CHARACTERIZATION OF NORTH DAKOTA HARD RED SPRING WHEAT FOR STEM AND STRIPE RUST RESISTANCE

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ABSTRACT

Puccinia graminis f. sp. *tritici* (*Pgt*) and *Puccinia striiformis* f. sp. *tritici* (*Pst*) are causal agents of devastating wheat stem and stripe rust diseases, respectively. Both diseases sporadically occur in North Dakota (ND), and stripe rust incidence has been increasing in the Midwest of United States over the last decade. Complete information of rust resistance in ND hard red spring wheat (HRSW) germplasm is not well-established. This study focused on the phenotypic characterization of ND HRSW germplasm for stripe and stem rust resistance and the identification of existing and novel rust resistance genomic loci in this population through genome-wide association study (GWAS). The GWAS has identified several marker-trait associations (MTAs) for both all-stage and adult plant resistance for each rust disease. This information will support the deployment of these resistant loci in wheat varieties by the NDSU HRSW breeding program.

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1. INTRODUCTION

Wheat is one of the most widely grown cereal crops and provides over 18% of daily caloric needs and approximately 21% of the daily dietary protein intake of the world (Shiferaw et al. 2013). Global wheat production yielded a total of 779 million tonnes in 2021/22, the third most produced food crop after sugarcane and maize (Foreign Agricultural Services/USDA 2022). From the total of 779 million tonnes, 44.7 million tonnes were produced in the United States which is the fifth largest wheat producer falling behind European Union, China, Russia, and India (Foreign Agricultural Services/USDA 2022). In the United States, six different types of wheat are grown including hard red spring wheat (HRSW), hard red winter wheat (HRWW), soft red winter wheat (SRWW), hard white wheat (HRW), soft white wheat (SWW), and durum wheat. From the total wheat production of 44.7 million tonnes, spring wheat production measured over 9 million tonnes accounting for 2.8 billion US dollars in 2021 (USDA NASS 2022). North Dakota is the leading producer of hard red spring wheat in the United States with total production of 4.75 million tonnes in 2021. North Dakota produces about 50.6% of the national hard red spring wheat which is valued at over 1.51 billion US dollars (USDA NASS 2022).

Spring wheat production in the United States is being challenged annually by biotic and abiotic diseases that cause severe yield losses. Wheat rust diseases including leaf, stem and stripe rust caused by *Puccinia triticina*, *Puccinia graminis* f. sp. *tritici* and *Puccinia striiformis* f. sp. *tritici*, respectively, are highly challenging diseases damaging global wheat production. (Chen 2005; Leonard and Szabo 2005; Kolmer et al. 2007). Stem rust epidemics used to be prevalent in the United States in the early 20th century where the average yield loss recorded 25.4% in Minnesota, 28.4% in North Dakota, and 19.3% in South Dakota (Singh et al. 2015). The

outbreaks of such devastating epidemics have been under control through the barberry eradication program and the deployment of resistance genes (Kolmer et al. 2007). The durable management of stem rust in the United States has suppressed the emergence of new virulent races resulting in prevalence of a limited number of stem rust races such as QFCSC (Upadhaya et al. 2022). However, the occurrence and migration of virulent stem rust races such as races of Ug99 lineage in wheat growing regions of other countries have become a potential threat to the U.S. wheat production. The sudden appearance of Ug99 race TTKSK and its derivatives have reduced effectiveness of many resistance genes such as *Sr31* and *Sr38* that have been widely deployed (Pretorius et al. 2000; Singh et al. 2015; Olivera et al. 2019). In 2013, the screening of 174 U.S. spring wheat germplasm for Ug99 race group under the field conditions in Njoro, Kenya demonstrated susceptibility in 91.4% of the population (Singh et al. 2015).

The frequency of wheat stripe rust epidemics including recent epidemics in 2021 have been increasing in the United States over the last two decades (Cereal Disease Lab, USDA 2022; Chen 2005; Wan and Chen 2014; Wang et al. 2022; Wan et al. 2016; Chen et al. 2002, 2021). From 2013 to 2017, 20 new races have been identified within only five year period indicating rapid emergence of new virulent races (Wang et al. 2022). Outbreaks of wheat stripe rust are not as prevalent in North Dakota as in the Western United States due to the inoculum availability, and onset of disease, and differences in climatic conditions. But yield losses have been reported in North Dakota in 2015 and 2016 due to higher stripe rust incidence. Genetic control such as resistance gene deployment is the most effective, economical, and environment-friendly strategy to maintain stable crop production (Wang et al. 2022).

The objectives of this research are to characterize North Dakota hard red spring wheat germplasm disease response to wheat stem and stripe rust and conduct Genome-Wide

Association Studies (GWAS) to identify genomic loci associated with rust resistance. This research will facilitate the development of genomic tools to support marker-assisted breeding and genomic selection pipeline for North Dakota State University hard red spring wheat breeding program.

2. LITERATURE REVIEW

2.1. Wheat taxonomy, evolution, and production

Modern bread wheat (*Triticum aestivum* L.) belongs to Poaceae family in the genus *Triticum* L. Wheat has been a staple crop to humans and cultivated for approximately 10,000 years originating in the Fertile Crescent (Haas et al. 2019). Domestication of wheat has focused on certain morphological and physiological traits that satisfy human needs such as larger grain size, a greater number of grains, less seed dormancy, and others. It has driven selection pressure on wild wheat to genetically develop for certain traits (Matsuoka 2011). In addition to selection pressure driven by domestication, natural hybridization caused allopolyploidization resulting in the emergence of tetraploid wheat (Matsuoka 2011). Evolutionary genetics studies have shown that another natural hybridization took place between tetraploid wheat and wild relative species, eventually giving rise to hexaploid wheat (Haas et al. 2019; Matsuoka 2011). Hexaploid wheat, also known as modern bread wheat comprises three subgenomes, A, B, and D (2n = 6x = 42, AABBDD genome), and was derived from hybridization of its progenitors and wild relatives (Haas et al. 2019; Matsuoka 2011; Goncharov 2011). The four most prominent *Triticum* species that were suggested to be highly attributed to wheat evolution, according to morphological and genetic studies, are *T. monococcum* (AA genome), *T. uratu* (AA genome), *T. turgidum* (AABB genome), *T. timopheevii* (AAGG genome) (Matsuoka 2011). Additionally, *Aegilops* species have made genetic contribution to the wheat evolution. *Aegilops speltoides* has the S genome which is known to be very similar to and the progenitor of the B genome of tetraploid wheat, *T. turgidum* (AABB genome), as many research studies have suggested that the initial hybridization took place with *T. monococcum* (AA genome) and *T. uratu* (AA genome) (Goncharov 2011; Haas et al. 2019; Kishii 2019; Matsuoka 2011; Miki et al. 2019). *Aegilops tauschii*, a wild relative of

wheat, is known to contain the DD genome and was proposed to be the main source of the D-genome donor to hexaploidy wheat (AABBDD genome) via hybridization with *T. turgidum* (AABB genome) (Ahmadi et al. 2018; Haas et al. 2019; Matsuoka 2011; Schneider et al. 2008).

Wheat is one of the most significant food crops as it provides approximately 18% of daily caloric needs as well as on average about 21% of the daily dietary protein intake (Shiferaw et al. 2013). Wheat ranked the third most produced food crop in the world with a total production of 779 million tonnes in 2021/22.

2.2. All-stage resistance and adult plant resistance

Genetic disease control, including deploying resistance genes in crops, has been often considered the most favored due to its effectiveness with lower input cost and environmental friendliness compared to chemical control. The constant search for novel sources of resistance to plant diseases caused by microbial pathogens is extremely important. On a molecular level, the pathogens secrete small proteins, called effectors, that interact with resistance proteins (R proteins) inside the host plant cell to induce either resistance or susceptibility depending on the types of interactions (Jones and Dangl 2006). The host resistance or susceptibility triggered by the interaction with effector(s) is called effector-triggered immunity (ETI) or effector-triggered susceptibility (ETS), respectively. In the presence of resistant host, the natural selection drives the pathogens to develop mechanisms to avoid the recognition by the R protein by altering their effector genes (Dangl and McDowell 2006; Jones and Dangl 2006; Van Der Hoorn and Kamoun 2008). The R proteins are known to have three main interaction mechanisms with pathogen effectors i.e. direct interaction, guard, and decoy. R proteins can interact with effector(s) by directly binding to them or act as a guard where R proteins monitor another plant protein that directly interacts with pathogen effector protein (Jones and Dangl 2006; Gururani et al. 2012). R

proteins can also act as a decoy to mimic the target of effectors and they cooperate with other R proteins to trigger the immune system once decoys interact with effectors (Gururani et al. 2012; Kourelis and Van Der Hoorn 2018).

Wheat plants have two major classes of resistance to wheat rust diseases. All-stage resistance (ASR), is mostly race-specific, qualitative, expressed at all stages, and also called seedling resistance. As ASR is race-specific, it fits the gene-for-gene model (Flor, 1971) where resistance is effective against a pathogen race that contains the corresponding effector gene. The host plant triggers a rapid localized cell-death called hypersensitive response (HR) when such interaction occurs between an R protein and a corresponding effector protein (Klement and Goodman 1967). The majority of identified seedling resistance genes encode nucleotide-binding sites (NBS) and leucine rich repeats (LRR) proteins (Gururani et al. 2012). Besides the NBS-LRR domains, coiled-coil (CC)and a toll/interleukin 1-like receptor (TIR) are common domains present in R proteins (Takken and Goverse 2012). All-stage resistance is described qualitative because it is mainly controlled by a single gene. Therefore, virulent rust races are capable of overcoming ASR genes in a relatively short period of time (Lin and Chen 2007; Chen 2013; Liu et al. 2018).

Adult plant resistance (APR) is more durable than ASR due to its different characteristics. Plants with APR genes are susceptible at seedling stage, but exhibit resistance in later growth stages of plant. Furthermore, the APR is non-race specific and is generally effective against all pathogen races. Plants with APR express reduced disease severity, for example slow-rusting against rust fungi, but still can show susceptible infection type (Chen 2013). APR is often a polygenic trait; thus, it is also sometimes characterized as a quantitative resistance. Additionally, the APR genes do not encode NBS-LRR domains in the protein products, whereas

the ASR proteins mainly consist of NBS-LRRs (Wilcoxson 1981; Li et al. 2006; Chen 2013; Flath et al. 2018). In contrast to ASR genes, identification of APR genes has been relatively difficult due to its quantitative nature and dependency of expression on external conditions (Liu et al. 2020). For rust diseases, only a few numbers of APR genes have been identified including *Lr34/Yr18/Sr57/Ltn1*, *Lr46/Yr29/Sr58/Ltn2*, and *Lr67/Yr46/Sr55/Ltn3*. It is very critical to continuously seek for not only novel ASR genes, but also durable APR genes so that sustainable wheat production can be practiced through gene pyramiding.

2.3. Wheat stem rust

Wheat stem rust is caused by the fungus, *Puccinia graminis* f. sp. *tritici* (*Pgt*), and can result in severe wheat yield losses. Stem rust has a prolonged history as it has been described in ancient Roman history where people held a festival, called Robigalia, to appease the rust gods to protect cereal crops (Leonard and Szabo 2005). Today, wheat stem rust continues to be one of the most devastating wheat diseases in wheat growing regions of the world.

