. The Spaniard colonialists introduced wheat into Mexico in 1529 and later the British introduced it in Australia in 1788.

The wonder crop didn't make it to the U.S until 1602. Olmstead and Rhode (2008) described how wheat became a major crop in North America. Wheat cultivars brought in by immigrants from Europe had to go through considerable modification to be able to grow in different parts of the U.S. The temperature at the time was not suitable for wheat growing. In New England, experimentation was done on cold and pest tolerant wheat. The experimentation successfully generated soft winter wheat which were later extensively cultivated in the northern prairies, Great Plains and the pacific coast. In the early 1800s, massive failure of winter wheat, spring wheat and soft winter wheat was recorded in the Great Plains and the Midwest (Olmstead and Rhode, 2008). The failure was primarily as a result of drought, freezing and insects. However, hard red winter wheat and spring wheat introduced by immigrants from frigid and arid areas of Eurasia were very successful (Olmstead and Rhode, 2008).

Wheat commercialization in North America reached its turning point when the governments started to invest in the crop by supporting wheat breeding programs. One notable example was the successful breeding of early-maturing, winter and summer adaptable wheat by William Sanders and his son, Charles Sanders in 1909. With the help of the Canadian

government, the two crossed *Red Fife* from Eastern Europe with *Red Calcutta* from India. The U.S government performed tests on the resulting progeny, *Marquis,* and subsequently released it to farmers in 1912-1913 (Pomeroy, 1956). *Marquis* was very successful and its cultivation spread rapidly across the U.S. spanning from Washington State to Northern Illinois (Clark et al., 1922). The icing on the cake of wheat breeding came with the work of Norman Borlaug that got started in the 1940s. While stationed in Mexico, Borlaug focused on breeding wheat that could survive almost anywhere. He bred wheat that was early ripening, rust resistant, and readily responsive to fertilizers. This wheat however had one problem; it grew tall and the stems broke easily. Borlaug later crossed *Norin* from Japan with *Brevor* from Washington State AES to produce dwarf lines of wheat that had strong straw, were high yielding and rust resistant. Borlaug's wheat changed the whole trajectory of wheat production in the world and was very instrumental in preventing hunger in other parts of the world like India and Pakistan (Borlaug, 1954; Dalrymple, 1986; Rajaram and Hettel, 1995).

Today, wheat is the leading export crop in the United States with about half of the total production exported (Plains Grains Inc., 2012; USDA-ERS, 2014). The crop is grouped into six classes in the United States based on hardness, kernel color and planting time, namely, Durum, Hard Red Spring, Hard Red Winter, Soft Red Winter, Hard White and Soft White. Hard red winter wheat is the most common class produced, accounting for 40% of total wheat production (Plains Grains Inc., 2012; USDA-ERS, 2014). The high demand for winter wheat on the world market can be attributed, among others, to its excellent milling and baking properties.

Agriculture is the backbone of economies of many states in the northern Great Plains of the United States. In North Dakota, agriculture is the largest economic industry with wheat as the topmost crop (www.nd.gov; North Dakota Wheat Commission, 2012). The state is the leading

producer of durum and hard red spring wheat in the nation and comes second, after Kansas, in total wheat production in the United States. Total wheat acreage covers one quarter of the state. Based on the market price of 2010, North Dakota collected an estimated $6.7 billion in direct cash and indirect commercial activity from wheat (North Dakota Wheat Commission, 2012). Now that North Dakota holds a strong grip on durum and hard red spring wheat, there is potential for the state to expand the production of winter wheat. There are numerous benefits to growing winter wheat. Winter wheat is higher yielding, allows efficient use of spring moisture, spreads out field work and most importantly conserves soil, moisture and wildlife (Salmon and McLelland, 2012; Roger Knapp, 2001). Nationwide, winter wheat acreage has been steady over the past decade, averaging around 42 million acres (USDA-NASS, 2014). Severe cold conditions and diseases such as the rusts are the major reasons why North Dakota winter wheat production is below the potential of the state. In recent years, producers have shown growing interest in winter wheat and the winter wheat acreage planted in the state has been increasing dramatically. In 2012 alone winter wheat acreage in the state increased by 75% over the previous year (Ducks unlimited, 2014; USDA-NASS, 2014). According to the National Agricultural Statistics Service, 800,000 acres of winter wheat was planted in North Dakota in 2014, nearly triple the acreage planted the previous year, and considered the highest ever (USDA-NASS, 2014). The historic achievement in winter wheat production in the state needs to be strengthened and maintained by developing winter wheat varieties adapted to this region. This will require the cooperation of all stakeholders in the state.

Wheat Rusts and Their Impact

Rusts of wheat (*Triticum aestivum* L.) are common diseases affecting wheat in all parts of the world where wheat is grown. There are three kinds of rust that are destructive to wheat.

Wheat leaf rust is caused by *Puccinia triticina (Pt),* wheat stem rust is caused by *Puccinia graminis f. sp. tritici (Pst),* and wheat stripe rust is caused by *Puccinia striiformis* f. sp. *tritici.* Rust fungi are obligate biotrophs that require living hosts to survive and reproduce (Hovmoller et. al, 2011). The life cycle of wheat rust pathogens is complicated and involves five spore stages that are broadly divided into sexual and asexual stages. The asexual phase requires a primary host (wheat) while the sexual stage requires an alternate host (*Berberis* spp. for stem rust and stripe rust and *Thalictrum* for leaf rust) (Jin et al., 2010). The disease-causing spores from the asexual phase of rusts are usually dispersed by wind and are the major causes of the diseases. Rusts of wheat are detrimental at all growth stages of wheat. They damage wheat plants by extracting nutrients from mesophyll cells through stomata and making wheat plants to increase transpiration and respiration and decrease photosynthesis. This results in a reduction in floret number and grain weight. Severe infection of stem rust on wheat stems can cause lodging and lead to substantial yield losses (Wiese, 1987; Roelfs et al., 1992; Marsalis and Goldberg, 2006).

Rusts of wheat can be managed by chemical fungicides, removal of alternate hosts, cultural practices and use of resistant cultivars. Fungicides are costly and may be harmful to the environment. Removal of the alternate host reduces the production of sexual aecia. Cultural practices such as planting early maturing cultivars and early sowing may allow wheat plants to escape infection while removing volunteer crops reduces the amount of inoculum. Cultural practices do not offer adequate control of wheat rusts since the effectiveness of each practice varies by epidemic and area. Growing resistant cultivars is the most economical and environmentally friendly strategy of managing wheat rusts (Roelfs et al., 1992; Roelfs and Bushnell, 1985). A wide variety of resistance genes are available (Mcintosh et al., 1995) and many are currently used in various wheat cultivars. Resistance has been broadly categorized into

all-stage resistance (also called seedling resistance) and adult plant resistance (APR) (Chen 2005). All-stage resistance is detected at the seedling stage and remains effective throughout plant growth. All-stage resistance is usually race-specific and involves gene-for-gene interaction (Flor, 1971). This type of resistance is not durable due to the constant evolution of virulent races of the rust pathogen. APR is expressed at later stages of plant growth and is mostly race-nonspecific and therefore more durable (Line, 2002). High temperature-adult plant resistance (HTAP), a type of APR, is race-non-specific and is more durable and stable (Line, 2002). HTAP is expressed in adult plants at high temperatures and is controlled by multiple genes (Qayoum and Line, 1985). The long term strategy to manage rusts is to improve the shelf life of cultivars by deploying both all-stage resistance and APR (Chen, 2005).

Leaf Rust

Leaf rust, also called brown rust, is the most common and widely distributed compared to stripe rust or stem rust (Bolton et al., 2008). The disease develops well at warmer temperatures (15-22℃) and high humidity. Symptoms of the diseases are visualized as round lesions, containing red-brown urediniospores, that are scattered all over the leaf and sometimes on leaf sheaths. Yield losses due to leaf rust vary depending on the susceptibility of the host, severity of infection and the stage of wheat development. In this region, yield loss is typically about 15%. However, severe losses of 30-40% have been reported when severe infection occurred at an early stage of wheat growth (McMullen et al., 2008). Genetic resistance is the preferred strategy to reduce losses due to leaf rust. Currently there are over 70 leaf rust resistance (*Lr*) genes that have been officially designated (McIntosh, et al., 2012). The majority of the *Lr* genes condition race-specific resistance in a gene-for-gene fashion (Flor, 1971). Though effective, race-specific resistance has not been durable due the constant emergence of virulent races of *Pt.* Many winter

wheat cultivars currently grown in North Dakota are susceptible to the local leaf rust pathogen population. Approximately 40-60 races of *Pt* are detected in the United States annually (Long et al., 2002; Kolmer et al, 2006). Some of these new races have overcome previously known resistant genes. The leaf rust race survey of 2011 found several races virulent to *Lr39/41, Lr21, Lr17* and other *Lr* genes that are found in many winter wheat cultivars grown in North Dakota (http://www.ars.usda.gov/). In 2012, leaf rust at Langdon, ND was mostly found on winter wheat (www.ars.usda.gov/mwa/cdl). The sources of race variability in the leaf rust pathogen in North America are mostly understood. Although *Thalictrum spp.*, the alternate host of *Pt,* is capable of contributing to new races of leaf rust, leaf rust infection on the alternate host is rare in North America. The alternate host is thus considered to be less important in the evolution of new races of leaf rust in North America (Samborski, 1985). The asexual stage is the main cause of leaf rust race changes in the United States. The primary source of leaf rust infection in North Dakota is from urediniospores carried along by wind currents that blow northwards every spring from states in the south. Leaf rust overwinters on winter wheat in the southern states where susceptible cultivars are mainly grown. This serves as reservoir of inoculum for the northern Great Plains. Every spring, wind blows inoculum from the south into the northern Great Plains as far as Manitoba (Samborski, 1985; Kolmer 2005; McMullen et al., 2008). The annual wind pattern adds complexity to the constantly changing races of leaf rust in North Dakota. New races brought in by the wind yearly makes it difficult to predict future pathotypes of *Pt* that will be found in the region. There is a need to constantly screen local winter wheat cultivars to track race changes and also deploy new effective resistance genes in local cultivars.

Stripe Rust

Stripe rust (commonly called yellow rust) causes significant yield losses in wheat globally. Stripe rust infection is characterized by round yellow pustules that usually occur in long stripes on the leaf surface. The pathogen, *Puccinia striiformis* f. sp. *tritici (Pst)*, normally requires cool temperatures to develop (10-15℃) so the disease is mostly common in areas with cooler climates. Stripe rust can be very damaging to wheat. Yield loss can be as high as 100%. Depending on severity of infection and cultivar susceptibility, yield losses usually range from 10-70% in a single field (Chen, 2005). Like leaf rust, utilization of genetic resistance is the preferred strategy to reduce losses due to stripe rust. Currently there are over 50 stripe rust resistance (*Yr*) genes designated (McIntosh et al., 2012). The majority of these genes condition race-specific resistance in a gene-for-gene fashion (Flor, 1971). In the United States stripe rust used to be a serious problem only in the Pacific Northwest and California where the climate is conducive for development of the disease. However, in 2000 stripe rust was reported in 25 states across the nation (Chen et al., 2002). The disease became more serious in the south central states and central Great Plains due to the emergence of new races of *Pst* (Chen, 2005; Markell and Milus, 2008). Stripe rust has now become an important disease throughout the United States because new races of the pathogen are adapted to high temperatures, are more aggressive (Chen, 2005; Milus et al., 2009, Chen et al., 2010) and some races are virulent on the previously resistant genes *Yr8* and *Yr9* (Chen et al., 2002) that are found in many winter wheat cultivars grown in the United States. In 2012 stripe rust was observed on winter wheat across North Dakota. Severe cases of stripe rust was observed on certain popular winter wheat varieties (Ideal, Jerry, Matlock) grown in the state (www.ars.usda.gov/mwa/cdl). Because stripe rust was very rare in the past in North Dakota, wheat varieties in the state have not been extensively screened

for resistance to stripe rust (McMullen et al., 2008). Therefore there is little information about stripe rust resistance in cultivars adapted to the state. Now that the disease has become a problem, North Dakota needs to be prepared for any stripe rust epidemics by screening local winter wheat varieties for stripe rust resistance and searching for new sources of resistance.

Association Mapping

Association mapping (AM), also called linkage disequilibrium (LD) mapping is a technique used to identify quantitative trait loci (QTL) that are strongly correlated with traits within a population. AM utilizes LD between alleles to identify trait-marker associations in a panel of diverse populations (Flint-Garcia et al., 2003). AM can be performed as genome-wide association (GWA) or candidate gene approach. GWA involves scanning markers across the entire genome for statistical significant associations between markers and specific phenotypes. Candidate gene approach identifies assocaitions between candidate genes and a phenotye where genome wide LD is limited (Hall et al., 2010). AM was initially used in medical research to understand human diseases such as cystic fibrosis (Kerem et al., 1989) and Alzheimer's disease (Corder et al., 1994). Recently, AM has been successfully used to identify marker-trait associations in higher plants including iron deficiency chlorosis in soybean (Mamidi et al., 2014; Mamidi et al., 2011), flowering time in maize (Thornsberry et al., 2001), and disease resistance in potatoes (Malosetti et al., 2007) and wheat (e.g. Stagonospora nodorum blotch, fusarium head blight, tan spot, stem rust) (Adhikari et al, 2011; Ghavami et al., 2011; Gurung et al., 2011; Yu et al., 2012).