Among three rust pathogens, *Pgt* is considered the most damaging due to its aggressiveness towards not only the leaf blades, but also other plant tissues such as stem, glumes, spike, and the leaf sheath (Eversmeyer 2000). In addition, *Pgt* prefers adequate moisture and higher temperature than other wheat rust fungi for optimum infection of hosts (Roelfs 1985; Eversmeyer 2000). *Pgt* is an obligate biotrophic, heteroecious fungus which requires two different hosts to complete its full macrocyclic lifecycle (McIntosh 2009). The lifecycle of stem rust includes five spore stages; urediniospore, teliospore, basidiospore, pycniospore, and aeciospore. A single-celled dikaryotic urediniospore contains two haploid nuclei and is the main spore that is produced on wheat and capable of re-infecting the same host via asexual propagation (Roelfs and Groth 1988). Urediniospores are often dispersed by wind and able to

travel thousands of miles to infect other wheat plants, referred to as ‘Puccinia Pathway’. Infected wheat plants show the symptoms of brick-red colored uredinia rupturing through the host epidermis of stems, leaf sheaths, and leaf surfaces containing asexual urediniospores (Leonard and Szabo 2005). At the end of the season on maturing hosts, uredial lesions are converted into black streaks producing two-celled teliospores. Initial ploidy of teliospores is dikaryotic, however it becomes diploid with the fusion of haploid nuclei via karyogamy. Teliospores undergo meiosis, germination, and form a germ tube that develops into basidium, a structure that gives rise to four basidiospores with two haplotypes (Roelfs 1985). De Bary first demonstrated that basidiospores can only infect the alternate host, common barberry (*Berberis vulgaris*) where the sexual stage of lifecycle occurs (Pipal 1918). Barberry plants exhibit most of infections on the upper leaf surface where the basidiospores germinate to form two mating types of pycnia (Roelfs 1985). Thereafter, the pycnia produce haploid, unicellular pycniospores that fertilize receptive hyphae of a different mating type to form dikaryotic aecia on the lower surface of barberry leaf. Dikaryotic aeciospores produced from aecia are capable of infecting wheat plants and create pustules called uredinia, subsequently repeating the entire lifecycle. The asexual stage of rust fungi occurs on wheat plants whereas the sexual stage takes place on barberry plants (Roelfs 1985, 1988; Roelfs et al. 1997; Leonard and Szabo 2005; Kolmer et al. 2007).

The emergence of new *Pgt* races result from sexual recombination and contribute to *Pgt* genetic diversity (Upadhaya et al. 2022). A number of major stem rust epidemics have outbroken in the early 1900s causing severe wheat yield losses, especially in the Midwestern states including ND, MN, and SD (Singh et al. 2015). Since the barberry eradication program started in the early 90s across the wheat growing regions of the U.S., there has been a huge decline in the emergence of new virulent stem rust population (Kolmer et al. 2007). The durable management

of stem rust disease was achieved by developing stem rust resistant wheat cultivars and implementing barberry eradication program (Leonard and Szabo 2005; Kolmer et al. 2007; Zhao et al. 2016). Consequently, stem rust race diversity in the United States has been limited to a few including the current predominant race QFCSC according to recent race surveys (Upadhaya et al. 2022). Despite the effective control of stem rust for decades, the sudden emergence of a new *Pgt* race in Uganda in 1998 has changed the dynamics of stem rust disease management. This new *Pgt* race was virulent to *Sr31* and *Sr38* which were widely deployed resistance genes, subsequently designated as Ug99 and racetyped as TTKSK (Pretorius et al. 2000; Singh et al. 2015). The Ug99 and other virulent races from the Eastern Africa are rapidly spreading through Africa, the Middle East, Asia, and even Europe. As the pathogen races continue to expand the geographical range, it is threatening the global wheat production (Singh et al. 2015; Olivera et al. 2018).

In 2013, there was an outbreak of a localized, but severe epidemic in Ethiopia by a stem rust race TKTTF. Consequently, nearly 100% yield loss across 10,000 ha on the most widely grown wheat cultivar 'Digalu' occurred (Singh et al. 2015). TKTTF has been reported in Turkey (Mert et al. 2012), the Middle East (Singh et al. 2015), eastern Africa (Olivera et al. 2015), Germany (Olivera Firpo et al. 2017), and England (Lewis et al. 2018). Although TKTTF is unrelated to Ug99 race group, it is still highly virulent to many deployed resistance genes (Olivera et al. 2019; Szabo et al. 2022). The growing concern is the continuous emergence of new virulent *Pgt* races via sexual recombination in the area, as well as the rapid intercontinental dispersal of these races. In fact, identification of TKTTF-related races in Georgia where wheat stem rust is prevalent and common barberry is extensively grown, increases the chances of

introducing new virulent *Pgt* races and potential epidemics (Olivera et al. 2019; Szabo et al. 2022).

Besides sexual recombination, the recent study has reported the somatic hybridization between an Australian isolate *Pgt21-0* and an unknown *Pgt* isolate exchanging whole haplotype nuclei and giving rise to a fully functional Ug99 isolate with virulence to *Sr31* (Li et al. 2019). *Pgt* has various ways to introduce new races that are virulent to many existing resistant *Sr* genes. Therefore, it is important to continue searching for novel sources of resistance in order to avoid potential epidemics.

2.4. Wheat stripe rust

Puccinia striiformis f. sp. *tritici* (*Pst*) is a causal agent of wheat stripe rust disease that belongs to the family of Pucciniaceae of Basidiomycetes. Since stripe rust was first described in 1777 (Hassebrauk 1965), it has gone through several nomenclatural changes, *Uredo glumarum* (Schmidt 1827), *Puccinia striaeformis* (Westerndorp 1854), *Puccinia straminis* (Fuckel 1860), *Puccinia glumarum* (Eriksson and Henning 1894), until the current name was revived (Hylander et al. 1953; Stubbs 1985). In North America, wheat stripe rust was first identified by Ravn in 1915 in the Western United States (Line 2002). Similar to other wheat rust fungi, *Pst* is also an obligate biotroph that requires living plant tissue in order to sustain its lifecycle (Schwessinger 2017).

Pst is more sensitive to environmental conditions for disease development compared to other wheat rust fungi (Chen 2005). The three primary factors that play the most essential role in disease development are moisture, temperature, and wind (Chen 2005). Minimum three hours of continuous moisture promotes effective spore germination, adhesion, and infection on plant surfaces (Rapilly 1979; Chen 2005; Rapilly and Foucault 1976; Chen et al. 2014). In most cases,

high moisture induces spore germination; however, excessive moisture can damage the viability of urediniospores (Chen 2005; Chen et al. 2014). Temperature is as significant factor as moisture in terms of spore germination, infection, survival, latent period, and host resistance. Generally, the optimum temperature for *Pst* infection and growth is in a range of 10-16℃ (Line 2002). High temperature not only the decreases the spore germination rate, but also shortens the latent period of *Pst* (Chen 2005; Milus and Seyran 2004). Studies have shown that unnecessarily low or high temperatures can result in fungal dormancy or inhibition of sporulation (Line 2002; Chen 2005; Sharma-Poudyal et al. 2014). Wind plays an essential role in dispersal of *Pst* spores, and it also reduces the excessive moisture in urediniospores to prolong viability (Chen 2005). In the past, outbreaks of wheat stripe rust were heavily concentrated in the Western United States. However, the occurrence of the disease has been increasing over the last few decades across the U.S. largely due to the increase in the diversity of *Pst* population, adaptation of *Pst* races into different climates, and the emergence of new races (Chen et al. 2021; Wang et al. 2022).

Wheat stripe rust has become one of the most widely distributed and significant wheat production constraint in the United States as well as global wheat growing regions (Wellings 2011). Historical stripe rust epidemics from 1950s to 2010 have resulted in severe yield losses of up to 80% in many countries including the United Kingdom, Australia, New Zealand, Iran, Chile, USA, China, Spain, South Africa, India, Pakistan, Italy, and Czechoslovakia (Wellings 2011). The recent epidemic in the Pacific Northwest U.S. in 2011 recorded average yield losses of 20.8% on commercial wheat cultivars which forced wheat growers to spend more than $70 million on fungicide applications (Wang et al. 2022). Additionally, stripe rust epidemic in 2015 averaged 11.2% yield loss throughout the entire U.S. damaging $8.7 million dollars (Lyon and

Broders 2017). In North Dakota, 5% and 1% of total wheat yield was lost due to the most recent stripe rust epidemics in 2015 and 2016, respectively (Evin et al. 2020).

Pst race distribution in the U.S. is often separated into 12 different epidemiological regions (Wang et al. 2022). The region R1 and R5, covering the state of Washington, have the most diverse *Pst* races due to the inoculum availability, the presence of diverse resistance gene pool, and the optimal climatic conditions (Wang et al. 2022). The predominant *Pst* races from 2019 to 2021 in the U.S. were PSTv-37, PSTv-41, and PSTv-47 (WSU extension resource for growers and researchers 2022). PSTv-37 has been the most predominant *Pst* race in the U.S. for nearly a decade with recent detection in 13 states in 2020 and 2021 (WSU extension resource for growers and researchers, 2022). Based on the virulence formula on a set of 18 *Yr* single-gene differentials, PSTv-37 is virulent to *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2*, but avirulent to *Yr1*, *Yr5*, *Yr10*, *Yr15*, *Yr24*, *Yr32*, *YrSP*, and *Yr76* (Table 3) (Wang et al. 2022). PSTv-41 is virulent to *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr10*, *Yr17*, *Yr24*, *Yr27*, *Yr32*, *Yr43*, *Yr44*, *YrTr1*, *YrExp2* and PSTv-47 is virulent to *Yr1*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2* (Wang et al. 2022).

North Dakota belongs to region R9 which has PSTv-37 and PSTv-52 as the two most frequent *Pst* races. The most recent epidemics of stripe rust in North Dakota was in 2015 and 2016 causing 5% and 1% yield losses of wheat for which PSTv-37 and PSTv-52 were very likely responsible (Hughes 2015; 2016; Wang et al. 2022). The virulence variation of PSTv-52 is not much different from PSTv-37. They both are virulent to the same set of *Yr* genes except for *YrTr1* to which PSTv-52 is avirulent (Wang et al. 2022). Other epidemiological regions also showed the dynamics of *Pst* virulence pattern similar to the Western U.S. and North Dakota indicating the predominant presence of PSTv-37, PSTv-41, and PSTv-52 (Wang et al. 2022).

The emergence of new *Pst* races occur almost every year, mostly in the Western U.S. During the period of 2013 to 2020, the total 25 new races were identified including a race (PSTv-284) from Texas and three races (PSTv-144, PSTv-293, and PSTv-323) were from Montana (Wang et al. 2022; WSU extension resource for growers and researchers 2022). The continuous emergence of new *Pst* races in the U.S. is speculatively caused by the geographical expansion of stripe rust disease toward a diverse range of host resistance (Lyon and Broders 2017). Therefore, the most of new races in the U.S. often show a slight modification in virulence to one or two genes from existing races. Sexual recombination of *Pst* occurs on barberry plants, but the its frequency in US *Pst* population is extremely low. Firstly, the barberry eradication program in the early 20th century has removed a large number of barberry population across the U.S. (Kolmer et al. 2007). Secondly, the climatic conditions are not the most suitable for the viability of *Pst* teliospores because teliospores are often produced in July/August and dry weather inhibits the teliospore germination (Wang and Chen 2015; Wang et al. 2015).

Stripe rust disease also occurs in more than a dozen countries including Canada, China, Ecuador, Egypt, Italy, Mexico, Morocco, and the United States. Since barberry plants are still present in many of these countries, the genetic diversity in *Pst* has been likely increasing via sexual recombination (Chen et al. 2021). The Himalayan and near-Himalayan regions of countries including China, Pakistan, and Nepal are often considered the epicenter of diversity of *Pst* due to the high susceptibility of barberry to *Pst* (Mehmood et al. 2019). Among the 138 races collected from Canada, China, Ecuador, Egypt, Italy, and Mexico, only 18 races have been reported and the remaining 120 races have not been reported in the U.S. (Chen et al. 2021; Bai et al. 2021). PSTv-220, a race with the highest number of virulence genes (13), was detected not only in Mexico, but also in the U.S. suggesting *Pst* can migrate thousands of miles between

North and South America (Chen et al. 2021). The detection of several identical races in China, Egypt, and Italy, particularly China and Italy sharing eight races, indicates that *Pst* is capable of migrating long distance via geographical expansion and infecting susceptible wheat plants in these new geographical regions (Bai et al. 2021; Chen et al. 2021). Migration and incursion of new virulent *Pst* races poses a potential threat to global wheat production. In order to prevent any future epidemics, identifying new resistance genes and developing resistance wheat cultivars must be continued as genetic control provides the most effective and efficient management to control stripe rust (Wang et al. 2022).

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3. CHARACTERIZATION OF NORTH DAKOTA SPRING WHEAT GERMPLASM FOR WHEAT STEM RUST RESISTANCE

3.1. Abstract

The objective of this study is to characterize North Dakota hard red spring wheat (HRSW) germplasm including advanced breeding and historical lines for seedling resistance against the North American native and foreign *Pgt* races under the greenhouse settings. Genome-wide association study (GWAS) was conducted to identify single-nucleotide polymorphism (SNP) markers associated with stem rust resistance in ND HRSW population. GWAS has identified a total of 52 significant SNP markers associated with resistance to four races, TTKSK, TTKTT+, TRTTF, and TTRTF. Some of the significant SNP markers associated with previously uncharacterized genomic regions suggest the possibility of novel stem rust resistance genes which can be deployed in wheat varieties by the NDSU HRSW breeding program.

3.2. Introduction

Global wheat production is challenged by stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) (Soko et al. 2018). Major stem rust epidemics occurred in the United States in the first half of 20th century (Kolmer et al. 2007). These epidemics were due to sexual recombination in *Pgt* populations leading to higher genetic diversity and new combinations of virulence genes (Zhao et al. 2016). Stem rust used to be the biggest priority in the Great Plains until the eradication of common barberry (Leonard and Szabo 2005; Kolmer et al. 2007). With the decline of stem rust virulence, the race structure has been shifting and the number of races were limited to three to five in 2002 (Kolmer et al. 2007). In the 1950s, stem rust race TPMK was predominant and was known to be virulent to *SrTmp*. After deploying *Sr6* in spring wheat, the frequency of TPMK started to decline in the wheat growing regions of the Great Plains (Kolmer

et al. 2007; Faris et al. 2008). The sudden emergence of *Pgt* race QCCJ in 1989 became a serious threat showing virulence to *Rpg-1*, a barley gene that has been providing resistance to *Pgt* since 1950s (Roelfs et al. 1997). QCCJ has expressed virulence to many *Sr* genes including *Sr5, Sr21*, and *Sr35*; however, the virulence of QCCJ started to decrease as spring wheat cultivars with *Sr6* and *Sr13* were resistant (Roelfs et al. 1997).

At global level, the emergence of a new stem rust race with virulence to resistance gene *Sr31* in 1998 in Uganda was reported and named as Ug99 (Pretorius et al. 2000). The very first Ug99 race TTKSK and its variants have become the most destructive stem rust races ever discovered. TTKSK was able to cause severe yield losses because *Sr31* and *Sr38*, widely deployed resistance genes in the region, were susceptible to this race (Singh et al. 2015). In 2013, the U.S. spring wheat varieties and breeding lines were screened for Ug99 and only 4% and 4.6% of the population was resistant at seedling and adult plant stage, respectively (Singh et al. 2015). Ug99 race group and other virulent foreign races has not been detected in the United States, but there is a possibility that these races could arrive considering its rapid migration in Africa, the Middle East, Asia, and Europe (Singh et al. 2015; Olivera et al. 2015, 2019). Interestingly, the majority of stem rust resistance genes identified thus far, including ones confer resistance to Ug99 races, are derived from progenitors and wild relatives of wheat (Olson et al. 2013; Olivera et al. 2018). For example, *Sr27, Sr35*, and *Sr50* that are known to confer resistance to some of the Ug99 races are derived from *Secale cereale* (rye) and *T. monoccocum* (einkorn wheat) (Anugrahwati et al. 2008; Zhang et al. 2010; Olson et al. 2013). Therefore, identifying novel sources of resistance to Ug99 must continue to prevent the devastating epidemics in the future.

Pgt is well known for its ability to migrate over thousands of miles and infect wheat plants, potentially causes an intercontinental disease. The emergence of new virulent races of *Pgt* is due to different mechanisms including asexual reproduction, sexual reproduction, and somatic hybridization (Li et al. 2019). The novel virulent races often render the existing resistance ineffective and become causal agents of stem rust epidemics. Although the *Pgt* race structure is not as diverse in the U.S. as in other wheat growing countries, the potential of new virulent races being introduced still exist. As HRSW is one of the most important crops in ND, evaluation for stem rust is needed in order to prevent future outbreaks of disease.

In the current study, the founder population of 380 North Dakota hard red spring wheat genotypes comprising advanced breeding lines and historical germplasm was evaluated for all stage resistance against one North America stem rust race QCCJ and Ug99 race group. The phenotypic data was subjected to genome-wide association analysis using SNP marker genotypic data to identify genomic loci associated with stem rust resistance.

3.3. Materials and methods

3.3.1. Plant materials and genotyping

The founder population of 380 North Dakota hard red spring wheat genotypes comprising advanced breeding lines and historical germplasm was used in stem rust disease evaluation and Genome-Wide Association Studies (GWAS) to identify novel and existing sources of rust resistance. The spring wheat population was provided by Dr. Andrew Green, spring wheat breeder at North Dakota State University (NDSU), Fargo, North Dakota. The founder population also includes six spring wheat varieties ‘Bolles’, ‘Prosper’, ‘Shelly’, ‘SY McCloud’, ‘SY Soren’, and ‘SY Valda’ which accounted for 21.1% of total acreage planted in North Dakota in 2021

(NASS, USDA 2021). Genotyping of the spring wheat panel was performed by Dr. Andrew Green's laboratory using Infinium iSelect 90K SNP bead chip array (Wang et al. 2014).

3.3.2. Stem rust seedling phenotyping

The 380 hard red spring wheat genotypes were phenotypically evaluated at two-leaf seedling stage with a North American *Pgt* race QCCJ and six foreign *Pgt* races including TTKSK, TTKTT+, TTRTF, TRTTF, TKTTF, and TKKTF+ of which some are known as Ug99 races. QCCJ screening for ASR in the spring wheat population was conducted at Agricultural Experiment Station Greenhouse Complex at North Dakota State University in Fargo, ND. For Ug99 races, the identical set of wheat genotypes was phenotypically evaluated in a biosafety level-3 facility at USDA-ARS Cereal Disease Laboratory in St. Paul, MN by Dr. Matthew Rouse. For each replication, five seeds of each line were planted in 50-cell seedling trays in a randomized complete block design (RCBD). The plants of each line as a whole was considered treatment and the experiment was replicated twice. The spring wheat line 'Morocco' and the barley line 'Steptoe' were used as susceptible checks in this trial. The urediniospores of *Pgt*, previously stored at -80℃, were heat shocked at 42-45℃ for 15 min and hydrated at 80% relative humidity with distilled water for 2 h in room temperature for revitalization (Roelfs et al. 1997). The reactivated spores were then suspended in Soltrol 170 Isoparaffin (Chevron Phillips Chemical Company, The Woodlands TX) and inoculated on the leaves of 'Morocco' and 'Steptoe' seedlings for spore increase. Fresh spores were used for inoculation immediately after being collected off the spore increase plants. A spore suspension at concentration 10^6 spores/ml was prepared in the same manner with Soltrol 170 oil and sprayed evenly on plants. The Soltrol 170 oil was allowed to evaporate from plants for 30 min before transferring them into a dark misting chamber overnight (Solanki et al. 2019). Thereafter, the plants were transferred in the

greenhouse room under a diurnal cycle of 25℃ and 18℃ (day/night) with a 16-hour photoperiod until the disease development.

Every plant of each genotype was evaluated for disease response at 14 days post-inoculation using the Stakman 0 to 4 infection type (IT) scale (Stakman et al. 1962). The IT scores were converted to a 9-point linear scale with ';' to '0', '1-' to '1', '1' to '2', '1+' to '3', '2-' to '4', '2' to '5', '2+' to '6', '3-' to '7', '3' to '8', '3+' to '9', and '4' to '9' to fit into the format of GWAS analysis (Zhang et al. 2014). The weighted mean was calculated depends on the number of plants with dominant IT. For example, if there were five infected plants and four of them were scored 8 and one plant was scored 4, then the weighted average would be 7.2 On linear scale, 0 to 6 were considered resistant and 7 to 9 were recorded as susceptible (Zhang et al. 2014).

3.3.3. Genome-wide association study (GWAS)

Prior to GWAS, the genotypic data were first filtered by >5% minor allele frequency (MAF), and the markers that failed to reach 95% call rate were excluded using the GenomeStudio 2.0 software (Illumina, San Diego CA). GWAS was conducted using general linear model (GLM) implemented in GAPIT R Software package (version 3) (Price et al. 2006; Wang and Zhang 2021). The GLM detects the association between a phenotype (y) and markers (Si) by adding a cofactor such as population structure (Q) where it accounts residuals (e) partially and adjust the effect of testing markers to reduce false positives (Wang and Zhang 2021). Marker-trait associations (MTAs) with the false discovery rate (FDR)-adjusted p-value less than 0.05 were selected as significant. The flanking sequences of SNP markers were BLAST searched against the Chinese Spring wheat genome sequence (IWGSC_RefSeq _v2.1) to identify physical locations of the markers. The identified MTAs from this study were compared to the

markers flanking previously known resistance genes. If the physical position of compared MTAs was within 10Mb of known resistant gene/region, they were considered as associated with the existing genes. But if the physical distance range was outside 10Mb or no comparison was observed, MTAs were considered newly identified.

3.4. Results

3.4.1. Disease response to *P. graminis* f. sp. *tritici*

At 10 to 12 days post-inoculation, the symptom started to develop with chlorotic flecking on the leaf surfaces, followed by the formation of brick red-colored pustules rupturing through the epidermal cell of leaf tissue producing stem rust urediniospores. As a result, 94% (356 genotypes) of population showed resistant reaction, whereas only 6% (22 genotypes) expressed susceptible reaction against QCCJ. From the resistant genotypes, 252 lines were rated between 0 to 3 infection and 104 lines were rated between 4 to 6 showing high resistance against QCCJ.

The percentage of HRSW genotypes resistant to foreign virulent *Pgt* races was very low except for TKTTF. The number of genotypes from the founder population resistant to TTKSK were 24 genotypes (7%), to TTKTT+ was 6 genotypes (2%), to TTRTF was 33 genotypes (8%), to TRTTF was 76 genotypes (20%), to TKTTF was 307 genotype (81%) and to TKKTF+ was 126 genotypes (33%). In contrast, the percentage of genotypes resistant to TKTTF was nearly 81% (307 genotypes). The six HRSW genotypes resistant to TTKTT+ were ‘Keene’, ‘ND832’, ‘NDSW16-12W-8’, ‘HY682’, ‘MN11394-6’, and ‘MN10055’.

Table 1. Number of HRSW genotypes evaluated for *Puccinia graminis* f. sp. *tritici*.

Reaction	QCCJ[a]	TTKSK	TTKTT+	TTRTF	TRTTF	TKTTF	TKKTF+
Genotypes screened	380	380	380	380	380	380	380
Resistant	358	24	6	33	76	307	126
Susceptible	22	356	374	347	304	68	253
Percentage of resistance	94%	7%	2%	8%	20%	81%	33%

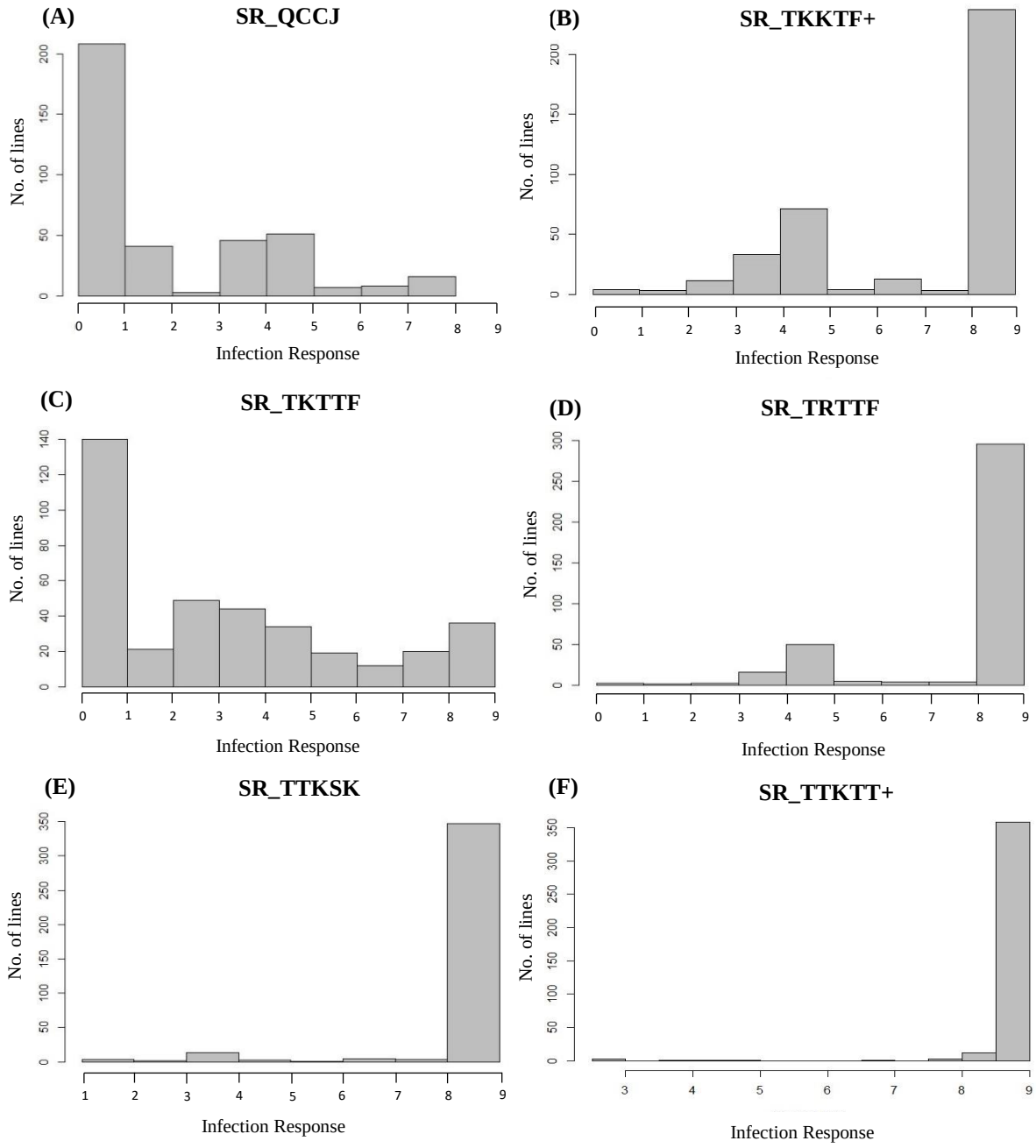

Figure 1. (A-G) The distributions of infection responses to seven *Pgt* races in 380 HRSW genotypes evaluated at seedling stage under greenhouse conditions. SR = Stem rust.