AM has a number of advantages compared to bi-parental QTL mapping, as the two main methods of identifying QTL in plants. AM can be applied directly to a panel of individuals thereby saving time and cost of developing mapping populations. Secondly, AM is capable of

detecting quantitative trait loci (QTL) with great resolution due to the higher level of recombination and genetic diversity in AM population panels (Neumann et al., 2010). Finally, multiple traits can be investigated simultaneously using the same AM population panel and same genotype information.

On the other hand AM has some limitations compared to bi-parental QTL mapping. The genetic diversity within AM population panel creates stratification which leads to spurious associations (Price et al., 2010). When testing marker-trait associations, models that take into account population structure and relatedness (kinship) are used to minimize false associations with stratification. These corrective models need to be used with caution otherwise it can lead to false negative or false positive results. Furthermore, allele frequency distribution within a population affects the ability to detect marker-trait association. The power to detect associations decreases at loci where Minor Allele Frequency (MAF) is below a certain level (Myles et al., 2009). Markers with low MAF are excluded from AM analysis, making it difficult to identify rare causative alleles. Similarly, compared to QTL mapping, AM has difficulty identifying QTL that explain small phenotypic variations (low heritability) as the signal is distributed across numerous QTL and alleles.

Despite this limitation, AM is a powerful alternative to bi-parental QTL mapping. With advances in sequencing technologies, methods and statistical tools have been developed to detect rare alleles while maintain high mapping resolution and reducing spurious associations caused by population structure. The methods utilize multiple parent intercross populations while combining both QTL mapping and AM. Examples of this approach include multiparent advanced generation intercross (MAGIC) in *Arabidopsis* (Kover et al., 2009) and wheat (Huang et al., 2012) and nested association mapping (NAM) population in maize (Yu et al., 2008).

Objectives of Research

The objectives of this project were to: 1) evaluate winter wheat varieties currently grown in the northern Great Plains and breeding genotypes for their reaction to locally prevalent races of the stripe rust and leaf rust pathogens. 2) Evaluate landraces to identify new or underutilized sources of resistance effective to prevalent races of leaf and stripe rust pathogens in the US Northern Great Plains.

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CHAPTER TWO. SCREENING OF WINTER WHEAT ACCESSIONS FROM THE U.S. NORTHERN GREAT PLAINS FOR RESISTANCE TO PREDOMINANT RACES OF *P. striiformis* f. sp. *tritici* (STRIPE RUST) and *P. triticina* (LEAF RUST)

Introduction

North Dakota has witnessed a dramatic increase in winter wheat production in recent years. According to the National Agricultural Statistics, 800,000 acres of winter wheat were planted in the state in 2014. This is nearly triple the acreage planted in the previous year and is considered the highest ever (USDA-NAAS, 2014). Severe cold conditions and diseases such as rusts are the major reasons why North Dakota winter wheat production is below the potential of the state.

Wheat leaf rust, caused by *Puccinia triticina (Pt),* and wheat stripe rust caused by *P. striiformis* f. sp. *tritici (Pst)* are important foliar diseases of wheat (*Triticum aestivum L*) in the United States. Leaf rust is a constant problem to winter wheat farmers in the northern Great Plains due to a disease conducive-environment during most of the growing season and the constant emergence of new, virulent races of *Pt* (Kolmer et al., 2006). Yield loss due to leaf rust is typically about 15% in this region. However, severe losses of 30-40% have been reported when severe infection occurred at an early stage of wheat growth (McMullen et al., 2008). Stripe rust used to be a major problem only in the Pacific Northwest and California until the year 2000. However since 2000, stripe rust became an important disease throughout the United States because of the emergence of new races of *Pst* that are more aggressive and adapted to high temperatures (Chen et al., 2010; Milus et al., 2009). Some of the new races are virulent on previously effective resistance genes *Yr8* and *Yr9* (Chen at al., 2002) that are found in many winter wheat cultivars grown in the United States. Stripe rust causes yield loss of 10-70%

depending on susceptibility of varieties and growth conditions but in severe cases 100% yield losses can occur (Chen, 2005)

Growing resistant varieties is the most economical and environmentally friendly strategy of managing wheat rusts (Roelfs and Bushnell, 1985; Chen, 2005). Resistance has been broadly categorized into all-stage resistance (also called seedling resistance) and adult-plant resistance (APR) (Chen 2005). All-stage resistance is expressed at all stages of plant growth. This type of resistance is usually race-specific and therefore not durable due to constant evolution of rust pathogen (Kolmer, 2005; Jin et al., 2010). Conversely, APR is effective at later stages of plant growth and is mostly race-nonspecific and more durable (Line, 2002). The long term strategy to manage rusts in wheat is to deploy varieties carrying multiple all-stage resistance and APR genes effective to the pathogen populations. Leaf rust and stripe rust present a unique challenge to breeding for resistance in North Dakota. Firstly, leaf rust overwinters in southern states where susceptible winter wheat cultivars are mainly grown. Every year spring winds from the south blow urediniospores into the northern Great Plains (Samborski, 1987; Kolmer, 2005). Winter wheat in the south therefore acts as a reservoir of inoculum and contributes to the emergence of new races of leaf rust in North Dakota. Secondly, winter wheat varieties in North Dakota have not been bred or extensively screened for stripe rust resistance since the disease previously was not a problem in the region. Now the evolution of stripe rust races that are adapted to warmer conditions may signal its emergence as a potentially important disease in North Dakota. These two major challenges necessitate screening of local winter wheat breeding material and incorporation of resistance in new varieties in order to safeguard winter wheat production in the state.

In this current greenhouse study we screened winter wheat cultivars grown in the northern Great Plains, as well as lines under trial, and breeding parents for seedling reaction to races of *Pt* and *Pst* that are predominant in the region. Additionally, we screened selected cultivars and lines with polymerase chain reaction (PCR) markers that are diagnostic for important resistance genes.

Materials and Methods

Plant material and pathogen isolates

A total of one hundred and seventy five winter wheat accessions consisting of 35 cultivars and 137 breeding genotypes were provided by Dr. Francois Marais, the winter wheat breeder at North Dakota State University. These accessions include cultivars adapted to the northern Great Plains, breeding parents and lines under trial in nurseries. The accessions originate from North Dakota, South Dakota, Montana, Nebraska and Canada. Five races of *Pt* (MCDL, MFPS, TDBG, THBL and TBDJ), and one race of *Pst* (PSTv-37) (Table 1.2), representing prevalent races of leaf rust and stripe rust pathogens in North Dakota, were used to screen the accessions at the seedling stage in the greenhouse.

Table 1.2. Virulence/avirulence of leaf rust and stripe rust races on differential sets.

Race	Virulent on genes	Avirulent on genes
PSTv-37[b]	6,7,8,9,17,27,43,44,Tr1,Exp2	1,5,10,15,24,32,SP,Tye
MCDL[a]	1,3,17,26,B	2a,2c,3ka,9,10,11,14a,16,18,24,30
MFPS[a]	1,3,3ka,10,14,17,24,26,30,B	2a,2c,9,11,16,18
THBL[a]	1,2a,2c,3,16,26,B	3ka,9,10,11,14a,17,18,24,30
TDBG[a]	1,2a,2c,3,10,24	3ka,9,11,14a,16,17,18,26,30,B
TBDJ[a]	1,2a,2c,3,10,17,14a	3ka,9,11,16,18,24,26,30,B

[a]Four letter for *Pt* race nomenclature used in North America (Long & Kolmer, 1989). [b]*Pst* race nomenclature based on differential lines in the United States (Wan & Chen, 2014).

Disease evaluation

The screening experiment was conducted at the North Dakota State Agriculture Experiment Station Greenhouse Complex in Fargo. The experiment was set up in a randomized complete block design with three replicates and the entire experiment was repeated twice for each race of rust pathogen. For each wheat genotype, five seeds were planted per cell in 50-cell trays containing sunshine mix #1 (Sungro Horticulture Distribution Inc., Quincy, MI, USA) and slow-release commercial fertilizer (Osmocote 15-9-12, N-P-K, Everris NA Inc., OH, USA) in a rust-free greenhouse set at 22℃/18℃ (day/night) with 16-hour photoperiod. Susceptible checks 'Little Club' and 'Avocet' were included in each tray for leaf rust and stripe rust, respectively. Foliar fertilizer, Peat Lite 20-20-20, was applied after seedling emergence followed by once a week thereafter. At 10 days after planting, seedlings at the two-leaf stage were spray inoculated with fresh rust spores suspended in Soltrol-170 oil (Phillips Petroleum, Bartlesville, OK, U.S.A) at a rate of 0.01g/mL and then left to air dry.

Leaf rust

Seedlings inoculated with races of the leaf rust pathogen (Table 1.2) were placed in a dark dew chamber for 16-24 hours at 20℃. The seedlings were then moved to the greenhouse until ready for disease scoring. Infection types (ITs) were scored 12-14 days post-inoculation using the 0-4 scale (McIntosh et al, 1995), where IT 0=no visible sign or symptom; 1=small uredinia with necrosis; 2=small to medium sized uredinia with green islands and surrounded by necrosis or chlorosis; 3=medium sized uredinia with or without chlorosis; 4=large uredinia without chlorosis. ITs of 0 to 2 were considered avirulent (resistant response), while 3 and 4 were considered virulent (susceptible response).

Stripe rust

Seedlings inoculated with race PSTv-37 (Table 1.2) were placed in a clean dark growth chamber for 16-24 hours at 13℃ at 98% humidity and then continued incubation in the growth chamber at 17℃/ 12℃ (day/night) and 16-hour photoperiod. Disease reaction was assessed 16-18 days after inoculation on a scale of 0-to-9 (Chen et al., 2002; Qayoum and Line, 1985; McIntosh et al, 1995), where IT 0=no visible signs or symptoms; 1=necrotic or chlorotic flecks with no sporulation; 2=necrotic and/or chlorotic blotches or stripes with no sporulation; 3=necrotic and/or chlorotic blotches or stripes with only a trace of sporulation; 4,5 and 6= necrotic and/or chlorotic blotches or stripes with light, intermediate and moderate sporulation, respectively; and 7,8 and 9= abundant sporulation with necrotic and/or chlorotic stripes or blotches, chlorosis behind the sporulation area, and no chlorosis or necrosis, respectively. Plants with ITs 0-3 were considered resistant, 4-6 were considered intermediate and 7-9 were considered susceptible.

Diagnostic marker analysis

Markers were used to screen for the presence of some all-stage and adult plant resistance genes that are still effective against races of *Pt* and *Pst* in the northern Great Plains of the U.S. Three diagnostic markers (Table 2.2) for resistance to leaf rust and stripe rust were used to screen a selected 64 winter wheat cultivars and lines from regional nurseries in the northern Great Plains. Information about primer pairs used in this study is found on the Wheat Applied Genomics Website (http://maswheat.ucdavis.edu/) and the GrainGenes Website (http://wheat.pw.usda.gov). The STS marker csLV34 (Lagudah et al., 2006) was used to test for the presence of APR gene *Yr18/Lr34*. For *Yr17/Lr37/Sr38*, VENTRIUP-LN2 primer pairs were used to amplify the N-allele of marker locus *Xcmwg682* (Helguera et al., 2003). The presence of

Lr21 was evaluated using the marker Ksud14 developed by Talbert and his colleagues (Talbert et al., 1994). The polymerase chain reaction (PCR) protocols used for each marker are summarized in Table 2.2.

Table 2.2. PCR protocol for three markers used for marker assisted selection.

Gene (marker)	*Lr37/Yr17/Sr38* (*Xcmwg682*)	*Lr21* (Ksud14)	*Lr34/YR18* (csLV34)
Forward primer	AGG GGC TAC TGA CCA AGG CT	CGC TTT TAC CGA GAT TGG TC	GTT GGT TAA GAC TGG TGA TGG
Reverse primer	TGC AGC TAC AGC AGT ATG TAC ACA AAA	TCT GGT ATC TCA CGA AGC CTT	TGC TTG CTA TTG CTA TTG CTG AAT AGT
Denaturing	94°C, 45s	94°C, 5mins	94°C, 5mins
Amplification	30 cycles 94°C, 45s 65°C, 30s 72°C, 60s	30 cycles 94°C, 60s 55°C, 60s 72°C, 2mins	40 cycles 94°C, 45s 58°C, 30s 72°C, 60s
Extension	72°C, 7mins	72°C, 5min	72°C, 7mins

A pair of sterile scissors was used to cut 5-10 cm sections of leaves from wheat seedlings at the two-leaf stage. The leaves were then folded, put in Eppendorf tubes and kept cold on ice. The leaves in tubes were transferred to a lyophilizer and left to freeze dry for 72 hours. Dried leaves were ground to a fine powder using a mechanical Mixer Mill MM300 (Retsch, Haan, Germany). DNA was extracted following the CTAB protocol described by Stewart and Via (1993). The concentration and purity of extracted DNA was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). PCR was performed on diluted DNA (30ng/uL) in 20uL reaction volume. Each 20µL reaction contained 2µL of 25mM $MgCl_2$, 1.5µL of 2.5µM dNTP, 0.5µL of 10µM of each reverse and forward primer, 0.1µL of Taq polymerase, 9.4µL of H_20, 2µL of DNA, and 4µL of 5x Green GoTaq® Flexi Buffer (Promega, Madison, WI). DNA was amplified in an Applied Biosystems® 2720 Thermal cycler (Life Technologies, Grand Island, NY, USA) as per protocol summarized in Table 2.2 for each

marker. Amplified products were separated at 80V for one hour on 2 % agarose gels prepared with GelRed dye. Gels were then visualized using a Benchtop 3UV™ Transilluminator (Ultra-Violet Products, Upland, CA, USA).