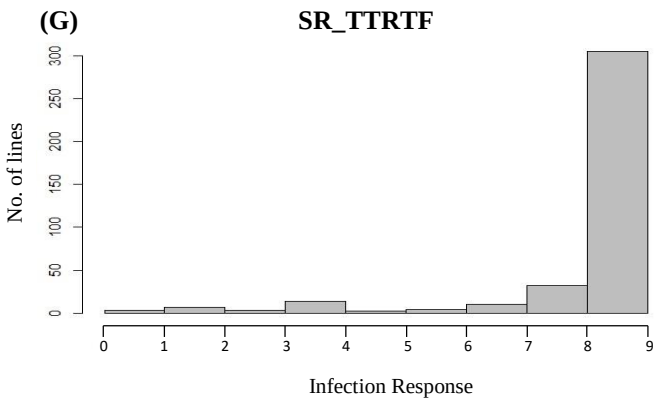

Figure 1. (A-G) The distributions of infection responses to seven *Pgt* races in 380 HRSW genotypes evaluated at seedling stage under greenhouse conditions (continued). SR = Stem rust.

3.4.2. Association analysis for race-specific resistance

After SNP markers with less than 5% MAF and 5% missing data were removed, 21,385 SNPs were used for further GWAS analysis. The GWAS analysis has identified a total of 52 marker-trait associations (MTAs) for all-stage resistance (ASR) against stem rust (Table 2). A number of significant MTAs were identified for ASR to stem rust races, TTKSK, TTKTT+, TRTTF, and TRTF, but unfortunately, there was no association observed above the significant threshold level in response to QCCJ, TKTTF, and TKKTF+.

For TTKSK, three MTAs, IWB64643, IWB29791, and IWA303, were observed on chromosomes 2A, 2D, and 5B, respectively. These MTAs were mapped to regions to which markers flanking previously reported resistance genes have not been mapped before. Despite the resistance in the least number of genotypes to TTKTT+, the highest number of MTAs were identified for ASR at seedling stage. A total of 25 MTAs was distributed on chromosomes 2A, 2B, 2D, 3A, and 7B. In particular, 10 MTAs within 2.5 Mb physical region were identified on chromosome 2A (Table 2). IWB32473, IWB54180, IWB58321, IWB27907, and IWB26148, five highly associated MTAs, were identified on chromosome 2B flanking a locus with a distance of 4.1Mb that has not been reported in the past. In addition, three MTAs, IWB72652,

IWA4075, and IWB56856 on chromosome 3A and 7B were mapped to a region that no existing gene-flanking markers have been reported.

In response to TRTTF, a total of 20 MTAs were identified and distributed across chromosomes 1B to 7A. From the total of 20 MTAs, 10 MTAs, IWA542, IWB22458, IWB7061, IWB43328, IWB63538, IWB73501, IWA6182, IWB882, IWB22191, and IWB22377, were associated with the potential novel resistance loci on chromosome 2A, 3B, 6A, 6B, 6D, and 7A, respectively.

There were only 4 MTAs identified to TTRTF, IWB1009, IWA1397, IWB6159, and IWB28338. Only IWB6159 on chromosome 2B has been a newly identified MTA, whereas the other three MTAs been reported to overlap with markers flanking previously published stem rust resistance genes. These three MTAs were also detected with response to TRTTF suggesting they are likely to be associated with the same gene. In regards to association with resistance to TKTTF and TKKTF+, no MTAs were observed with the p-values above the critical threshold.

Table 2. Marker-trait association (MTA) with response to four *Puccinia graminis* f. sp. *tritici* races in the hard red spring wheat population using general linear model.

MTAs[a]	**Race**	**Chromosome**	**Position**[b]	**Marker alleles**[c]	**P-value**[d]	**MAF**[e]	**Marker effect**[f]
IWB64643*	TTKSK	2A	565,311,161	G/**A**	1.17 X 10^{-2}	0.15	-0.52
IWB29791*	TTKSK	2D	705,823,441	**T**/C	1.17 X 10^{-2}	0.15	0.51
IWA303*	TTKSK	5B	411,930,486	**T**/G	1.58 X 10^{-2}	0.08	0.67
IWB32397	TTKTT+	2A	15,451,882	T/**C**	9.97 X 10^{-4}	0.05	-0.47
IWB36119	TTKTT+	2A	15,612,817	C/**T**	9.97 X 10^{-4}	0.06	-0.40
IWB61256	TTKTT+	2A	15,623,703	C/**T**	9.97 X 10^{-4}	0.05	-0.41
IWB14543	TTKTT+	2A	15,883,378	C/**A**	9.97 X 10^{-4}	0.05	-0.43
IWB9989	TTKTT+	2A	16,404,735	**T**/C	9.97 X 10^{-4}	0.06	0.40
IWB32169	TTKTT+	2A	16,433,372	T/**C**	9.97 X 10^{-4}	0.11	-0.37
IWB5947	TTKTT+	2A	16,672,502	G/**A**	9.97 X 10^{-4}	0.06	-0.40
IWB5959	TTKTT+	2A	16,672,508	C/**T**	9.97 X 10^{-4}	0.05	-0.41
IWB240	TTKTT+	2A	17,001,366	G/**T**	9.97 X 10^{-4}	0.05	-0.44

Table 2. Marker-trait association (MTA) with response to four *Puccinia graminis* f. sp. *tritici* races in the hard red spring wheat population using general linear model (continued).

MTAs[a]	Race	Chromosome	Position[b]	Marker alleles[c]	P-value[d]	MAF[e]	Marker effect[f]
IWB35719	TTKTT+	2A	18,017,808	C/**G**	9.97 X 10^{-4}	0.05	-0.42
IWB2305	TTKTT+	2B	16,421,075	**A**/G	1.05 X 10^{-4}	0.04	0.57
IWB32473*	TTKTT+	2B	24,239,830	C/**A**	9.97 X 10^{-4}	0.05	-0.41
IWB54180*	TTKTT+	2B	26,100,866	A/**G**	9.97 X 10^{-4}	0.05	-0.43
IWB58321*	TTKTT+	2B	26,337,247	**A**/C	9.97 X 10^{-4}	0.05	0.41
IWB27907*	TTKTT+	2B	28,368,510	**A**/G	9.97 X 10^{-4}	0.05	0.41
IWB26148*	TTKTT+	2B	28,368,534	G/**A**	9.97 X 10^{-4}	0.05	-0.41
IWB61793	TTKTT+	2D	13,908,108	**A**/G	9.97 X 10^{-4}	0.05	0.40
IWB12495	TTKTT+	2D	14,254,638	C/**T**	9.97 X 10^{-4}	0.05	-0.41
IWB42130	TTKTT+	2D	14,413,782	**A**/G	9.97 X 10^{-4}	0.06	0.40
IWB13316	TTKTT+	2D	15,875,881	**T**/C	9.97 X 10^{-4}	0.06	0.40
IWB35702	TTKTT+	2D	17,656,661	**A**/C	9.97 X 10^{-4}	0.06	0.40
IWB55908	TTKTT+	2D	27,892,957	**G**/A	9.97 X 10^{-4}	0.05	0.41
IWB72652*	TTKTT+	3A	543,178,759	A/**G**	9.97 X 10^{-4}	0.06	-0.41
IWA4075*	TTKTT+	3A	543,178,759	C/**T**	9.97 X 10^{-4}	0.06	-0.40
IWB56856*	TTKTT+	7B	643,353,125	A/**G**	1.05 X 10^{-4}	0.09	-0.44
IWB45439	TRTTF	1B	7,039,325	T/**C**	8.92 X 10^{-4}	0.13	**-0.85**
IWB1009	TRTTF	1D	8,185,547	**G**/T	1.80 X 10^{-3}	0.19	0.61
IWA1397	TRTTF	1D	8,185,547	**C**/A	2.33 X 10^{-3}	0.13	**0.91**
IWA542*	TRTTF	2A	692,755,867	T/**C**	1.59 X 10^{-3}	0.31	-0.58
IWB22458*	TRTTF	2A	705,826,664	**C**/T	1.59 X 10^{-3}	0.06	**1.10**
IWB7061*	TRTTF	3B	745,733,304	**G**/A	2.41 X 10^{-3}	0.17	0.76
IWB72429	TRTTF	6A	6,596,918	**C**/A	1.59 X 10^{-3}	0.22	0.62
IWB43805	TRTTF	6A	6,732,787	C/**T**	1.59 X 10^{-3}	0.15	-0.68
IWB72958	TRTTF	6A	6,733,332	C/**T**	1.59 X 10^{-3}	0.15	-0.69
IWB72957	TRTTF	6A	6,733,400	C/**T**	1.59 X 10^{-3}	0.15	-0.68
IWA7913	TRTTF	6A	6,739,607	A/**G**	2.27 X 10^{-3}	0.14	-0.70
IWB28338	TRTTF	6A	12,392,199	**C**/T	1.59 X 10^{-3}	0.10	**0.86**
IWB43328*	TRTTF	6A	462,631,624	T/**C**	2.51 X 10^{-3}	0.24	-0.55

Table 2. Marker-trait association (MTA) with response to four *Puccinia graminis* f. sp. *tritici* races in the hard red spring wheat population using general linear model (continued).

MTAs[a]	Race	Chromosome	Position[b]	Marker alleles[c]	P-value[d]	MAF[e]	Marker effect[f]
IWA1098	TRTTF	6A	606,767,947	A/**G**	1.59 X 10^{-3}	0.18	-0.65
IWB63538*	TRTTF	6B	576,850,582	G/**A**	1.55 X 10^{-3}	0.24	-0.61
IWB73501*	TRTTF	6B	576,850,582	G/**A**	1.59 X 10^{-3}	0.23	-0.60
IWA6182*	TRTTF	6B	606,765,822	T/**C**	1.59 X 10^{-3}	0.18	-0.64
IWB882*	TRTTF	6D	6,734,033	**G**/T	2.27 X 10^{-3}	0.15	0.68
IWB22191*	TRTTF	6D	12,393,183	**G**/A	1.45 X 10^{-3}	0.07	**1.17**
IWB22377*	TRTTF	7A	47,444,468	C/**A**	1.59 X 10^{-3}	0.22	-0.64
IWB1009	TTRTF	1D	8,185,547	**G**/T	1.44 X 10^{-2}	0.20	0.55
IWA1397	TTRTF	1D	8,185,547	**C**/A	1.58 X 10^{-2}	0.13	**0.80**
IWB6159*	TTRTF	2B	72,610,551	**G**/A	1.58 X 10^{-2}	0.17	0.55
IWB28338	TTRTF	6A	12,392,199	**C**/T	1.58 X 10^{-2}	0.10	0.70

[a] Marker-trait association (MTA) in bold is associated with more than a single *Pgt* race. MTAs with an asterisk (*) have not been reported to overlap with previously published stem rust resistance genes or flanking markers.