Results

Evaluation of cultivars and breeding genotypes with leaf rust and stripe rust

Cultivars

Thirty-five cultivars were evaluated for seedling resistance to five races of *Pt* (MCDL, THBL, TDBG, TBDJ and MFPS) and one race of *Pst* (PSTv-37). The information on virulence and effective genes from a differential set for each race is summarized in Table 1.2. The disease reactions for all cultivars are summarized in Table 3.2. A total of 16 (45.7%), 16 (45.7%), 12 (34.3%), 11 (31.4%), 10 (28.6%) and 8 (22.9%) were resistant to MCDL, THBL, TDBG, TBDJ, MFPS and PSTV-37, respectively (Figs. 1.2, 2.2, 3.2a). The majority of resistant cultivars had infection type of 2 for all the five races of the leaf rust pathogen (Fig. 1.1). Seven (20%) cultivars were resistant to all five races of *Pt* while 13 (37%) were susceptible to the five races. All of the eight cultivars resistant to PSTv-37 were moderately resistant (Fig. 3.1a). Only two cultivars; WB Grainfield and Ideal were resistant to all races of *Pt* and *Pst* tested (Table 3.2).

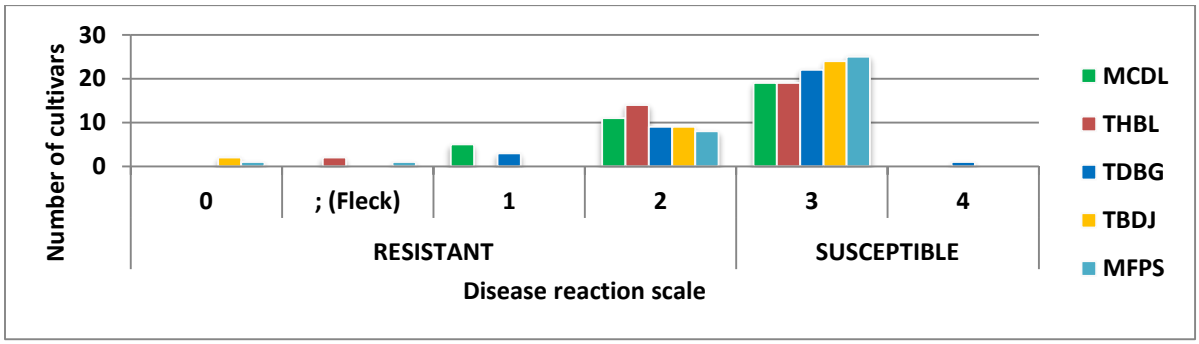

Figure 1.2. Frequency distribution of infection type (IT) values for 35 winter wheat cultivars screened with five races of *P. triticina*. IT scores are based on the median obtained for three replicates, five plants per replicate and two experiments. Cultivars with IT≤2± were considered resistant and those with IT≥3 were considered susceptible.

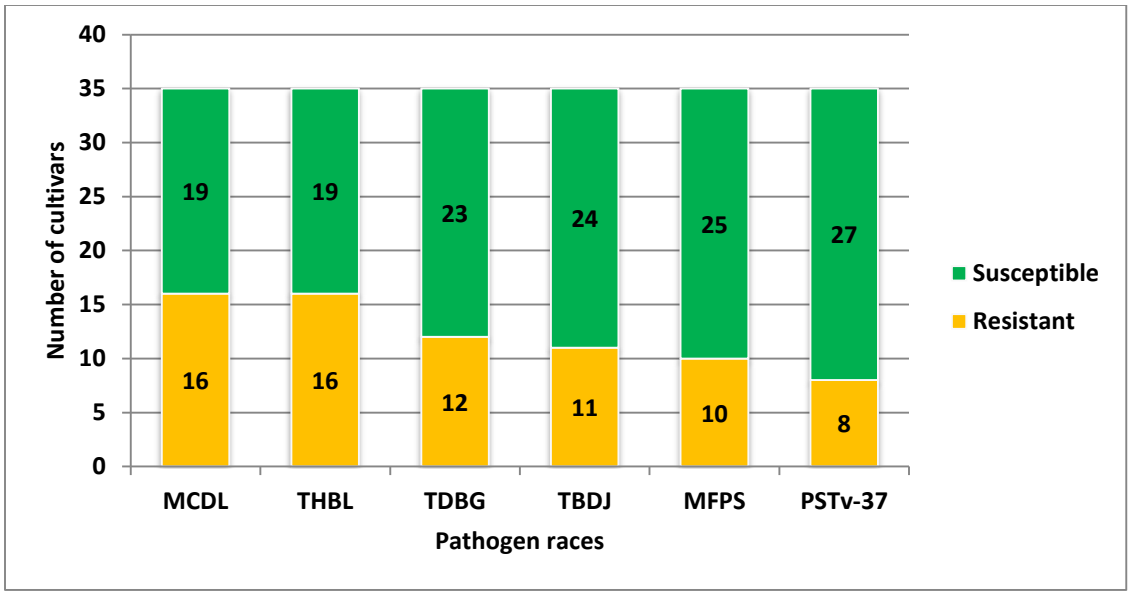

Figure 2.2. Number of cultivars resistant and susceptible to each race of *P. triticina* and *P. striiformis* tested. Thirty-five cultivars were screened against five races of *P. triticina* (MCDL, THBL, TDBG, TBDJ and MFPS) and one race of *P. striiformis* (PSTv-37). For leaf rust, cultivars with IT≤2± were considered resistant and those with IT≥3were considered susceptible. For stripe rust, IT≤6 was considered resistant and IT≥7 was susceptible.

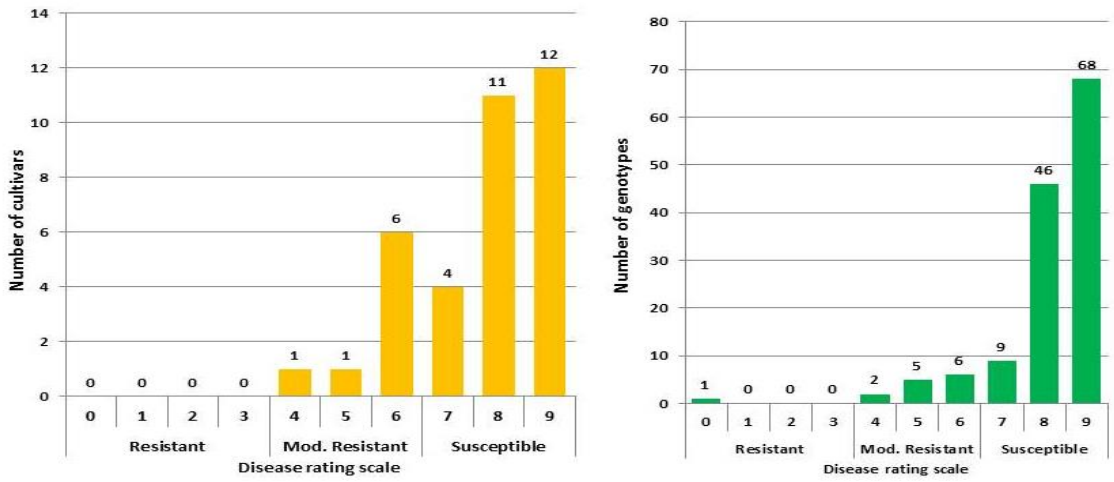

Figure 3.2. Frequency distribution of 35 cultivars (a) and 137 genotypes (b) evaluated for seedling resistance to *P. striiformis* f. sp. *tritici* in the greenhouse.

Table 3.2. Infection type (IT) and description of 35 winter wheat cultivars screened with five races of *P. triticina* (TDBG, MFPS, THBL, MCDL and TBDJ) and one race of *P. striiformis* f. sp. *tritici* (PSTv-37). Disease reactions recorded here are the median ITs for two experiments with three replicates per experiment.

Genotype	Breeder or Origin[a]	Year	Stripe rust[1]	Leaf rust[1]	Genes postulated[2]/present	TDBG	MFPS	THBL	MCDL	TBDJ	PSTv37
AC Broadview[5]	Canada	2011	MS	R	*Lr21, Lr24/Sr24*	1	2	2	1	3	7
AC Emerson[3]	Canada	2012	R	R	*Sr38/Lr37/Yr17*	3	3	3	3	3	8
Accipiter	Western Ag	2008	NA	MS		3	3	2	2	3	9
Art	Agripro	2008	R	R	*Lr16,Lr17*	1	3	2	2	2	6
Bigsky	Montana State University	2000				3	3	3	3	3	9
Boomer	WestBred	2009	MS	MR		2	2	2	2	2	8
Carter	WestBred	2010	S	NA		3	3	3	3	2	6
CDC Buteo	WesrBred	2004	NA	MS		3	3	2	2	3	9
CDC Falcon	WestBred	2000	MS	MS		3	3	2	2	3	9
Darrel	South Dakota State Univ.	2006	NA	S		3	3	3	3	3	8
Decade[5]	Montana State University/North Dakota State Univ.	2010	S	VS	*Lr24*	3	3	2	1	3	8
Expedition	South Dakota State Univ.	2002	MS	MS		3	3	3	3	3	9
Flourish[5]	Canada	2011	MR	MS	*Lr24*	3	3	3	2	3	6
Hawken	Agripro	2007	S	MR	*Lr17, Lr26*	3	3	2	3	2	9

Table 3.2. Infection type (IT) and description of 35 winter wheat cultivars screened with five races of *P. triticina* (TDBG, MFPS, THBL, MCDL and TBDJ) and one race of *P. striiformis* f. sp. *tritici* (PSTv-37) (continued). Disease reactions recorded here are the median ITs for two experiments with three replicates per experiment.

Genotype	Breeder or Origin[a]	Year	Stripe rust[1]	Leaf rust[1]	Genes postulated[2]/present	TDBG	MFPS	THBL	MCDL	TBDJ	PSTv37
Ideal[5]	South Dakota State Univ.	2011	NA	R	*Lr34*[4]*, Lr24*	2	2	2	1	2	6
Jerry[5]	North Dakota State Univ.	2001	MR	MR	*Lr24*	2	2	2	2	2	8
Kharkof			NA	NA		3	3	3	3	3	8
Lyman[5]	South Dakota State Univ.	2008	MS	R	*Lr16,Lr24*	2	;	;	1	0	8
McGill	ARS-Univ. of Nebraska	2010	MS	MS	*Lr17, +*	3	2	3	3	2	9
Moats	Canada	2011	NA	R		3	3	3	3	3	6
Norstar	Canada	1977		S		4	3	3	3	3	9
Overland	University of Nebraska	2006	MR	MR/R	*Lr16, Lr24, Lr17*	2	2	2	2	2	8
Peregrine	Western Ag	2008	R	MR		3	3	3	3	3	9
Radiant[5]	Canada	2005	R	S	*Yr10*	3	3	3	3	3	5
Robidoux	ARS-Univ. of Nebraska	2010	MR	MS		3	3	3	3	3	7
Roughrider	North Dakota State Univ.	1975	NA	S		3	3	3	3	3	9
Striker	WestBred	2009	MS	MR		3	2	2	1	3	8
Sunrise	Canada	2011	R	MR		3	3	3	3	3	8
SY Wolf	Agripro	2010	MS	MR	*Lr26,Lr34,Lr37*	1	2	2	2	2	9

Table 3.2. Infection type (IT) and description of 35 winter wheat cultivars screened with five races of *P. triticina* (TDBG, MFPS, THBL, MCDL and TBDJ) and one race of *P. striiformis* f. sp. *tritici* (PSTv-37) (continued). Disease reactions recorded here are the median ITs for two experiments with three replicates per experiment.

Genotype	Breeder or Origin[a]	Year	Stripe rust[1]	Leaf rust[1]	Genes postulated[2]/present	TDBG	MFPS	THBL	MCDL	TBDJ	PSTv37
WB Grainfield	WestBred	2013	MS	MS		2	0	;	2	0	6
WB-Matlock	WestBred	2010	MS	MS		3	3	3	2	3	8
Wendy	South Dakota State Univ.	2004		MS		2	3	3	3	3	9
Wesley	Univ. of Nebraska/South Dakota State Univ./Wyoming	2000	MR	MS	*Lr26?,+*	2	3	2	3	3	7
Willow Creek	Montana State University	2006				2	3	3	3	3	7
Yellowstone	Montana State University	2005	R	S		3	3	3	3	3	4

[1]Field data from variety trials (Ransom *et al*., 2013); R=resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible, VS=very susceptible, NA=not available. [2]Gene postulation (Cereal Disease Lab, 2014), [3]Gene presence reported in Graf *et al* 2013, [4]Gene presence reported in Berzonsky and Vorst, 2012, [5]Information on genes present obtained from Dr. Francois Marais (personal communication, 2014). [a]ARS-NE=USDA Agricultural Research Service.

Breeding genotypes

Of the 137 genotypes screened with five races of *Pt* and one race of *Pst*, 70 (51.1%), 71 (51.8%), 57 (41.6%), 54 (39.4%), 49 (35.8%) and 14 (10.2%) were resistant to MCDL, THBL, TDBG, TBDJ, MFPS and PSTv-37 respectively (Fig. 4.2, 5.2, 3.2b). Twenty-nine (21.1%) genotypes were resistant to all five races of *Pt* while 42 (30.7%) were susceptible to the five races. Among the 14 genotypes resistant to *Pst*, only one was immune (IT=0) while the rest were moderately resistant (Fig. 3.2b). A total of six genotypes (4.4%) were resistant to all the races of leaf rust and stripe rust pathogens tested.

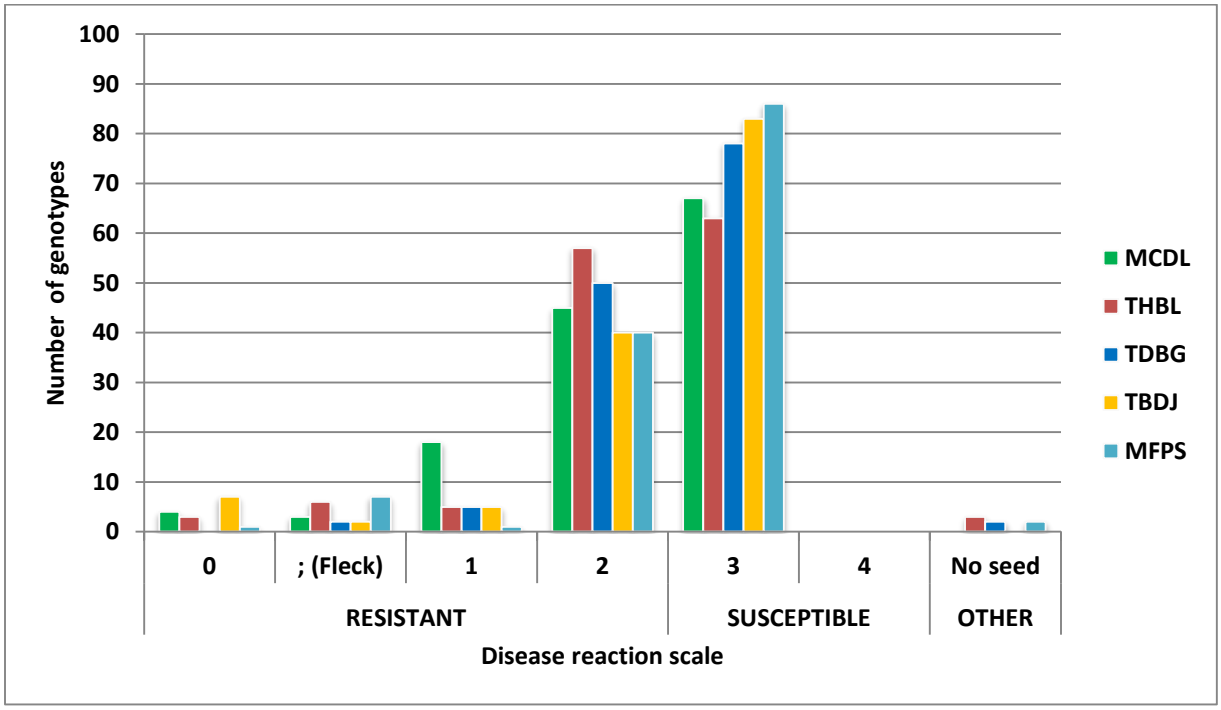

Figure 4.2. Frequency distribution of infection type (IT) values for 137 winter wheat genotypes screened with five races of *P. triticina*. IT scores are based on the median obtained for three replicates, five plants per replicate and two experiments. Genotypes with IT≤2± were considered resistant and those with IT≥3were considered susceptible.

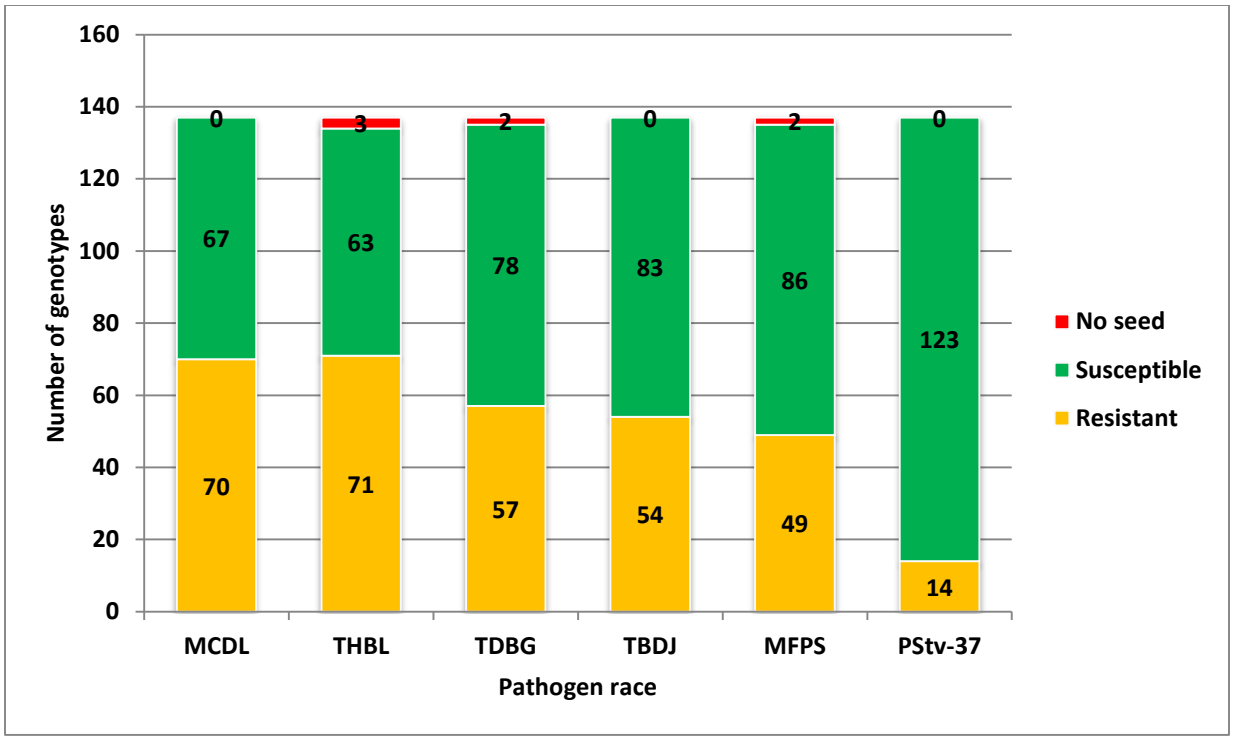

Figure 5.2. Number of winter wheat genotypes resistant and susceptible to each race of *P. triticina* and *P. striiformis* tested. One hundred and thirty seven cultivars were screened against five races of *P. triticina* (MCDL, THBL, TDBG, TBDJ and MFPS) and one race of *P. striiformis* (PSTv-37). For leaf rust, genotypes with IT≤2± were considered resistant and those with IT≥3 were considered susceptible. For stripe rust, IT≤6 was considered resistant and IT≥7 was considered susceptible.

Screening with diagnostic PCR markers

Fourteen and seven genotypes were positive for seedling resistance markers for *Lr37/Yr17/Sr38* and *Lr21*, respectively. Additionally 11 genotypes tested positive for APR maker csLV34 linked to *Lr34* (Table 4.2). No genotype tested positive for all three markers. Four genotypes had both *Lr34* and *Lr37*, two had both *Lr21* and *Lr34* and no genotype had both *Lr21* and *Lr37*. Cultivar WB Grainfield that showed resistance to all the races of *Pt* and *Pst* tested in this project (Table 2.2) was also positive for *Lr34* and *Lr37*.

Table 4.2. Winter wheat genotypes that were positive for at least one of the three markers tested.

Genotype	*Lr37/Yr17/Sr38*	*Lr34/Yr18*	*Lr21*
	Xcmwg682	csLV34	Ksud14
NE10589	+	-	-
SD10026-2	+	-	-
SD10257-2	+	-	-
NE06545	+	-	-
Moats	+	-	-
AC Emerson	+	-	-
Peregrine	+	-	-
Hawker	+	-	-
WB Grainfield	+	+	-
SD07184	+	+	-
SY Wolf	+	+	-
Art	+	+	-
Carter	-	+	-
N10MD2073	-	+	-
NX10MD2300	-	+	-
NE10418	-	-/+	-
SD08080	-	+	-
NW10487	-	+	+
NX10M02216	-	+	+
Lyman	-	-	+
MT08172	-	-	+
NE10442	-	-	+
NH10665	-	-	+
Sunrise	+	-	+

Discussion

This study provides important information on the status of seedling (all-stage) resistance of winter wheat genotypes in the northern Great Plains of the United States to wheat leaf and stripe rust pathogens. The races used in the study represent prevalent races in the region. The majority of cultivars and genotypes evaluated were susceptible to many of the races. Susceptibility in some of the genotypes may be the result of lack of adaptation to the local pathogen population as some of them have not necessarily been bred specifically for leaf and stripe rust resistance for the Northern Great Plains. Additionally, the genotypes were only evaluated at the seedling stage which prevented evaluation of adult-plant resistance. In the case of the winter wheat cultivars evaluated, that are currently grown in our region, most of the resistance genes previously postulated in these cultivars are race-specific all-stage resistance which can lead to pathogen easily evolving to overcome the resistances (Kolmer et al., 2007). Another major factor is the frequent introduction of new races of rust pathogens into the Northern Great Plains, by the annual spring winds that blow spores from the south (Kolmer 2005; McMullen et al., 2008).

In the case of stripe rust it is not surprising that majority of accessions tested are susceptible since stripe rust used not to be a major problem in the Great Plains due to prevalent non-conducive environmental conditions for stripe rust epidemics to develop. This is reflected in the absence of cultivars highly resistant to the isolate of race PSTv-37 used for screening in this study (Fig.3.1a). This race has a similar virulence phenotype to the isolates belonging to the 'newer' races of *Pst* that were detected after the year 2000. The races from this population of *Pst* are virulent and adapted to warmer conditions (Chen, 2005). PSTv-37, is one of the most predominant and virulent races according to recent surveys across the United States. Stripe rust

resistance genes *Yr1, Yr5, Yr10, Yr15, Yr24, Yr32, YrSP* and *YrTye* are effective against PSTv-37 (Table 1.2). Extensive information on how many of these genes are common in cultivars and breeding lines tested in this study is not available. However, the cultivar Radiant that is moderately resistant to PSTv-37 is known to have the resistance gene *Yr10* (Marais, personal communication). Further studies are needed to test for the presence of *Yr* genes that are effective against PSTv-37. Similarly, there is limited information about the presence of *Lr* genes effective against the races of leaf rust pathogen listed in Table 1.2. The presence of *Lr and Yr* genes in wheat varieties can be postulated based on their pedigree and their reaction to various races of stripe rust and leaf rust pathogens. Diagnostic markers are available for only a few of the genes including *Lr37, Lr21,Lr34/Yr18* and *Yr15* and their usefulness may be limited by, among others, the genetic backgrounds in wheat genotypes. Table 3.2 includes some *Lr* and *Yr* genes that have been postulated (Cereal Disease Lab, http://www.ars.usda.gov/main/docs.htm?docid=10342).

Sixty-four winter wheat genotypes were screened with PCR markers for leaf rust and stripe rust resistance genes that are still effective in many winter wheat cultivars (Table 4.2). The results show that the majority of the genotypes have none of the resistance genes tested. Cultivar Emerson has been reported to have *Sr38/Lr37/Yr17* (Graf et al., 2013). The present analysis with a marker diagnostic for *Sr38/Lr37/Yr17* verifies this (Table 4.2). However, seedling screening results show Emerson to be susceptible to leaf rust races THBL and TDBG that are avirulent on *Lr37* (Table 3.2). This kind of unexpected outcome has been reported previously and was attributed to suppression of *Lr37* activity by background genes (Helguera et al., 2003).

This study evaluated winter wheat genotypes for seedling resistance to leaf rust and stripe rust. Although the majority of genotypes were susceptible, they may possess adult plant resistance. Available field data from previous screening by Ransom et al.,(Table 3.2) shows that

some of the genotypes susceptible to races of the leaf rust and stripe rust pathogens at the seedling stage show a level of resistance in the adult stage (Ransom et al., 2013). This suggests that these genotypes possibly have adult plant resistance. Screening for adult plant resistance will therefore provide important additional information about these genotypes. Additionally, marker assisted screening can be used to detect particular adult plant resistance genes.

Acknowledgements

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CHAPTER THREE. IDENTIFICATION OF QTL FOR STRIPE RUST AND LEAF RUST RESISTANCE IN WINTER WHEAT LANDRACES USING ASSOCIATION MAPPING

Introduction

Wheat leaf rust, caused by *Puccinia triticina (Pt)*, and wheat stripe rust caused by *P. striiformis* f. sp. *tritici* (*Pst*) are important foliar diseases of wheat (*Triticum aestivum L*) worldwide (Bolton et al., 2008; Kolmer, 2005)**.** Genetic resistance is the preferred method of protecting against yield losses due to these diseases (Chen, 2005; Roelfs & Bushnell, 1985). Resistance has been broadly categorized into all-stage resistance (also called seedling resistance) and adult-plant resistance (APR) (Chen, 2005). Seedling resistance is expressed at all stages of plant growth. This type of resistance is mostly race-specific and offers a high level resistance yet it is easily overcome by changes in virulence of rust pathogens (Jin, et al., 2010; Kolmer, 2005). Conversely, APR is effective at later stages of plant growth and is mostly race-nonspecific and more durable (Line, 2002). The constant evolution of new virulent races of leaf rust and stripe rust pathogens has rendered many wheat varieties susceptible (Chen, 2005; Chen et al., 2002; Kolmer, et al., 2006; Markell & Milus, 2008). Therefore there is a need to find new sources of resistance to protect the yield of wheat.