[b] Physical position of the single-nucleotide polymorphism (SNP) markers based on the Chinese Spring reference genome sequence version 2.1, a hexaploidy wheat, available on International Wheat Genome Sequencing Consortium (IWGSC).

[c] Major/minor allele of corresponding MTA. The allele in bold is associated with stem rust resistance.

[d] False discovery rate (FDR)-adjusted P-value.

[e] Minor allele frequency of the SNPs.

[f] Positive allelic effect indicates major allele is associated with stem rust resistance, whereas negative allelic effect indicates minor allele is associated with stem rust resistance. The absolute value of highest allelic effect indicates the MTA has the greatest effect on association with resistance among other markers. The absolute value of allelic effect ≥ 0.8 was considered relatively high.

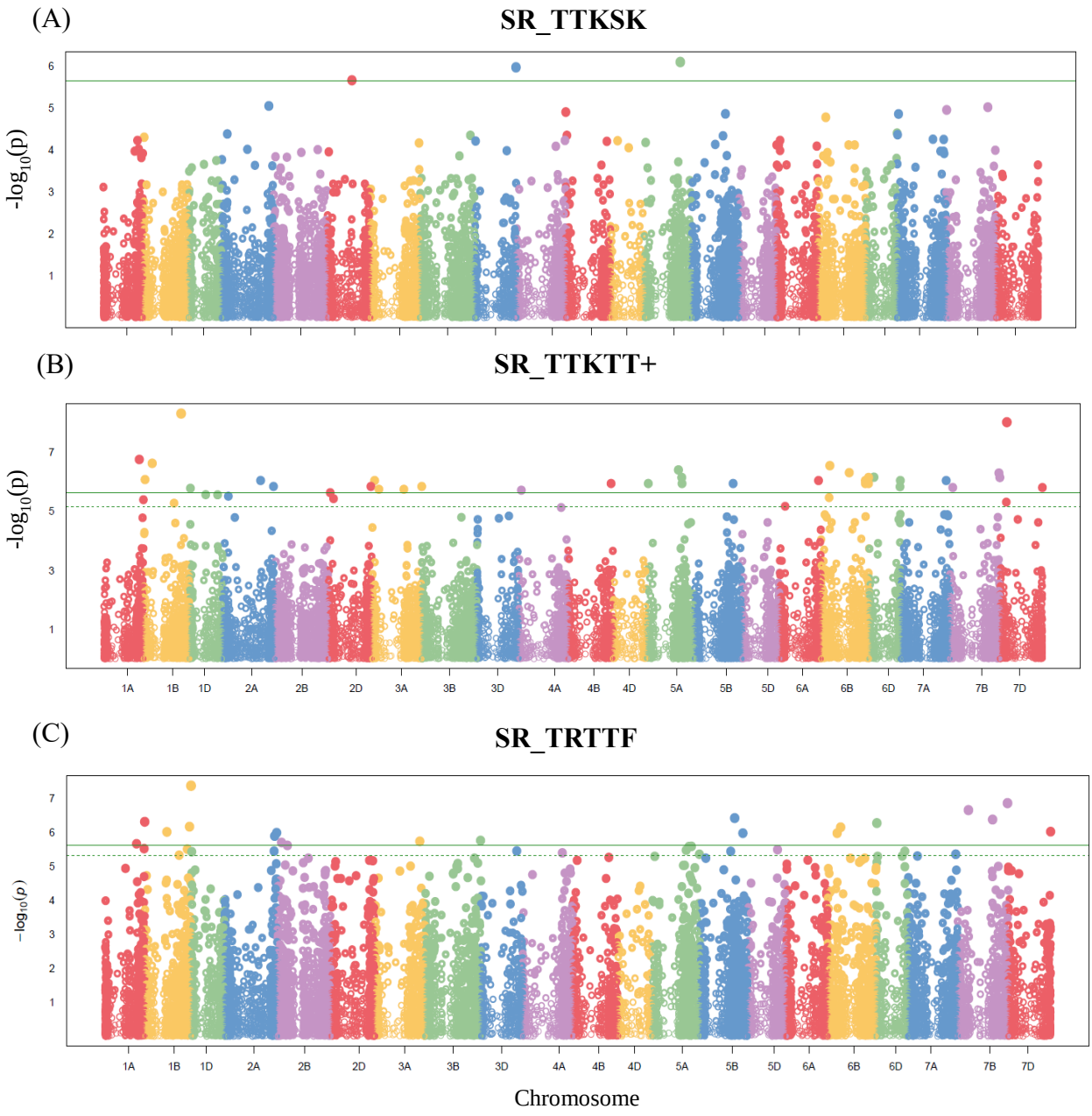

Figure 2. (A-D) Manhattan plots of marker-trait associations (MTAs) with disease responses in ND HRSW genotypes to four *Pgt* races. The horizontal green line indicates significance level at P-value ≤ 0.001. The horizontal dotted green line indicates significance level at FDR ≤ 0.05.

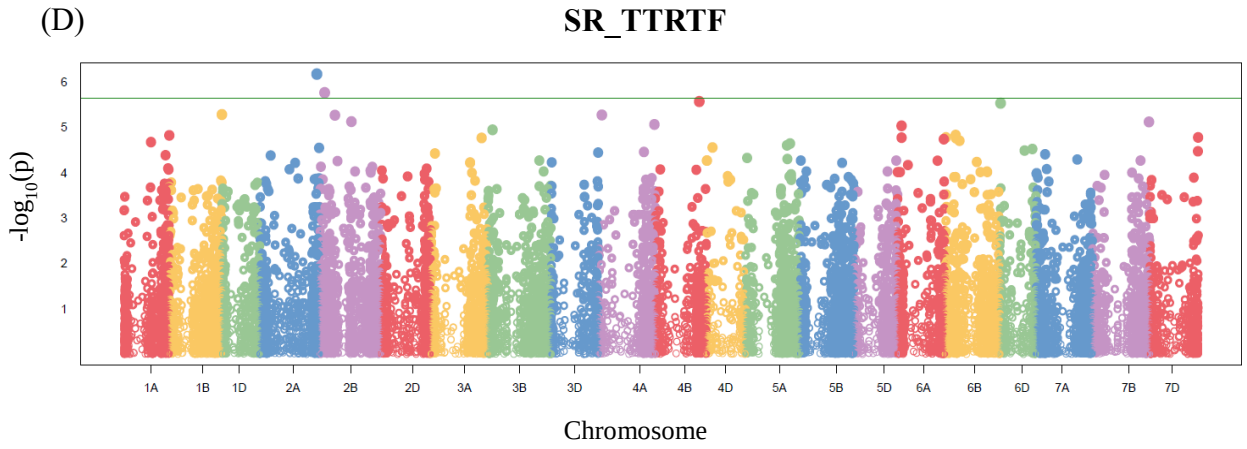

Figure 2. (A-D) Manhattan plots of marker-trait associations (MTAs) with disease responses in ND HRSW genotypes to four *Pgt* races. The horizontal green line indicates significance level at P-value ≤ 0.001. The horizontal dotted green line indicates significance level at FDR ≤ 0.05 (continued).

3.5. Discussion

In this study, the founder population of 380 spring wheat genotypes was phenotypically evaluated for stem rust resistance against one US race and six foreign races including Ug99 group. *Pgt* race QCCJ was first noticed in 1989 when it showed high virulence on barley plants with *Rpg-1* (Roelfs et al. 1997). In contrast to its virulence on barley and a winter wheat cultivar 'Karl' (Sears et al. 1991), QCCJ was avirulent on most of HRSW in the northern Great Plains (Roelfs et al. 1997; Jin 2011). The two postulated reasons for high resistance in ND HRSW genotypes to QCCJ are QCCJ has been present in northern Great Plains for decades and HRSW in the upper Midwest has always exhibited resistance to QCCJ.

Because the Ug99 and other virulent races prevalent in Eastern Africa and the Middle East are rapidly spreading out towards other wheat growing regions such as Europe and Asia, it is necessary to identify new sources of resistance to prevent the potential epidemics of stem rust disease (Singh et al. 2015; Olivera et al. 2015). MTAs identified on chromosome 2D for resistance to TTKTT+ are IWB61793, IWB12495, IWB42130, IWB13316, and IWB35702 with

the physical position at 13.9 Mb, 14.2 Mb, 14.4 Mb, 15.8 Mb, and 17.6 Mb, respectively. These MTAs covered the total length of 3.7 Mb and were only 0.3 Mb to 3 Mb away from the marker Xwmc111 flanking *Sr46*. The MTAs were also located within a 0.4 Mb to 2.3 Mb range to a SNP marker IWB8481 flanking a QTL (*QSr.abr-2D*) which was found in RIL population crossed between a common wheat landrace PI 374670 and LMPG-6 (Babiker et al. 2015). Therefore, these MTAs are likely to be highly associated with *Qsr.abr-2D*. GWAS has also identified 10 MTAs (Table 2) on chromosome 2A covering a QTL with a total length of 2.5 Mb, only distant about 6.8 Mb to 9.0 Mb from a DArT marker wPt-5839 flanking *Sr34*. Interestingly, IWB2305 was the only MTA mapped in the vicinity of a SNP marker, BS00049499_51, flanking *Lr16* which is also closely linked with *Sr23* (McIntosh and Luig, 1973; Kassa et al. 2017). Therefore, IWB2305 is likely to be associated with *Sr23* which supposedly confers resistance to TTKTT+, as well as, to be present in the germplasm. For resistance to TRTTF, IWA7913 which was previously identified to flank *Sr8a,* was also detected in this association analysis along with five additional MTAs including IWB72429, IWB43805, IWB72958, and IWB28338 at 1.2 Mb and 4.3 Mb on chromosome 6A. These MTAs are highly likely to be associated with *Sr8a* as previously reported (Hiebert et al. 2017). The identical MTAs for *Sr8a* were also in close proximity with a *Sr8155B1* flanking SNP marker IWB10558 about 4.3 Mb away. According to the Cereal Disease Laboratory, *Sr8155B1* is not as prevalent in common wheat as in durum wheat, but is rarely found in CIMMYT bread wheat (Nirmala et al. 2017). IWB45439 was a notable MTA that was located 5.2 Mb away from a *Sr31* flanking marker wPt-8949 on chromosome 1B (Yu et al. 2014). *Sr31* is an alien gene originated from a rye chromosome 1RS, but it has been introgressed into wheat and widely used to improve disease resistance. Unfortunately, the 1RS translocation also includes the transfer of *Sec-1* locus which contains

genes encoding ω-secalins that deteriorates the end-use quality (Li et al. 2016). Hence, important to evaluate lines containing positive allele of IWB45439.

A total of four MTAs was detected in association with TTRTF, but also three of them (IWB1009, IWA1397, and IWB28338) were observed in response to TRTTF. IWB1009 and IWA1397 were mapped to a region in vicinity of markers flanking *Sr33*. IWB28338 showed association with markers flanking *Sr8155B1*, but the speculation on the presence of this gene was eliminated due to its ineffectiveness to TTRTF. For TTKSK, three MTAs (IWB64643, IWB29791, and IWA303) were identified which are located in the genomic region with no previously identified stem rust resistance genes.

The majority of known *Sr* genes, particularly those conferring resistance to Ug99 race group are originated from wild or close relatives of hexaploidy wheat such as *Aegilops, Triticum*, and *Secale* species. Although GWAS has identified an abundance of MTAs that have been closely located to previously reported resistance genes or the markers flanking, but most of the postulated *Sr* genes are from the wheat wild relatives. Therefore, the MTAs have high probability of being another allelic form of known resistance gene or likely to be novel sources of resistance.

In conclusion, our study identified stem rust resistant germplasm in the HRSW population and molecular markers associated with resistance. This research provides basic guidance for fine-mapping of the resistance QTL and support marker-assisted selection to deploy novel rust resistance genes in spring wheat varieties by the NDSU HRSW breeding program.

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4. CHARACTERIZATION OF NORTH DAKOTA SPRING WHEAT GERMPLASM FOR WHEAT STRIPE RUST RESISTANCE

4.1. Abstract

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most important diseases damaging global wheat production. Similar to *Pgt*, *Pst* is a heteroecious fungus and infects barberry, the alternate host, to complete its full lifecycle. The incidence of stripe rust disease has been increasing in the U.S. due to the introduction of new virulent *Pst* races into the vulnerable wheat growing regions. To this day, complete information of stripe rust resistance in ND hard red spring wheat (HRSW) germplasm in unavailable. The objectives of this study are to evaluate ND HRSW germplasm for stripe rust resistance at both seedling and adult plant stages under the greenhouse and field conditions; as well as, to identify previously characterized and novel genomic loci associated with stripe rust all-stage resistance (ASR) and adult plant resistance (APR) through genome-wide association study (GWAS). The association analysis for ASR has identified seven marker-trait associations (MTAs) to PSTv-37 and four MTAs toPSTv-52. On the other hand, a total of six MTAs was observed for APR. This research data will provide support for genomic selection tools and marker-assisted breeding by the NDSU HRSW breeding program.

4.2. Introduction

Puccinia striiformis f. sp. *tritici* (*Pst*), like many other *Puccinia* species, is a macrocyclic heteroecious fungus that requires two distinctive host plants in order to complete its sexual lifecycle (Wellings 2011). For many years, the complete lifecycle of *Pst* has remained unknown because of the unidentified alternate hosts of the fungus. But, the study by Jin et al. (2010) identified that the basidiospores of *Pst* were able to infect the leaf blade of *Berberis chinensis*

and formed aecia producing aeciospores followed by aeciospore infection producing uredinia on wheat and Kentucky bluegrass. Similar to other rust fungi, *Pst* is an obligate biotrophic fungus that requires living plant tissue to survive and cannot be cultured on artificial media (Schwessinger 2017). Environmental conditions are extremely important for the stripe rust fungus and disease development. The optimal conditions conducive for stripe rust are slightly different from other wheat rust fungi as temperature and moisture play critical roles (Chen 2005). Lower temperature in the range 10 to 16°C with high moisture is the most optimal for stripe rust spore germination and host infection, whereas other wheat rust fungi favor higher temperature with adequate moisture (Chen 2005). Geographically, the weather climate and the topography of the state of Washington is the most suitable for stripe rust to thrive, hence the stripe rust in Washington is the most diverse and concentrated compared with other wheat growing regions (Wang et al. 2022).

Because *Puccinia striiformis* f. sp. *tritici* favors cool climates and high moisture environment, the occurrence of stripe rust disease in North Dakota is limited. From 2009 to 2019, stripe rust was reported in seven years including 2015 and 2016 with the highest prevalence in North Dakota. Stripe rust infection on spring wheat was detected at tillering stage, resulting in 5% and 1% yield losses in 2015 and 2016, respectively (Evin et al. 2020). The U.S. stripe rust distribution is separated into 12 different regions based on the epidemiological locations. According to race survey from 2013 to 2017 by Wang et al. (2022), North Dakota is clustered with Minnesota and South Dakota in region R9 and the top four races in R9 are PSTv-37 with 44.4%, PSTv-52 with 28.6%, PSTv-198 with 15.9%, and PSTv-14 with 3.2. Both PSTv-37 and PSTv-52 were the two most frequent races detected in the United States with virulence to the identical nine genes except for virulence of PSTv-37 to one additional gene, *YrTr1* (Wang et

al. 2022). R9 race group expressed virulence to *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2* (Wang et al. 2022). The most of known *Yr* genes that confer resistance to these races are ASR which is race-specific and provides effective resistance, but it becomes susceptible soon after the pathogen overcomes (Line 2002). On the other hand, APR provides more durable resistance than ASR. However, only a few numbers of APR genes have been identified thus far including *Yr18, Yr29,* and *Yr46*. It is hard to identify APR genes due to their nature of being quantitatively inherited and suppressed expression by environmental condition (Liu et al. 2020). Therefore, the identification of *Yr* genes in ND HRSW population is essential for not only pyramiding genes, but also to mitigate the yield losses by stripe rust disease in the future.

4.3. Materials and methods

4.3.1. Plant materials

In this study, the founder population of 380 ND HRSW germplasm was evaluated for stripe rust disease screening to characterize the resistance to *Pst*. The GWAS was conducted to identify novel and known *Yr* genes that confer ASR or APR. This founder population includes advanced breeding lines, commercial varieties, and historical germplasm. Some of spring wheat varieties including ‘Bolles’, ‘Prosper’, ‘Shelly’, ‘Sy McCloud’, ‘Sy Soren’, and ‘Sy Valda’ were responsible for 21.1% of total acreage planted in North Dakota in 2021 (NASS, USDA 2021).

4.3.2. Seedling and adult plant stripe rust disease evaluation

Seedlings of the 380 hard red spring wheat genotypes were phenotypically evaluated with two United States *Pst* races PSTv-37 and PSTv-52 at two-leaf stage at Agricultural Experiment Station Greenhouse Complex at North Dakota State University in Fargo, ND. PSTv-37 and PSTv-57 are not only the widely distributed races across the U.S., but also the two most

predominant races in North Dakota from 2013 to 2017 (Wang et al. 2022). PSTv-37 isolate 'Yr2017-16' and PSTv-52 isolate 'Yr2016-37' were used in this screening. Both 'Yr2017-16' (PSTv-37) and 'Yr2016-37 (PSTv-52) were collected from hard red spring wheat in Stutsman County, North Dakota by Dr. Andrew Friskop laboratory in 2017 and 2016, respectively. PSTv-37 is avirulent to *Yr1*, *5*, *10*, *15*, *24*, *32*, *SP*, and *76*, whereas PSTv-52 avirulent to *Yr1*, *5*, *10*, *15*, *24*, *32*, *SP*, *Tr1*, and *76* (Table 2). To confirm the virulence phenotypes of these *Pst* races, two isolates were racetyped using 18 single *Yr* gene wheat differential lines (Table 3) and an octal system (Wan and Chen 2014; Wan et al. 2016).

For the experimental design of seedling screening, planting five seeds of each line in 50-cell seedling trays in randomized complete block design (RCBD) was considered as one replication. The plants of each line as a whole were examined as treatment and the experiment was replicated twice. The wheat check cultivar 'Avocet S' was included in each 50-cell tray as susceptible checks. After the seeds were planted, every tray was fertilized with Multicote (4) 15-7-15 + 2MgO + Micronutrients (Haifa Group, Israel) and grown in a rust-free greenhouse at 23/19℃ (day/night) with a 16 h photoperiod until inoculation.

The urediniospores of two *Pst* races were removed from -80℃ and heat shocked at 45℃ for 5 min and hydrated at 80% relative humidity with distilled water for 2h under a room temperature condition (McDonald and Strange 1976). The heat shocked spores were suspended in Novec™ 7100 (3M, St. Paul MN) and inoculated on the leaves of susceptible wheat check cultivar 'Prosper' and 'Avocet S' for inoculum increase. Freshly collected spores from the susceptible plants were immediately sprayed in Novec 7100 suspension onto the leaves of 380 wheat genotypes followed by distilled water spray. The inoculum was suspended in Novec 7100 because it has less lethal concentration 50 value and higher evaporation rate than Soltrol 170

(Chevron Phillips Chemical Company, The Woodlands TX). Therefore, it is unnecessary to wait for the leaves to dry after inoculation as the evaporation occurs quickly to enhance the adhesion of spores to the leaf surface. Furthermore, each tray was covered with a plastic dome to maintain 100% relative humidity and placed in a dark growth chamber (Percival Scientific, Perry IA) at 10℃ for first 24 h (Buck et al. 2010). Subsequently, the chamber setting was switched to a diurnal cycle at 14/10℃ (day/night) with a photoperiod of 16 h.

The infection types (ITs) of stripe rust were evaluated 17 to 20 days post-inoculation using 0 to 9 scale where 0 = no visible signs or symptoms, 1 = necrotic or chlorotic flecks with no sporulation, 2 = necrotic or chlorotic blotches or stripes with no sporulation, 3 = necrotic or chlorotic blotches or stripes with trace sporulation, 4 = necrotic or chlorotic blotches or stripes with light sporulation, 5 = necrotic or chlorotic blotches or stripes with intermediate sporulation, 6 = necrotic or chlorotic blotches or stripes with moderate sporulation, 7 = necrotic or chlorotic blotches or stripes with abundant sporulation, 8 = chlorosis behind sporulating areas with abundant sporulation, and 9 = no necrosis or chlorosis with abundant sporulation. Wheat plants expressing ITs in a range of 0 to 6 and 7 to 9 were rated as resistant and susceptible, respectively (Line and Qayoum 1992).

The APR greenhouse trial for stripe rust was carried out in Fargo, ND, and the APR field trials were conducted in Prosper and Langdon, ND, as well as, in Mt. Vernon and Pullman, WA. The 380 HRSW germplasm that were previously used in seedling phenotyping, were also tested for APR screening. For the evaluation of adult plant disease response to stripe rust, infection types (ITs) and disease severity (DS) were measured separately. IT is a degree of rust phenotype which is characterized by the size of sporulating pustules and the presence of chlorosis around the pustules depending on the host resistance using 0 to 9 scale (Line and Qayoum 1992; Chen et

al. 2014). DS is described as the percentage of infected area covering flag leaves (Line and Qayoum 1992; Liu et al. 2020a). Disease reaction was evaluated twice, a week apart, when the susceptible checks were showing more than 60% DS.

The WA field trial was carried out by Dr. Xianming Chen and his laboratory. The HRSW plants were evaluated for stripe rust disease at two different growth stages based on the Feekes scale (Miller 1992). The disease responses to stripe rust were measured at Feekes 3 and 10.53 under natural infection. For ND field experiment, two replications of the HRSW lines were planted in a RCBD and a hill plot method with 15 to 20 seeds of each line. In addition to field trials, the APR screening was also conducted at AES-Greenhouse Complex at NDSU in Fargo, ND. Five seeds of each 380 HRSW lines were planted in a single cone-shaped pot and were maintained at 25℃ and 18℃ (day/night) with a 16-hour photoperiod until the plants were grown to the Feekes scale 10.0 to 10.5 (Miller 1992). The inoculum for both North Dakota field and greenhouse APR trial used the mixture of multiple isolates of a *Pst* race PSTv-37 that were collected from different field locations in recent years. The inoculum was suspended in Soltrol 170 (Chevron Phillips Chemical Company, The Woodlands TX) and Novec™ 7100 (3M, St. Paul MN) for field and greenhouse, respectively, and sprayed onto the adaxial and abaxial sides of leaves at booting-stage. Particularly for greenhouse trial, the plants were sprayed with distilled water afterwards and covered in black, non-transparent plastic bags to maintain high humidity and darkness for 24 h at 12℃. Thereafter, the plants were maintained at 18/14℃ (day/night) with a photoperiod of 16 h until further disease evaluation.

Table 3. Virulence and avirulence phenotypes of Puccinia striiformis f. sp. tritici races.

Race	Year of collection	Isolate location	Virulent to *Yr* genes	Avirulent to *Yr* genes
PSTv-37	2017	ND	*Yr6*, *7*, *8*, *9*, *17*, *27*, *43*, *44*, *Tr1*, and *Exp2*	*Yr1*, *5*, *10*, *15*, *24*, *32*, *SP*, and *76*
PSTv-52	2016	ND	*Yr6*, *7*, *8*, *9*, *17*, *27*, *43*, *44*, and *Exp2*	*Yr1*, *5*, *10*, *15*, *24*, *32*, *SP*, *Tr1*, and *76*

ND, North Dakota.

4.3.3. Genome-wide association study (GWAS)

After 380 HRSW lines were genotyped with iSelect 90K SNP bead chip array, the SNP markers were first filtered by >5% minor allele frequency (MAF), then markers that failed to reach 95% call rate were removed using GenomeStudio 2.0 software (Illumina, San Diego CA). GWAS (version 3) was conducted using general linear model (GLM) implemented in GAPIT R Software package (version 3) (Price et al. 2006; Wang and Zhang 2021). The GLM detects the association between a phenotype (y) and markers (Si) by adding a cofactor such as population structure (Q) where it accounts residuals (e) partially and adjust the effect of testing markers to reduce false positives (Wang and Zhang 2021). Marker-trait associations (MTAs) with the false discovery rate (FDR)-adjusted p-value less than 0.05 were selected as the significant SNP markers. The flanking sequences of SNP markers were BLAST against the Chinese Spring wheat genome sequence (IWGSC_RefSeq _v2.1) to obtain the physical locations of the identified MTAs. The identified MTAs from this study were compared to the markers flanking previously known *Yr* genes. If the physical position of compared MTAs was within 10Mb, they were considered as associated with the existing genes. But if the physical distance range was outside 10Mb or no comparison was observed, MTAs were considered newly identified.