There are currently over 70 leaf rust resistance (*Lr*) and over 50 stripe rust resistance (*Yr*) genes that have been officially designated (Mcintosh et al., 2012). The majority of these genes condition race-specific resistance in a gene-for-gene fashion (Flor, 1971) and many of these genes have been overcome by the emergence of virulent races. The most effective strategy of protecting wheat from rust is by deploying both seedling and adult plant resistance genes. APR such as high temperature-adult plant resistance (HTAPR) is crucial for protecting plants at the

critical stage of development and at high temperatures (Qayoum and Line, 1985; Chen, 2005). On the other hand, the use of seedling resistance is necessary to protect plants in the early stages of development in growing areas where environmental conditions conducive for disease development occur early in the season. For many years molecular markers for resistance to leaf rust and stripe rust have been characterized using bi-parental populations obtained from crossing resistant and susceptible wheat genotypes. Many molecular markers for resistance genes to these two plant diseases have been identified using bi-parental populations (Uauy et al., 2005; William et al., 2003). Though it has been successful, bi-parental QTL mapping requires considerable time to develop the mapping population and gene discovery is limited by resistance genes present in the two parents.

Association mapping (AM) is an alternative to bi-parental linkage mapping since it uses natural populations thereby eliminating the need for developing mapping populations. AM is credited for detecting quantitative trait loci (QTL) with great resolution from populations of diverse origins (Neumann et al., 2010). AM uses linkage disequilibrium (LD) between alleles within diverse populations to identify markers associated with particular traits (Flint-Garcia et al., 2003). This approach was initially used in medical research to understand human diseases such as cystic fibrosis (Kerem et al., 1989) and Alzheimer's disease (Corder et al., 1994). Recently, AM has been successfully used to identify marker-trait associations in many crops including iron deficiency chlorosis in soybean (Mamidi et al., 2014; Mamidi et al., 2011), flowering time in maize (Thornsberry et al., 2001), and disease resistance in wheat (Adhikari et al, 2011; Ghavami et al., 2011; Gurung et al., 2011; Patel et al., 2013) and potatoes (Malosetti et al., 2007).

Wheat landraces are an important potential source of new resistance genes since they have been exposed to high levels of disease pressure and other stresses over a long period of time. This co-existence of rust pathogens and wheat may have resulted in the accumulation of diverse resistance in wheat (Newton et al, 2010). Studies have demonstrated that landraces can be a good source of resistance to leaf rust and stripe (Bux et al., 2012; Newton et al., 2010), and an effort to harness the genetic diversity in landraces continues. We therefore anticipate that new or underutilized resistance genes against the leaf rust and stripe rust pathogens may exist in winter wheat landraces. The USDA National Small Grain Collection (USDA-NSGC) in Aberdeen, ID has accessions of wheat landraces from different geographic regions of the world. The objective of this research was to evaluate 575 winter wheat landrace accessions from USDA-NSGC for seedling resistance to five races of *Pt* and one race of *Pst* that are predominant in the U.S northern Great Plains.

Materials and Methods

Wheat germplasm and pathogen isolates

Five hundred and seventy five winter wheat landrace accessions (*Triticum aestivum* L.) from a core population obtained through single plant selection were provided by the USDA National Small Grain Collection (USDA-NSGC) located in Aberdeen, ID, U.S.A. These 575 landraces were from 44 countries; representing diverse geographic regions of the world (Fig. 1.2). Five races of *Pt* (MCDL, MFPS, TDBG, THBL and TBDJ), and one race of *Pst* (PSTv-37), representing prevalent races of the leaf rust and stripe rust pathogens in North Dakota were used to screen these accessions at the seedling stage in a greenhouse (Table 1.3; Long & Kolmer, 1989).

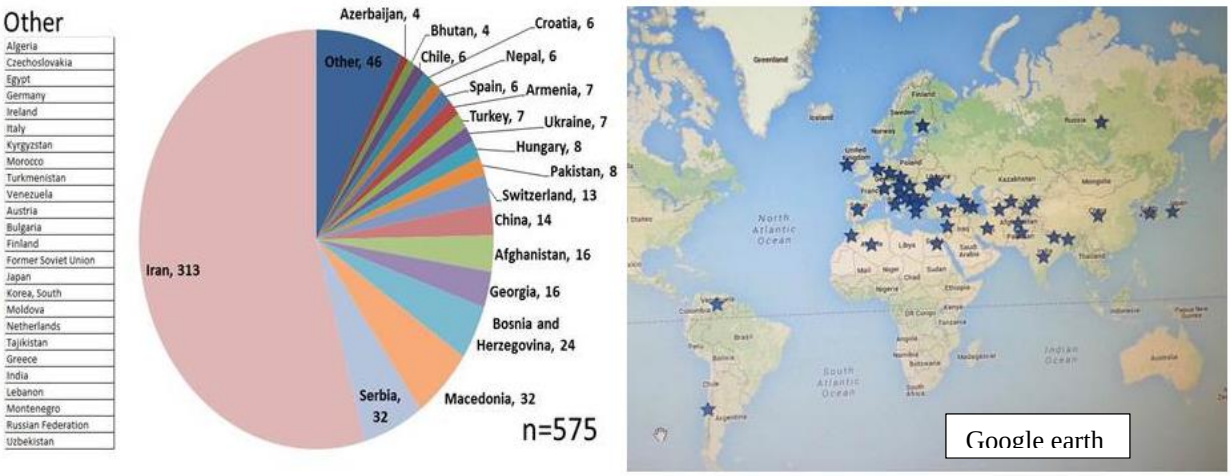

Figure 1.3. Pie chart and map showing the distribution of 575 landrace accessions by their origin. Origins with ≤2 accessions are grouped as "other".

Table 1.3. Virulence/avirulence of leaf rust and stripe rust races on differential sets.

Race	Virulent on genes	Avirulent on genes
PSTv-37[b]	6,7,8,9,17,27,43,44,Tr1,Exp2	1,5,10,15,24,32,SP,Tye
MCDL[a]	1,3,17,26,B	2a,2c,3ka,9,10,11,14a,16,18,24,30
MFPS[a]	1,3,3ka,10,14,17,24,26,30,B	2a,2c,9,11,16,18
THBL[a]	1,2a,2c,3,16,26,B	3ka,9,10,11,14a,17,18,24,30
TDBG[a]	1,2a,2c,3,10,24	3ka,9,11,14a,16,17,18,26,30,B
TBDJ[a]	1,2a,2c,3,10,17,14a	3ka,9,11,16,18,24,26,30,B

[a]Four letter for *Pt* race nomenclature used in North America (Long & Kolmer, 1989). [b]*Pst* race nomenclature based on differentials lines in the United States (Wan & Chen, 2014)

Seedling disease screening

All the screening experiments were conducted at the North Dakota State University Agricultural Experiment Station Greenhouse Complex (NDAES) in Fargo. The experiment was set up in a randomized complete block design with three replicates and the entire experiment was repeated twice for each race of rust pathogen. For each wheat genotype, five seeds were planted per cell in 50-cell trays containing sunshine mix #1 (Sungro Horticulture Distribution Inc., Quincy, MI, USA) and slow-release commercial fertilizer (Osmocote 15-9-12, N-P-K, Everris NA Inc., OH, USA) in a rust-free greenhouse set at 22℃/18℃ (day/night) with 16-hour photoperiod. Susceptible checks 'Little Club' and 'Avocet' were included in each tray for leaf

rust and stripe rust, respectively. Foliar fertilizer, Peat Lite 20-20-20, was applied after seedling emergence followed by once a week thereafter. At 10 days after planting, seedlings at the two-leaf stage were spray inoculated with fresh rust spores suspended in Soltrol-170 oil (Phillips Petroleum, Bartlesville, OK, U.S.A) at a rate of 0.01g/mL and then left to air dry.

Leaf rust

Seedlings inoculated with races of the leaf rust pathogen were placed in a dark dew chamber for 16-24 hours at 20℃. The seedlings were then moved to a greenhouse until ready for disease scoring. Infection types (ITs) were scored 12-14 days post-inoculation using the 0-4 scale (McIntosh et al., 1995) where IT 0=no visible sign or symptom; 1=small uredinia with necrosis; 2=small to medium sized uredinia with green islands and surrounded by necrosis or chlorosis; 3=medium sized uredinia with or without chlorosis; 4=large uredinia without chlorosis. ITs of 0 to 2 were considered avirulent (resistant response), while 3 and 4 were considered virulent (susceptible response).

Stripe rust

Seedlings inoculated with race PSTv-37 were placed in a clean dark growth chamber for 16-24 hours at 13℃ at 98% humidity and then incubated in a growth chamber at 17℃/ 12℃ (day/night) and 16-hour photoperiod. Disease reaction was assessed 16-18 days after inoculation on a scale of 0-to-9 (Chen et al., 2002; McIntosh et al., 1995; Qayoum & Line, 1985) where IT 0=no visible signs or symptoms; 1=necrotic or chlorotic flecks with no sporulation; 2=necrotic and/or chlorotic blotches or stripes with no sporulation; 3=necrotic and/or chlorotic blotches or stripes with only a trace of sporulation; 4,5 and 6= necrotic and/or chlorotic blotches or stripes with light, intermediate and moderate sporulation, respectively; and 7,8 and 9= abundant sporulation with necrotic and/or chlorotic stripes or blotches, chlorosis behind the sporulation

area, and no chlorosis or necrosis, respectively. Plants with ITs 0-3 were considered resistant, 4-6 were considered intermediate and 7-9 were considered susceptible.

Genotyping

Five hundred and sixty seven winter wheat accessions were genotyped by the Triticeae Coordinated Agricultural Project (T-CAP) using the Illumina iSelect 9K wheat chip (Cavanagh et al., 2013). The remaining eight accessions were not used in association analysis since they had no genotype data.

Association mapping

Imputation, population structure and kinship

Missing genotype data for the 567 accessions were imputed using fastPhase 1.3 (Scheet & Stephens, 2006) software with default settings. Markers with a minor allele frequency (MAF) of <0.05 were removed since the power of association with the phenotype are low for these alleles (Myles et al., 2009).

Principal component (PC) analysis to control for population structure (Q-matrix) (Price et al., 2006) was estimated using the PRINCOMP procedure in SAS 9.3® (SAS Institute, Cary, NC). Principal components that explain 25% and 50% cumulative variation were used in mixed models for association analysis. Also an identity-by-state matrix (K-matrix) (Zhao et al., 2007) which is estimated as a centered relatedness matrix in Gemma 0.92 (Zhou & Stephens, 2012) was used to control for population relatedness.

Marker-trait association

Four linear regression models were used to test for marker-trait associations. For a simple model without population structure and kinship, a Wilcoxon rank sum test was performed in SAS 9.3® using the npar1way procedure (Mamidi et al., 2011). The other models that contain

kinship in them (kinship, PC2+kinship, PC20+kinship) were performed in Gemma 0.92 (Zhou & Stephens, 2012; Gurung et al., 2014). The best model was selected using two criteria: Mean Squared Difference (MSD) between observed and expected *p*-values (Sujan Mamidi et al., 2011) and plots of expected *p*-values versus observed *p*-values (Q-Q plots). The model with the smallest MSD was considered the best since random marker p-values follow a uniform distribution (Yu et al., 2006). The best model with smallest MSD is also equivalent to the model with Q-Q plot closest to the diagonal line of best fit. The expected *p*-values were calculated by dividing rank with number of markers. A marker was considered significant if its *p*-value (pFDR) was less than 0.1 for the best model after false discovery rate (FDR) multiple comparison correction (Benjamini & Yekutieli, 2001) calculated using the multtest procedure in SAS 9.3. Furthermore, stepwise regression was performed on all significant markers using the REG procedure in SAS 9.3 in order to determine the minimum number of SNPs independently associated with disease resistance (Gurung et al., 2014; Mamidi et al., 2014). The selected markers from stepwise regression explain the most phenotypic variation similar to variation explained by all markers considered together for each trait.

Results

Seedling screening

The majority of accessions had infection type (IT) scores of 3 for each of the five races of *Pt.* Infection type scores ranged from 1 to 4 (Fig. 2.2). Fifteen (2.6%), 11 (1.9%), 28 (4.7%), 20 (3.5%), and 12 (2.1%) accessions were resistant to races, THBL, MCDL, TDBG, TBDJ and MFPS, respectively (Fig. 3.2). For each of these races, the largest numbers of resistant accessions have IT scores of 2. Among the 575 accessions screened with the stripe rust pathogen race PSTv-37, disease reaction ranged from immunity (IT=0) to complete susceptibility (IT=9)

(Fig. 4.2). Sixty-nine (12%), 73 (12.7%), and 433 (75.3) accessions were very resistant (IT=0-3), moderately resistant (IT=4-6) and susceptible (IT=7-9), respectively. Among the accessions that were very resistant, three accessions were immune and originated from Georgia, Egypt and Chile, respectively. There were six accessions that were resistant to all five races of *Pt* and the one race of *Pst* tested in this experiment (Table 2.3). All the six resistant accessions originated from Iran.

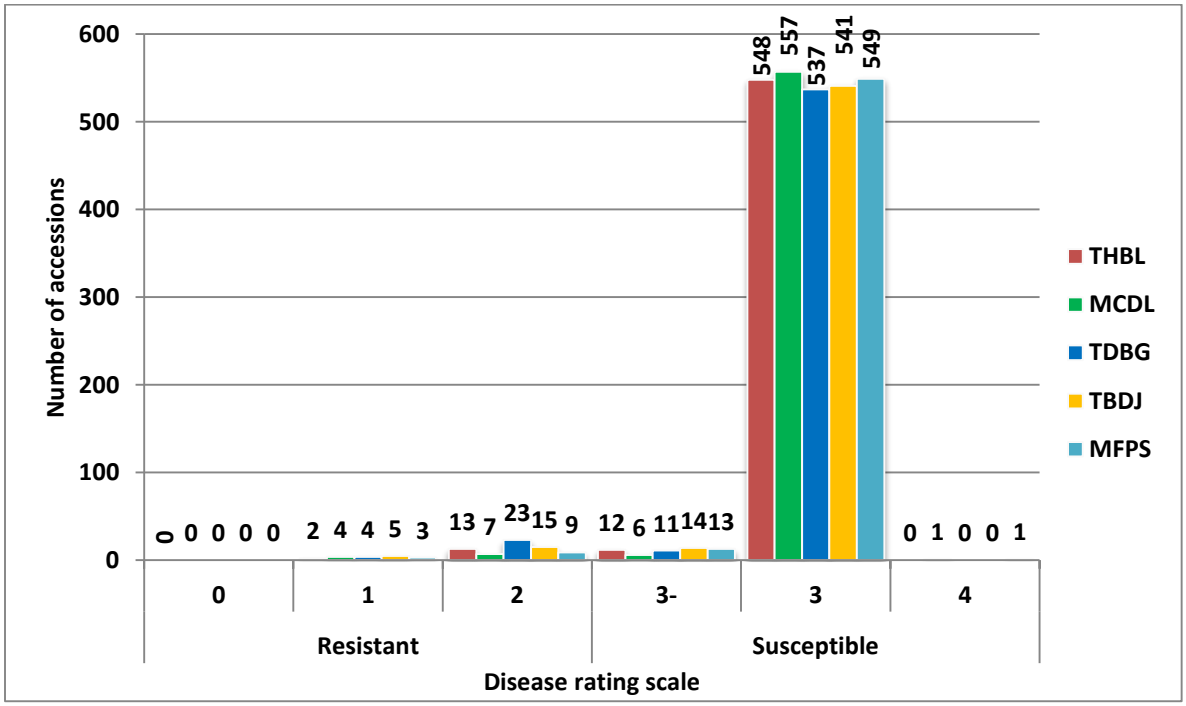

Figure 2.3. Phenotypic distribution of infection types to five races of the leaf rust pathogen. The number of lines is shown on the y-axis and the disease rating scale on the x-axis. Disease was scored on a scale of 0-4.

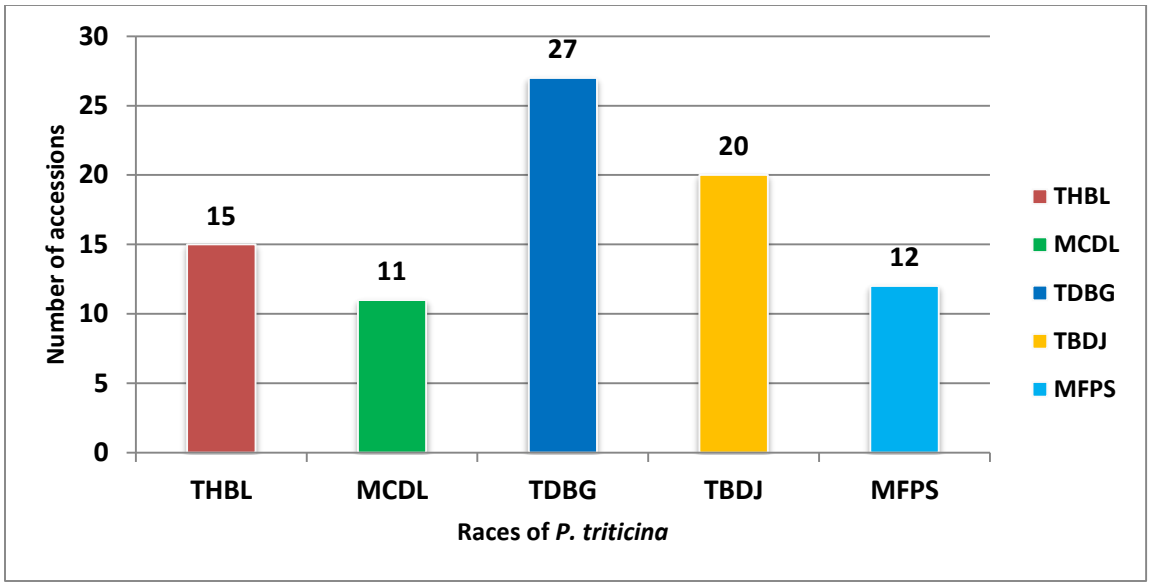

Figure 3.3. Number of resistant lines for each race of the leaf rust pathogen tested. A total of 575 lines were screened at the seedling stage with five races of the leaf rust pathogen. A score of ≤2+ was considered resistant and ≥3 was considered susceptible.

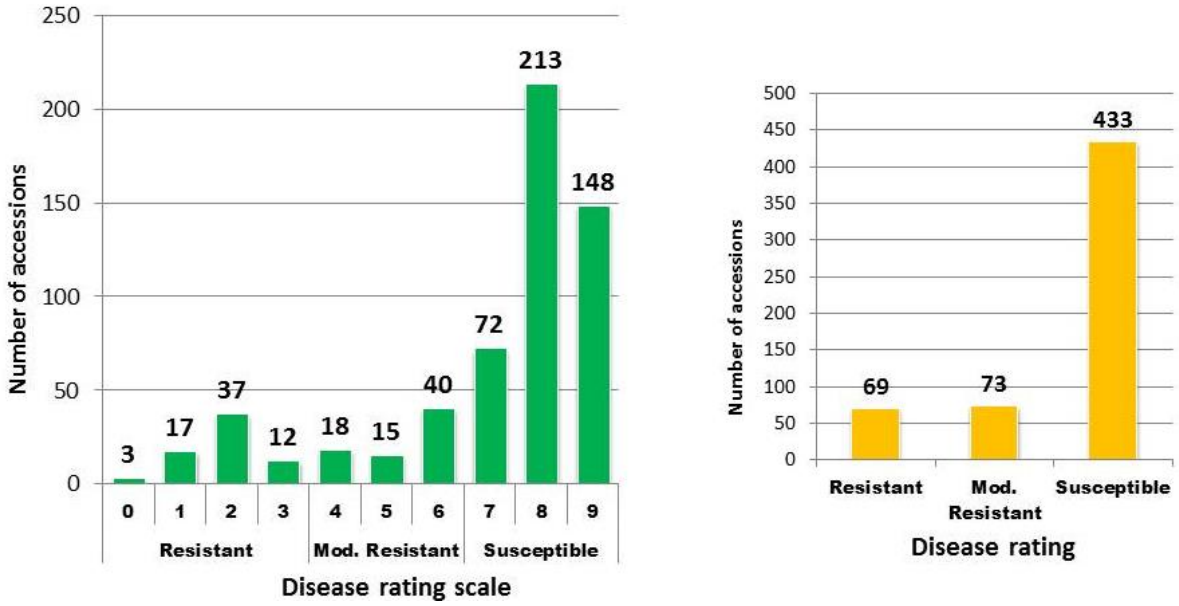

Figure 4.3. Phenotypic distribution of infection types produced by the stripe rust pathogen race PSTv-37. The number of lines is shown on the y-axis and the disease rating scale on the x-axis. Disease was scored on a scale of 0-9. A score of ≤6 was considered resistant and ≥7 was considered susceptible.

Table 2.3. Infection type of six accessions that show resistance to all five races of the leaf rust pathogen and one race of the stripe rust pathogen.

Accession	Origin	MCDL	MFPS	THBL	TDBG	TBDJ	PSTv-37 (stripe)
PI621539	Iran	2	2	2-	;2	2/3	4
PI621674	Iran	1	2-	2+	2-	1/2	6
PI622111	Iran	2	1	2-	1	12-	1
PI622129	Iran	1	1	1	1	1	1
PI622243	Iran	1	2	2-	2	12-	1
PI622246	Iran	1	1	1	1	1	1

(-) indicates slightly smaller uredinia than the standard, (+) indicates slighter larger uredinia, two infection types (IT)(such as 12-) indicates a mixed reaction on the same leaf, two IT separated by slash (such as 2/3) indicates varying reaction among seedling plants of the same accession (some seedlings are 2, other seedlings are 3).

Imputation, population structure, kinship and model selection

A total of 5633 high quality SNPs were obtained from 9K chip. The 1.4% missing SNP data were imputed and 4234 markers with MAF of greater than 5% were selected for further analysis. These markers covered the whole wheat genome as summarized in Figure 5.3. The chromosomal locations of some markers are unknown because they were not polymorphic among all the mapping populations used to construct a consensus map. Principal component (PC) analysis showed that 20 PCs explained a cumulative 50.43% genotype variation. The first two PCs that explained about 25% cumulative genotype variation were chosen for further analysis of this population. The two PCs showed that the population grouped in two major clusters. One cluster contained accessions mainly from Asia and the other cluster had accessions mainly from Europe. The few accessions from Africa and South America grouped with accessions from Europe (Figure 6.3). Based on MSD values of the four linear models tested, no single model was best for all traits. The best model is described as one with smallest MSD value and whose QQ plot is closest to the diagonal line of best fit. The best models were as follows; Kinship for TDBG and THBL, PC2+Kinship for MCDL and MFPS and PC20+Kinship for TBDJ and PSTv-37 (Table 3.3, Figure 7.3).

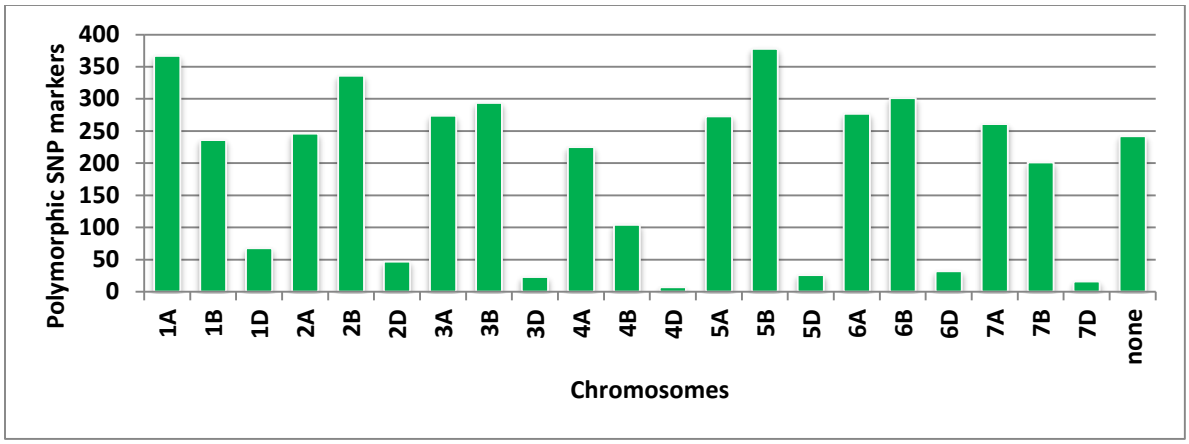

Figure 5.3. Distribution of 4234 SNPs on wheat chromosomes. SNPs with unknown chromosome positions are grouped in "none".

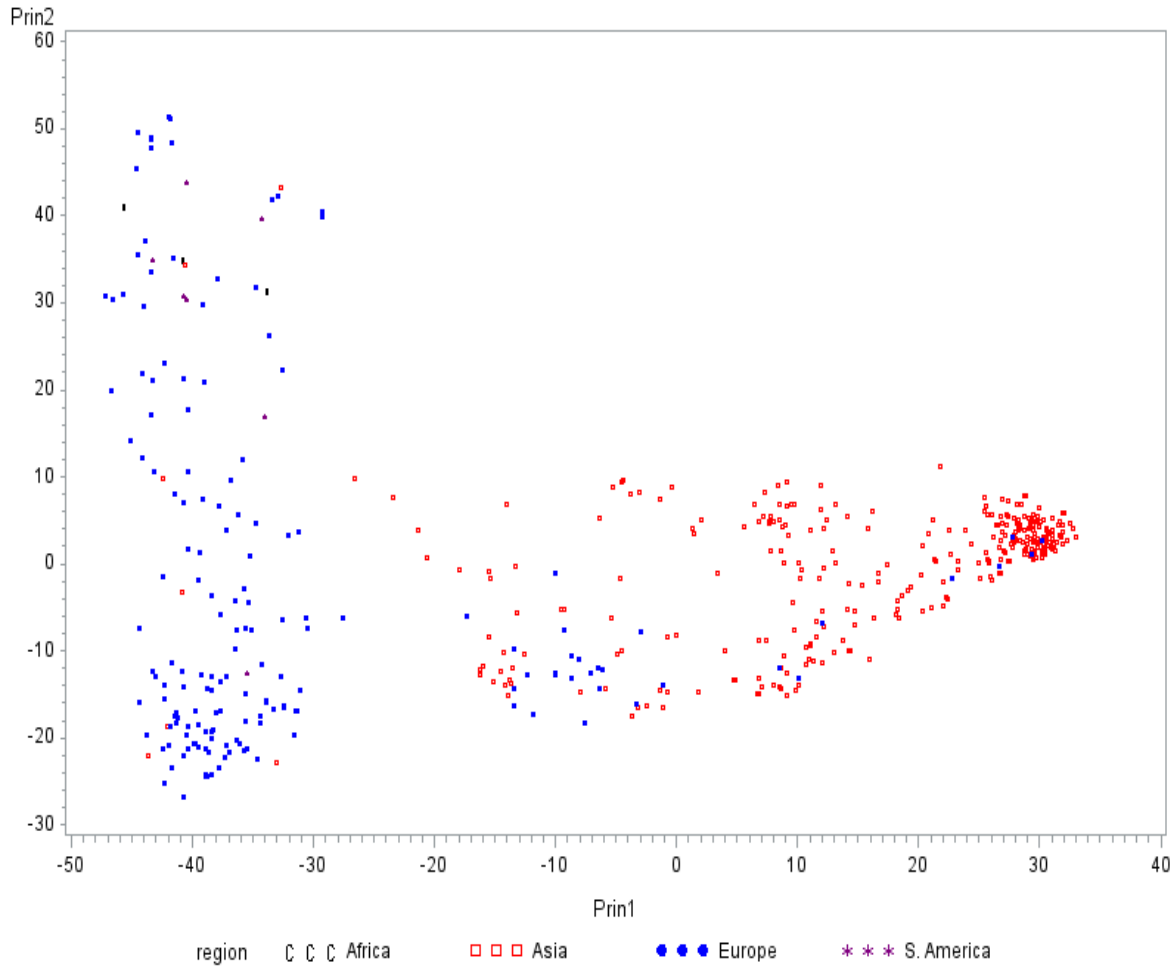

Figure 6.3. A graph showing two principal components obtained from 4234 polymorphic SNPs. PC1 and PC2 explain 19.41% and 5.22% variation, respectively.