4.4. Results

4.4.1. Phenotypic evaluation of hard red spring wheat seedlings

The visible symptoms of stripe rust like flecking and circular-shaped chlorosis started to appear at about 14 to 16 days after they were incubated in growth chamber. At nearly 20 days post-inoculation, the yellow to orange-colored pustules were fully developed on the upper leaf surface producing urediniospores.

Evaluation of the HRSW seedlings showed susceptibility to PSTv-37; whereas, the HRSW seedlings displayed relative resistance to PSTv-52 (Table 4). Based on 0 to 9 infection type scale (Line and Qayoum 1992), the plants ranged from 0 to 6 were considered as resistant. As a result of phenotyping, 24% and 59% of the spring wheat genotypes were resistance to races PSTv-37 and PSTv-52, respectively. Among several resistant varieties, 'LCS Nitro', 'Buck Pronto', 'Sy McCloud', 'OKLEE', 'SD4393', 'KWS Cochise', 'ND804', '11YUYR1', and 'SD4607' were consistently resistant to both stripe rust races with ITs ranging from 1 to 2. On the other hand, some of the most planted varieties in ND in 2021 including 'Bolles', 'Prosper', 'Sy Soren', 'Sy Valda' were highly susceptible to both *Pst* races. 'Shelly', another top grown spring wheat cultivar in ND, was susceptible to PSTv-37 (IT = 8.5) but resistant to PSTv-57 (IT = 4.0).

Table 4. Number of HRSW genotypes evaluated for *Puccinia striiformis* f. sp. *tritici*.

Trait	PSTv-37	PSTv-52
Genotypes screened	380	380
Resistant	125	237
Susceptible	255	143
Percentage of resistance	33%	62%

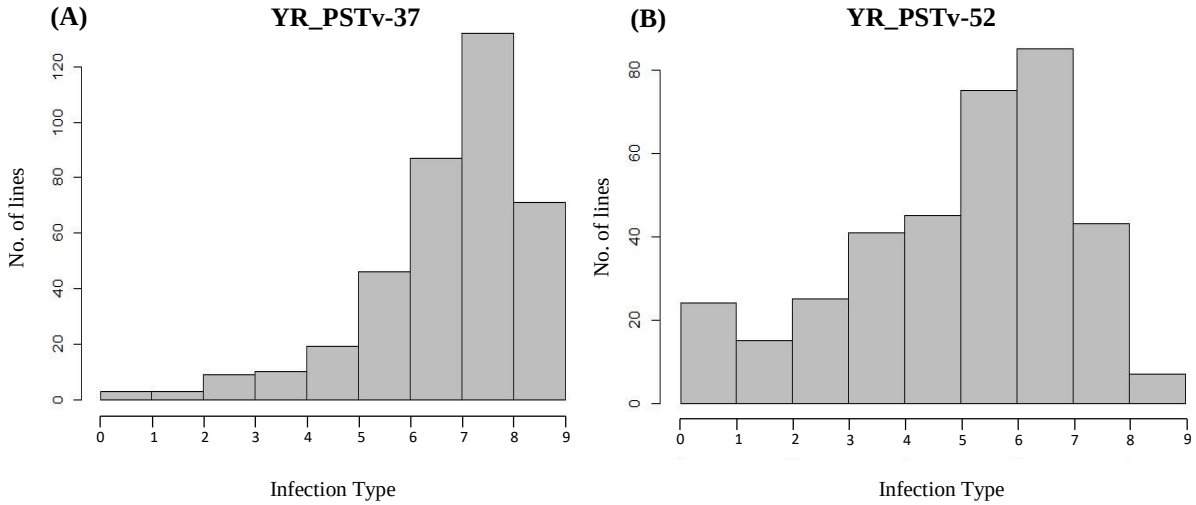

Figure 3. (A-B) The distributions of infection types to two *Pst* races in 380 HRSW genotypes evaluated at seedling stage under the greenhouse settings. YR = Stripe rust.

4.4.2. Phenotyping adult plant resistance

Under the greenhouse conditions, the adult plants started to display the visible signs and symptoms of stripe rust infection at approximately 20 days post-inoculation in growth chamber and the yellow to orange-colored pustules completely formed on the flag leaves about 23 days post-inoculation. The infection type (IT) and disease severity (DS) were separately measured for the adult plant resistance screening. For the greenhouse trial in Fargo, ND, 13% of the 380 HRSW lines were resistant for IT and 70% of them were resistant for DS. Several HRSW lines including ‘Reeder’, ‘ND813’, ‘00S0219-10W’, ‘F9N12-0152’, ‘NDHRS16-13-98’, ‘NDSW0601’, ‘NDSW0914’, and ‘NDHRS16-16-554’ showed high resistance to stripe rust with low percentage of DS.

In the APR field trial in Mt. Vernon, WA, 32% of the HRSW genotypes were resistant to stripe rust in regards to IT. Also, 34% of the HRSW genotypes were resistant in regards to DS. The HRSW lines that were resistant under the greenhouse conditions were also resistant to stripe

rust under natural infection in Mt. Vernon field trial. Also. Several HRSW genotypes with resistance at adult plant stage were susceptible when infected at 3.0 Feekes scale growth stage. Stripe rust disease was not present in Prosper and Langdon, ND and Pullman, WA due to severe heat and drought. Therefore, the disease response of adult plants was not evaluated.

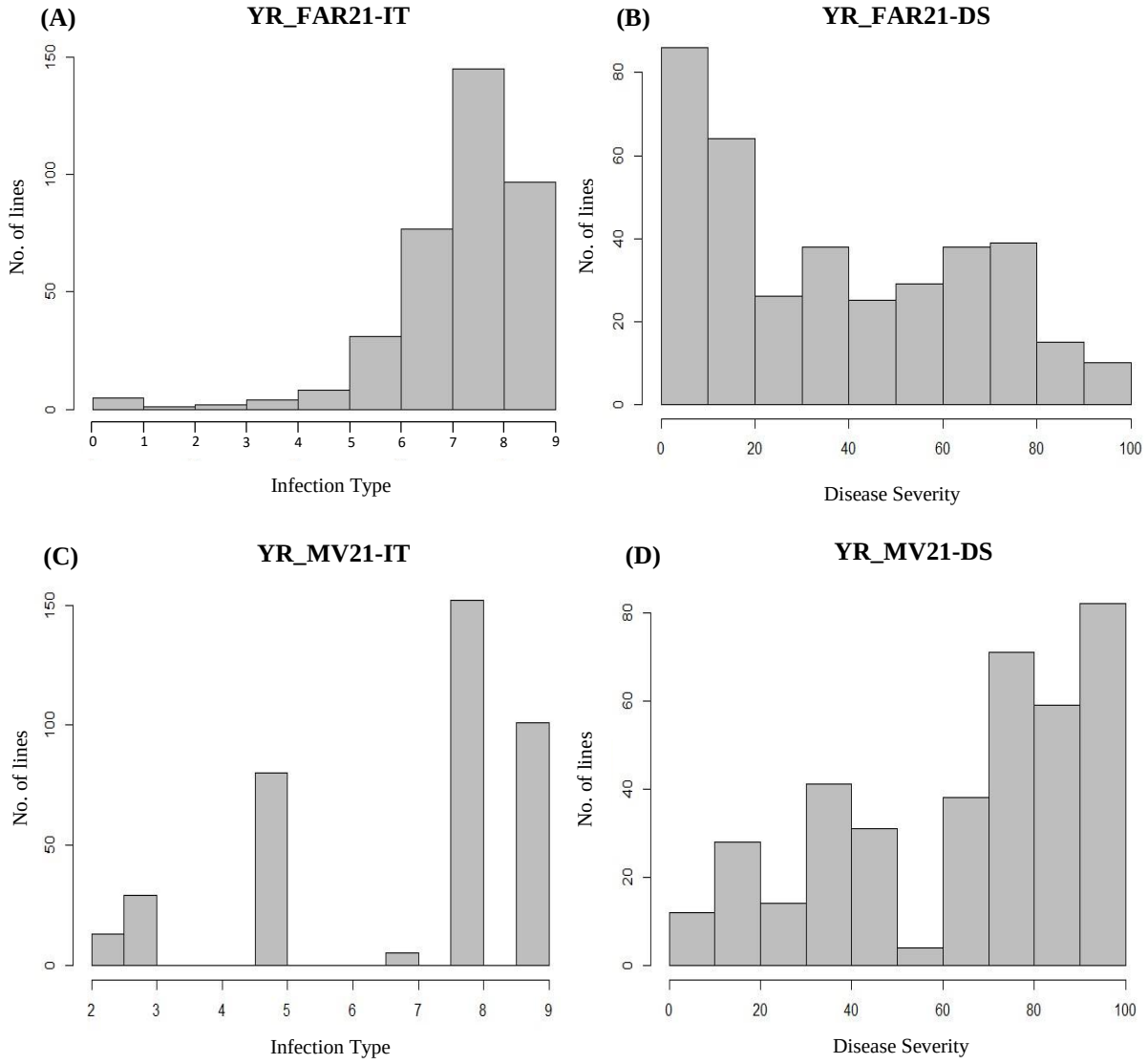

Figure 4. (A-D) The evaluation of adult plant resistance (APR) to stripe rust in the 380 HRSW germplasm under the greenhouse and field settings. APR trial in Fargo was conducted in the greenhouse, whereas the trial in Mt. Vernon was done in the field. YR = Stripe rust; FAR21 = Fargo, ND in 2021; MV21=Mt. Vernon, WA in 2021; IT = Infection type; DS = Disease severity.

4.4.3. Association analysis for race-specific resistance

Association analysis based on the IT to PSTv-37 at seedling stage identified seven significant MTAs on chromosome 1D, 2B, 2D, 5A, and 7B for ASR (Table 6). IWB7806 was detected on near 11.5 Mb of chromosome 1D. IWB57806, IWB36415, and IWB4009 between 232.3 Mb and 234.6 Mb on chromosome 2B covered a QTL with a total length of 2.2 Mb. Furthermore, IWB43416, IWB35977, and IWB9373 were identified on chromosome 2D, 5A, and 7B, respectively. In response to PSTv-52, a total of four MTAs was identified and they all were located very closely on chromosome 7A (Table 6). The four MTAs (IWA7755, IWA4845, IWB55310, and IWA4109) were associated with a locus covering 2.1 Mb.

Table 5. Marker-trait association (MTA) for all-stage resistance to two *Puccinia striiformis* f. sp. *tritici* races in the hard red spring wheat population using general linear model.

MTAs[a]	Race	Chromosome	Position[b]	Marker alleles[c]	P-value[d]	MAF[e]	Marker effect[f]
IWB7806*	PSTv-37	1D	11,498,873	**G**/A	1.05×10^{-2}	0.08	0.72
IWB57806*	PSTv-37	2B	232,348,210	**C**/T	1.05×10^{-2}	0.08	0.67
IWB36415*	PSTv-37	2B	233,268,742	**C**/T	1.05×10^{-2}	0.08	0.67
IWB4009*	PSTv-37	2B	234,636,215	**G**/A	1.05×10^{-2}	0.08	0.67
IWB43416*	PSTv-37	2D	31,806,260	T/**C**	1.05×10^{-2}	0.07	-0.73
IWB35977*	PSTv-37	5A	569,469,628	**A**/G	6.39×10^{-3}	0.17	0.71
IWB9373*	PSTv-37	7B	684,458,037	T/**C**	1.05×10^{-2}	0.44	-0.43
IWA7755	PSTv-52	7A	623,451,341	**G**/A	3.01×10^{-3}	0.18	0.75
IWA4845	PSTv-52	7A	623,451,405	**G**/A	3.01×10^{-3}	0.18	0.76
IWB55310	PSTv-52	7A	623,451,405	**G**/A	3.01×10^{-3}	0.18	0.75
IWA4109	PSTv-52	7A	625,640,119	**C**/T	3.01×10^{-3}	0.18	0.75

[a] Marker-trait associations (MTAs) with an asterisk (*) have not been reported to overlap with previously published stripe rust resistance genes or flanking markers.