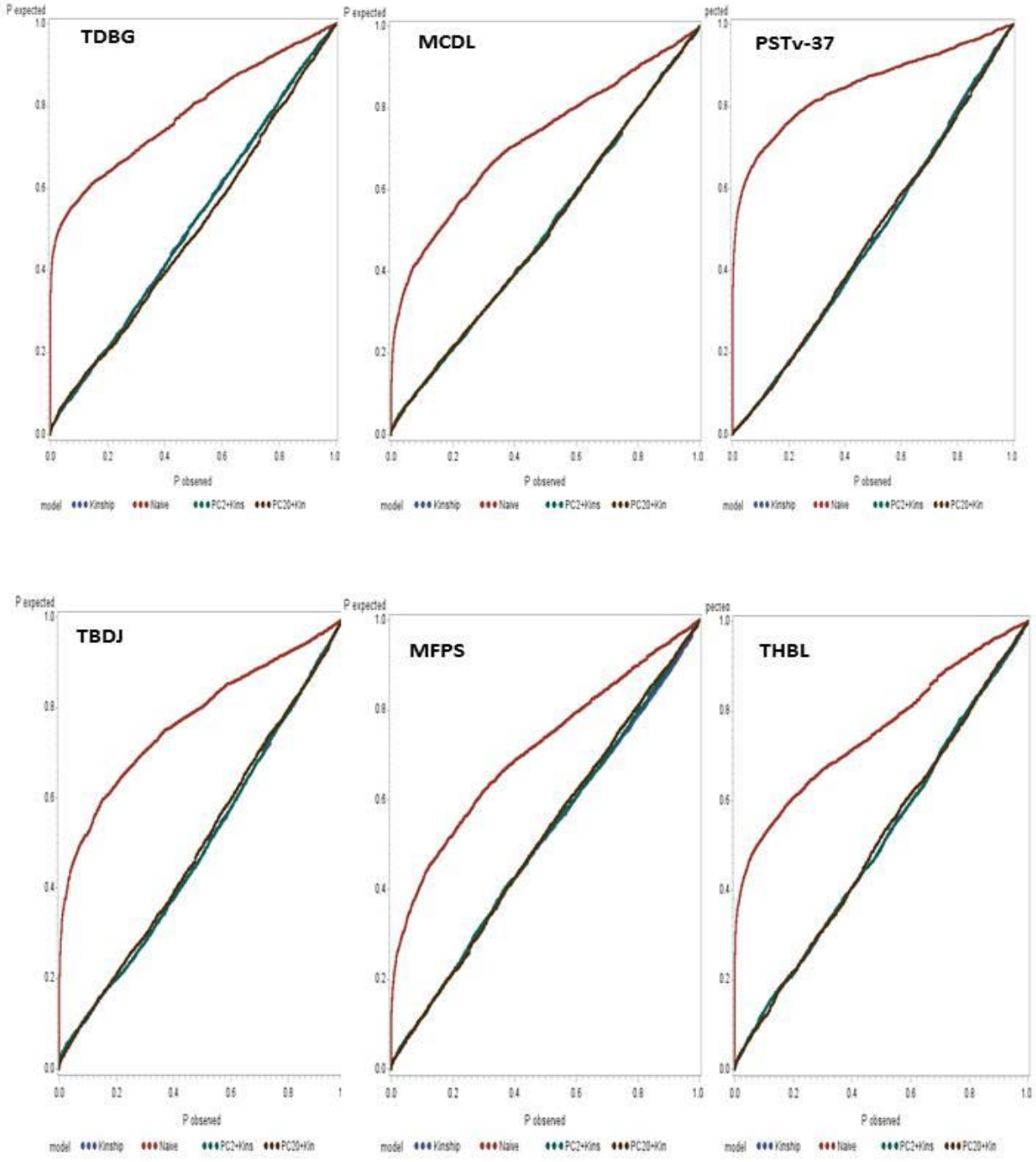

Figure 7.3. Q-Q plots for four models tested for each pathogen race. The observed P-value was plotted against the expected p-value. The best model of regression is closer to the diagonal line.

Table 3.3. Mean square difference (MSD) for each disease race and model. The best model with lowest MSD for each trait is indicated in bold.

	Disease traits					
Model	MCDL	MFPS	TBDJ	TDBG	THBL	PSTv37
Naïve	6.23E-02	5.45E-02	9.39E-02	1.00E-01	8.11E-02	1.57E-01
Kinship	1.03E-04	2.80E-04	2.99E-04	**1.54E-04**	**1.48E-04**	5.32E-04
PC2+Kinship	**9.82E-05**	**2.69E-04**	2.69E-04	1.75E-04	1.63E-04	5.03E-04
PC20+Kinship	1.24E-04	2.84E-04	**8.78E-05**	2.24E-04	1.51E-04	**3.82E-04**

Marker-disease resistance associations

The cutoff point for markers significantly associated with disease resistance was set at pFDR <0.1 after multiple testing correction. Based on this cutoff a total of 73 markers were found associated with one or more of the five races of leaf rust and one race of stripe rust. Seven markers were associated with both leaf rust races MCDL and TBDJ. The 73 markers are distributed on all wheat chromosomes except chromosomes 1D, 2D, 3D, 5D, and 7D. The number of associated markers by race were distributed as follows; 34 for MCDL, 2 for MFPS, 17 for TBDJ, 18 for TDBG, one for THBL and 8 for PSTv-37 (Table 4.2). Stepwise regression carried out to find the minimum number of significant markers for each trait reduced the number of significant markers to 31 distributed as follows; 11 for MCDL, 1 for MFPS, 8 for TBDJ, 10 for TDBG, 1 for THBL and 3 for PSTv-37. Three markers (*wsnp5977, wsnp2126, wsnp8375*) located on chromosomes 3A, 4B and 5B, respectively were significantly associated with both races MCDL and TBDJ. The selected markers from stepwise regression explain the same amount of phenotypic variation explained by all the significant markers obtained for each trait. These results, including the chromosomal positions of significant markers, are summarized in Table 4.3.

Table 4.3. Significant markers associated with each race of rust pathogen. Markers labelled with 'x' were chosen after stepwise regression. #N/A indicates unknown.

Trait	Marker	Chrom[a]	cM[b]	-log$_{10}$(p-value)	pFDR[c]	SNP	MAF[d]	Stepwise Regression
MCDL	wsnp5702	1A	57.95091	5.11	4.61E-03	[T/C]	9.17	
MCDL	wsnp2768	1AS	72.53472	3.19	8.60E-02	[T/C]	6.35	x
MCDL	wsnp295	2B	76.0234	3.25	8.38E-02	[A/C]	5.11	
MCDL	wsnp762	2B	76.0234	3.25	8.38E-02	[A/G]	5.11	
MCDL	wsnp2887	2B	76.0234	3.25	8.38E-02	[T/C]	5.11	
MCDL	wsnp5977	3AL	47.74788	3.47	6.14E-02	[T/C]	39.15	x
MCDL	wsnp6244	3BL	71.14209	5.80	1.65E-03	[T/C]	40.74	x
MCDL	wsnp4030	4A	4.062322	17.18	9.20E-15	[A/G]	38.45	
MCDL	wsnp2816	4A	74.81151	65.56	3.79E-63	[A/G]	15.87	
MCDL	wsnp3756	4AL	93.49469	58.36	6.11E-56	[T/C]	15.70	x
MCDL	wsnp7859	4AL	151.3215	5.70	1.65E-03	[A/G]	31.22	x
MCDL	wsnp2126	4B	16.37032	4.22	2.78E-02	[T/C]	12.70	x
MCDL	wsnp3815	4D	52.44132	5.23	4.05E-03	[T/C]	9.52	
MCDL	wsnp286	4D	52.81113	3.23	8.38E-02	[T/C]	9.17	
MCDL	wsnp8375	5B	82.62131	3.67	5.92E-02	[T/C]	39.51	x
MCDL	wsnp6694	5BL	168.7408	3.22	8.38E-02	[A/G]	34.22	x
MCDL	wsnp6737	6A	89.87239	3.31	8.38E-02	[A/G]	40.74	x
MCDL	wsnp185	6B	73.7018	3.50	5.92E-02	[A/C]	11.82	
MCDL	wsnp3131	6B	73.7018	3.50	5.92E-02	[T/C]	11.82	
MCDL	wsnp3133	6B	73.7018	3.50	5.92E-02	[A/G]	11.82	
MCDL	wsnp5785	6B	73.7018	3.80	5.92E-02	[A/C]	11.99	
MCDL	wsnp6142	6B	73.7018	3.50	5.92E-02	[A/G]	11.82	
MCDL	wsnp6825	6B	73.7018	3.50	5.92E-02	[A/G]	11.82	
MCDL	wsnp6826	6B	73.7018	3.50	5.92E-02	[T/C]	11.82	
MCDL	wsnp7873	6B	73.7018	3.50	5.92E-02	[A/C]	11.82	

Table 4.3. Significant markers associated with each race of rust pathogen (continued). Markers labelled with 'x' were chosen after stepwise regression. #N/A indicates unknown.

Trait	Marker	Chrom[a]	cM[b]	-log$_{10}$(p-value)	pFDR[c]	SNP	MAF[d]	Stepwise Regression
MCDL	wsnp8192	6B	73.7018	3.50	5.92E-02	[T/C]	11.82	
MCDL	wsnp596	6B	83.04075	3.15	9.23E-02	[T/C]	41.80	
MCDL	wsnp55	#N/A	#N/A	3.57	5.92E-02	[A/G]	10.05	
MCDL	wsnp287	#N/A	#N/A	3.57	5.92E-02	[A/G]	10.05	
MCDL	wsnp397	#N/A	#N/A	3.09	9.92E-02	[A/C]	5.64	x
MCDL	wsnp2121	#N/A	#N/A	3.23	8.38E-02	[A/G]	9.17	
MCDL	wsnp2122	#N/A	#N/A	3.57	5.92E-02	[A/G]	10.05	
MCDL	wsnp6340	#N/A	#N/A	3.12	9.48E-02	[A/G]	6.88	
MCDL	wsnp8186	#N/A	#N/A	4.32	2.51E-02	[T/C]	40.04	x
MFPS	wsnp5418	1BS	47.53401	4.34	9.78E-02	[T/C]	5.82	x
MFPS	wsnp7466	1B	47.53401	4.34	9.78E-02	[T/C]	5.82	
PSTV37	wsnp4240	1AL	0	4.27	5.98E-02	[A/G]	28.75	x
PSTV37	wsnp7331	1BL	10.97925	5.64	4.89E-03	[T/G]	20.28	x
PSTV37	wsnp6853	6A	193.6831	4.02	5.98E-02	[A/G]	5.82	
PSTV37	wsnp2416	6A	98.97873	3.95	5.98E-02	[T/G]	8.11	
PSTV37	wsnp3526	6A	98.54792	3.95	5.98E-02	[T/C]	8.11	
PSTV37	wsnp3527	6A	98.54792	3.95	5.98E-02	[T/C]	8.11	
PSTV37	wsnp8110	6A	99.62919	3.95	5.98E-02	[T/G]	8.11	
PSTV37	wsnp62	#N/A	#N/A	7.01	4.17E-04	[A/G]	13.23	x
TBDJ	wsnp3160	1AS	51.12473	6.00	1.54E-03	[T/C]	16.23	x
TBDJ	wsnp435	1BL	30.46871	5.17	3.53E-03	[T/C]	8.47	x
TBDJ	wsnp574	2AS	103.393	5.18	3.53E-03	[T/G]	45.68	x
TBDJ	wsnp295	2B	76.0234	5.65	1.54E-03	[A/C]	5.11	
TBDJ	wsnp762	2B	76.0234	5.65	1.54E-03	[A/G]	5.11	
TBDJ	wsnp2887	2B	76.0234	5.65	1.54E-03	[T/C]	5.11	
TBDJ	wsnp3824	2B	77.53357	5.07	3.91E-03	[A/G]	5.47	
TBDJ	wsnp2557	2B	76.37349	4.86	5.77E-03	[A/G]	5.29	
TBDJ	wsnp3546	3AS	118.0674	5.74	1.54E-03	[T/C]	7.76	x
TBDJ	wsnp5977	3AL	47.74788	3.43	9.16E-02	[T/C]	39.15	x

Table 4.3. Significant markers associated with each race of rust pathogen (continued). Markers labelled with 'x' were chosen after stepwise regression. #N/A indicates unknown.

Trait	Marker	Chrom[a]	cM[b]	-log$_{10}$(p-value)	pFDR[c]	SNP	MAF[d]	Stepwise Regression
TBDJ	wsnp54	4A	192.365	3.75	5.28E-02	[T/G]	11.29	
TBDJ	wsnp285	4A	192.365	3.75	5.28E-02	[T/G]	11.29	
TBDJ	wsnp8389	4A	192.365	3.59	7.06E-02	[A/G]	11.46	
TBDJ	wsnp2126	4B	16.37032	5.70	1.54E-03	[T/C]	12.70	x
TBDJ	wsnp8375	5B	82.62131	3.43	9.16E-02	[T/C]	39.51	x
TBDJ	wsnp619	6D2S	45.41536	3.84	5.05E-02	[T/C]	5.11	x
TBDJ	wsnp6340	#N/A	#N/A	4.75	6.68E-03	[A/G]	6.88	
TDBG	wsnp6290	1BL	30.46871	6.43	1.37E-03	[A/G]	7.58	x
TDBG	wsnp2195	2A	97.14017	3.36	8.92E-02	[A/G]	18.69	
TDBG	wsnp7429	2A	91.42041	3.36	8.92E-02	[A/G]	18.69	
TDBG	wsnp3924	2B	110.8466	3.68	7.68E-02	[A/G]	9.88	
TDBG	wsnp5005	3A	69.46751	3.46	8.92E-02	[T/C]	6.00	
TDBG	wsnp5006	3A	68.77375	3.46	8.92E-02	[T/C]	6.00	
TDBG	wsnp5786	3AS	72.50424	3.37	8.92E-02	[A/G]	7.05	x
TDBG	wsnp1900	4AL	198.8437	3.69	7.68E-02	[A/C]	5.82	x
TDBG	wsnp7014	5A	53.70673	5.43	6.82E-03	[A/G]	23.46	x
TDBG	wsnp2445	5A	122.7194	4.30	3.70E-02	[A/C]	6.70	x
TDBG	wsnp7361	5A	184.8948	3.63	7.75E-02	[A/G]	8.29	
TDBG	wsnp3996	5A	87.89319	3.36	8.92E-02	[T/C]	23.28	x
TDBG	wsnp8395	5B	71.11065	3.44	8.92E-02	[A/G]	5.47	
TDBG	wsnp3699	6BS	95.6662	4.17	4.10E-02	[A/G]	24.87	x
TDBG	wsnp7506	6BS	106.4728	3.99	4.69E-02	[T/C]	8.29	x
TDBG	wsnp7616	6D2S	69.19544	4.52	3.70E-02	[A/G]	11.99	x
TDBG	wsnp5526	7AS	102.8454	4.11	4.11E-02	[A/G]	8.47	x
TDBG	wsnp5000	7B	129.5085	4.33	3.70E-02	[A/C]	6.17	
THBL	wsnp6512	1BS	140.723	8.51	1.29E-05	[T/C]	14.29	

[a]Chrom=Chromosome; [b]cM=Marker position on consensus map; [c]pFDR=Positive false discovery rate; [d]MAF=Minor allele frequency

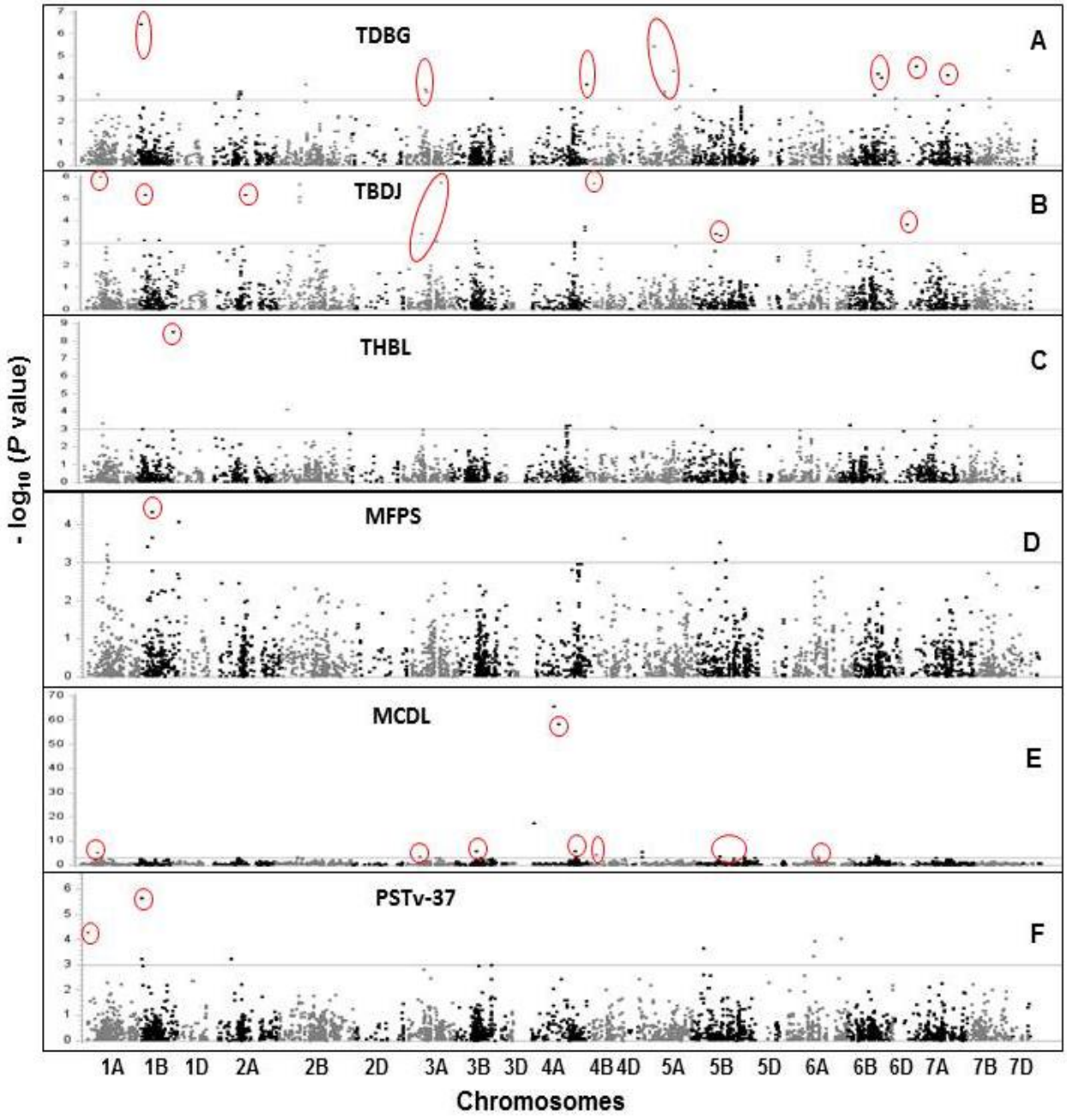

Figure 8.3. Manhattan plots showing SNPs associated with disease resistance. Chromosomes are ordered on the x-axis. The horizontal black line indicate a cutoff point at p-value=0.001. SNPs included in stepwise regression are circled in red.

Discussion

The constant evolution of new races of the leaf rust and stripe rust pathogens continues to threaten winter wheat production in the northern Great Plains of the United States. Host resistance is the preferred strategy for managing these diseases and over 70 and 50 resistance genes against leaf rust and stripe rust, respectively have been identified (Chen, 2005; Mcintosh et al., 2012). The majority of these genes have been overcome by virulent populations of the pathogens. Therefore the search for new sources of resistance to these destructive fungal pathogens is essential. In this study we used association mapping to identify seedling resistance to leaf and stripe rust in winter wheat landraces from diverse geographical origins.

This study identified six landrace accessions that were resistant to all five races of *Pt* and one race of *Pst* tested at the seedling stage. All six accessions originated from Iran and were collected in the same year. Four accessions were collected from Mazandaran province in northern Iran while the other two accessions were each collected from Tehran and Hamadan provinces located in northern and western Iran, respectively. Two of the accessions from Mazandaran (PI 622243 and PI 622246) were collected from the same exact location but they exhibit differential reactions to races of *Pt* tested in this study and dwarf bunt tested elsewhere (X. M. Chen, http://www.ars-grin.gov/npgs/acc/acc_queries.html). This suggests that these two accessions are not duplicates. Field evaluations at two locations in Washington, USA, where stripe rust is a major constraint to wheat production, showed these accessions to be highly resistant to the local stripe rust pathogen population (X. M. Chen, http://www.ars-grin.gov/npgs/acc/acc_queries.html). The identification of highly resistant accessions from Iran is not surprising as Iran is located in the Fertile Crescent which is known as the center of origin and diversity of wheat (Salamini et al., 2002). Additionally, rust epidemics are highly common in

this region which could provide an opportunity for natural selection and maintenance of resistant genotypes by farmers in the region. This also suggests that we might expect to obtain many accessions from Iran with resistance to leaf rust and stripe rust since the co-existence of rust pathogens and wheat is believed to result in accumulation of diverse resistance in wheat (Newton et al., 2010). Though phenotypic and genotypic data show these accessions as different, performing allelism test will confirm if in fact these accessions carry the same or different resistance genes.

Association mapping can produce spurious marker-trait associations if not corrected for population structure and relatedness among individuals (Price et al., 2010; Yu et al., 2006). Population structure analysis grouped the winter wheat accessions in this study into two major subpopulations suggesting population structure could influence marker-trait association. Therefore we tested multiple models taking into consideration relatedness (K) and population structure (Q). Model analysis revealed that the best models are those that accounted for familial relatedness (K) and/or population structure (Q). Also, multiple testing corrections were performed as False Discovery Rates (FDR) to further eliminate false positive associations. Initially, Manhattan plots of p-values showed many significant markers associated with resistance to each race of rust pathogen tested even at a more stringent significant cutoff point of p-value=0.001 (Figure 8.3). After multiple testing corrections, only few markers were found significantly associated implying that many markers obtained before multiple testing correction were false positives. We further applied the power of stepwise regression to identify the minimum number of markers for each race of rust pathogen that explains nearly the same amount of variation explained by all the markers considered together.

Association analysis identified a total of 31 SNP markers in winter wheat landrace accessions associated with seedling resistance to leaf rust and stripe rust. These markers were located on chromosomes 1A, 1B, 2A, 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6B, 6D and 7A. Two markers, *wsnp424 and wsnp73310* associated with resistance to *Pst* race Pstv-37 were located on the long arms of chromosomes 1A and 1B, respectively. Chromosome 1B contains several known stripe rust resistance genes originally from *Triticum aestivum*, which are located on the long arm (*Yr29*) or not assigned to a specific arm (*Yr3a, Yr3b, Yr3c* and *Yr21*). *Yr29* confers adult plant resistance and thus unlikely to be detected at seedling stage, however it can't be ruled out at this point. Therefore the association of marker *wsnp73310* with stripe rust resistance could possibly represent resistance genes *Yr3a, Yr3b, Yr3c, Yr21,* and *Yr29* or a new resistance locus. On chromosome 1A, only seedling resistance gene temporarily designated as *YrDa1* from *T. aestivum* has been previously identified but not assigned to a specific chromosome arm (Chen et al, 1995; Cereal Disease Lab, http://www.ars.usda.gov/main/docs.htm?docid=10342). This suggests *wsnp424* could be a marker representing *YrDa1* or a novel resistance locus for *Pst.* Further investigation using bi-parental population QTL mapping will provide more information about the relationship between *YrDa1* and this locus identified in chromosome 1AL.

Of the 13 chromosomes that contained markers associated with resistance to one or more races of *Pt,* four chromosomes 3A, 4A, 5A and 6D have not been previously known to contain any leaf rust resistance genes originally from *T. aestivum* (McIntosh et al., 1995; Cereal Disease Lab, http://www.ars.usda.gov/main/docs.htm?docid=10342). Therefore the eleven markers identified in these chromosome regions (*wsnp5977, wsnp3546, wsnp5786, wsnp7014, wsnp2445, wsnp3996, wsnp619, wsnp7616, wsnp3756, wsnp7859,* and *wsnp1900*) appear to be associated with novel sources of resistance and could be useful for the identification of leaf rust seedling

resistance genes. Based on the chromosome locations of previously identified leaf rust resistance genes in *T. aestivum* and their effectiveness on *Pt* races used in this study, markers identified in chromosomes 1A, 2A, and 4B could possibly be for *Lr10, Lr11* and *Lr30*, respectively. The markers identified in the other chromosomes could possibly be for seedling resistance genes *Lr31* (4BS), *Lr33* (1BL) and *Lr52* (5BS) that are not included in the differential set but have been previously identified in *T. aestivum.* Comparison to composite wheat map (Saintenac et al., 2013) indicated that marker *wsnp3160* is located in the chromosome region where *Lr10* is mapped. Similarly, markers *wsnp435* and *wsnp6290* are within the region of *Lr33*. Mapping information for *Lr11, Lr30, Lr31,* and *Lr52* are not available to allow for comparison with markers found in chromosomes where these resistance genes are located.

In summary, six winter wheat landraces were identified to have seedling resistance to leaf rust and stripe rust. These accessions can be utilized for resistance breeding of adapted winter wheat cultivars in the United States. A total of 31 markers were found associated with resistance to leaf rust and stripe rust in winter wheat landraces from diverse geographical regions. Of those, 20 markers were identified in chromosomes previously known to contain resistance genes while 11 markers were found in new locations. The markers identified can be useful in marker-assisted selection (MAS). Additional studies are needed to determine whether the markers identified in chromosomes previously known to contain resistance genes correspond to any of the previously characterized leaf and stripe rust resistance genes. Further studies are needed to validate these markers on biparental population so that they can be more useful for resistance breeding.

Acknowledgements

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