[b] Physical position of the single-nucleotide polymorphism (SNP) markers based on the Chinese Spring reference genome sequence version 2.1, a hexaploidy wheat, available on International Wheat Genome Sequencing Consortium (IWGSC).
[c] Major/minor allele of corresponding MTA. The allele in bold is associated with stem rust resistance.
[d] False discovery rate (FDR)-adjusted P-value.
[e] Minor allele frequency of the SNPs.
[f] Positive allelic effect indicates major allele is associated with stem rust resistance, whereas negative allelic effect indicates minor allele is associated with stem rust resistance. The absolute value of highest allelic effect indicates the MTA has the greatest effect on association with resistance among other markers

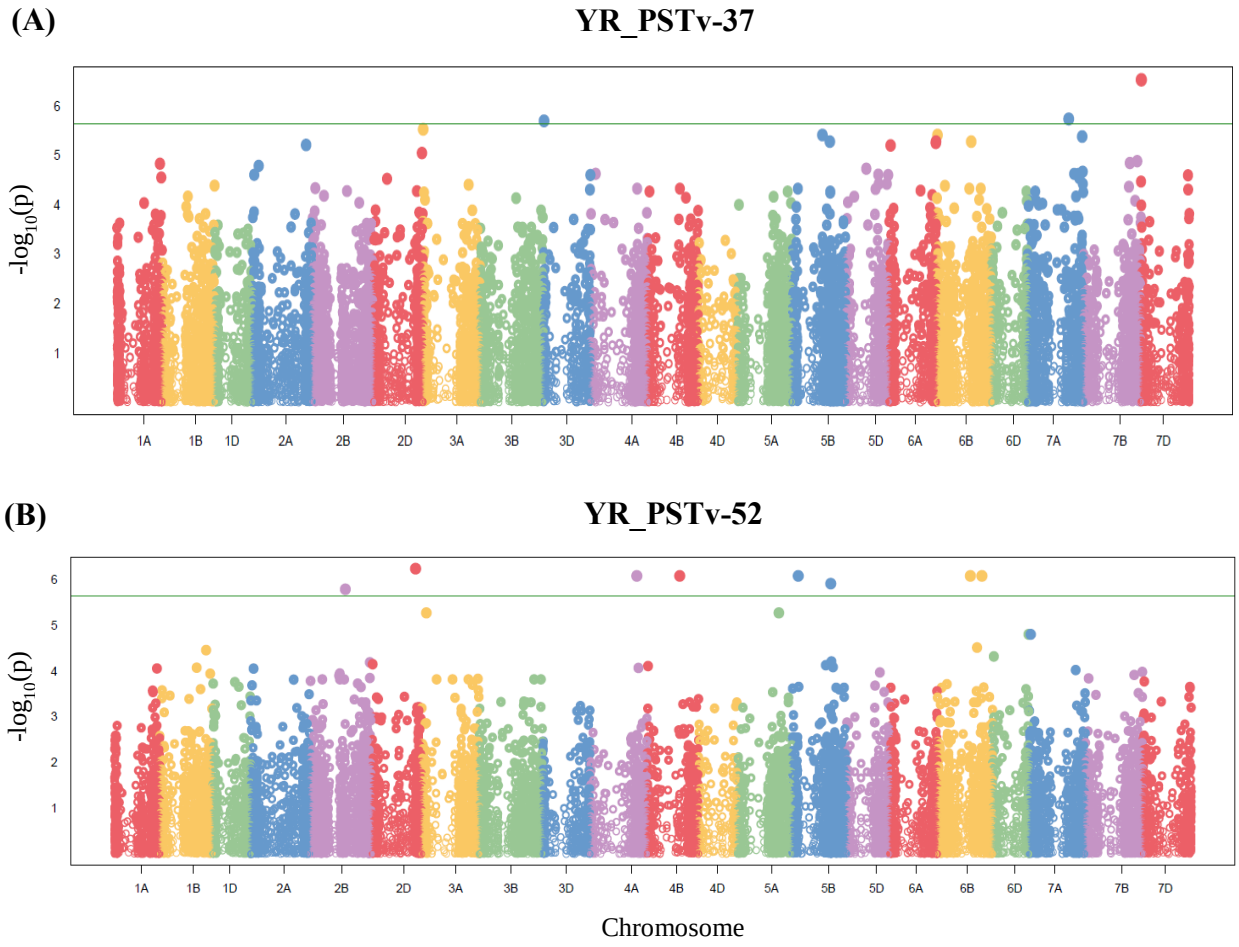

Figure 5. (A-B) Manhattan plots of marker-trait associations (MTAs) with disease responses in ND HRSW genotypes to two *Pst* races at seedling stage. The horizontal green line indicates significance level at P-value ≤ 0.001. The horizontal dotted green line indicates significance level at FDR ≤ 0.05. YR = Stripe rust.

4.4.4. Association analysis for adult plant resistance

GWAS for APR identified four MTAs to IT and two MTAs to DS, based on the Mt. Vernon disease evaluation data. There were no significant MTAs identified related to APR evaluation under greenhouse conditions in Fargo, ND. Based on IT, four MTAs were detected on chromosome 1D, 5A, and 6A. IWB6668 and IWB49537 were only 192 bp apart from each other and were detected on 37.2 Mb of chromosome 1D. IWB44837 and IWB6465 were identified on chromosome 5A and 6A, respectively. The APR association based on DS, IWB72466 was mapped to chromosome 1B. The same MTA, IWB49537, that was associated with IT was also detected for DS on the same location of chromosome 1D.

Table 6. Marker-trait association (MTA) for Mt. Vernon adult plant resistance to *Puccinia striiformis* f. sp. *tritici* in the hard red spring wheat population using general linear model.

MTAs[a]	Trait[b]	Chromosome	Position[c]	Marker alleles[d]	P-value[e]	MAF[f]	Marker effect[g]
IWB6668*	IT	1D	37,220,945	T/C	1.30×10^{-2}	0.06	-1.13
IWB49537*	IT	1D	37,221,137	G/T	1.30×10^{-2}	0.07	-1.13
IWB44837*	IT	5A	569,452,729	T/C	1.30×10^{-2}	0.06	1.10
IWB6465	IT	6A	24,049,619	G/A	1.30×10^{-2}	0.08	1.42
IWB72466	DS	1B	41,642,360	G/A	2.64×10^{-2}	0.50	6.96
IWB49537*	DS	1D	37,221,137	G/T	2.64×10^{-2}	0.07	-14.49

[a] Marker-trait association (MTA) in bold is associated with more than a single *Pst* APR trait. MTAs with an asterisk (*) have not been reported to overlap with previously published stripe rust resistance genes or flanking markers.
[b] Two traits of APR, infection type (IT) and disease severity (DS), were evaluated separately in the field trial of Mt. Vernon, WA.
[c] Physical position of the single-nucleotide polymorphism (SNP) markers based on the Chinese Spring reference genome sequence version 2.1, a hexaploidy wheat, available on International Wheat Genome Sequencing Consortium (IWGSC).
[d] Major/minor allele of corresponding MTA. The allele in bold is associated with stripe rust resistance.
[e] False discovery rate (FDR)-adjusted P-value.
[f] Minor allele frequency of the SNPs.
[g] Positive allelic effect indicates major allele is associated with stripe rust resistance, whereas negative allelic effect indicates minor allele is associated with stripe rust resistance. The absolute value of highest allelic effect indicates the MTA has the greatest effect on association with

resistance among other markers. The absolute value of allelic effect ≥ 0.8 was considered relatively high.

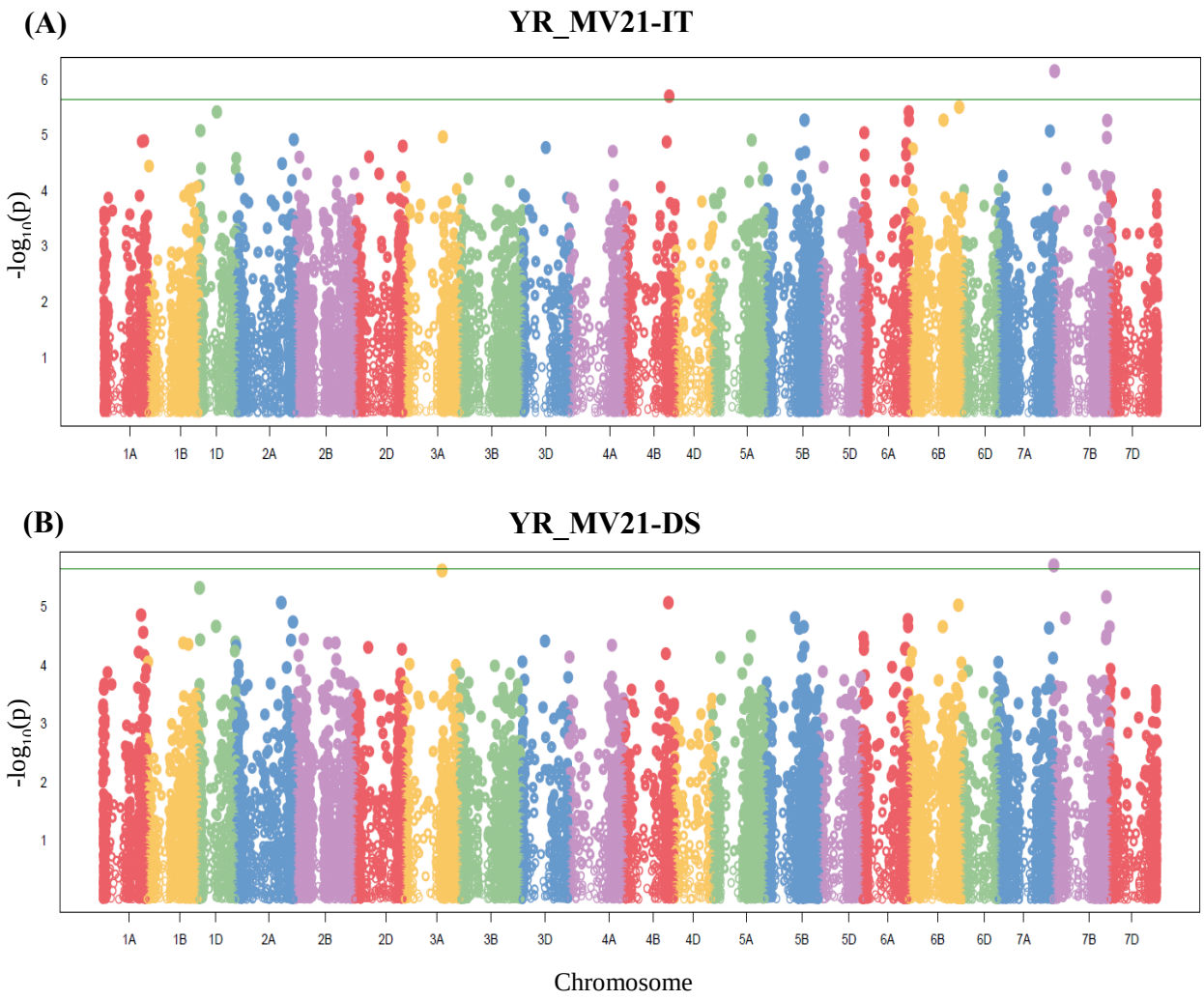

Figure 6. (A-B) Manhattan plots of marker-trait associations (MTAs) for adult plant resistance (APR) in ND HRSW genotypes to stripe rust under the field conditions. The horizontal green line indicates significance level at P-value ≤ 0.001. The horizontal dotted green line indicates significance level at FDR ≤ 0.05.

4.5. Discussion

From 2009 to 2019, the occurrence of stripe rust was identified in North Dakota in seven years including 2015 and 2016 (Evin et al. 2020). Prior to 2010s, stripe rust disease was rarely observed; however, the incidence has been increasing since 2011 suggesting the possible

appearance of virulence *Pst* isolates adapting to the ND climate. Isolates from post 2000 showed they have adapted into warmer climates than the isolates prior to 2000 (Milus et al. 2009). Prior to 2000, stripe rust was problematic to mainly the Western U.S. But since the severe epidemic in Arkansas and Louisiana in 2000, the frequency of stripe rust epidemics started to rise in the central U.S. since the early 2000s (Milus et al. 2009).

PSTv-37 has steadily been the most prevalent race across the United States over the last nine years even in 2021 with 76.4% frequency, whereas PSTv-52 was number two from 2013 to 2017, but remained in top five in 2019 with 3.2% frequency (Wang et al. 2022). PSTv-37 is known to be virulent to *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2*, and PSTv-52 is known to be virulent to *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, and *YrExp2*. The evaluation of disease response in 380 ND HRSW germplasm for ASR at seedling stage showed 76% of the population were susceptible to PSTv-37 and 38% of the population were susceptible to PSTv-52. In response to PSTv-52, 44% of the germplasm had IT range 4 to 6 which was 20% increase from the same IT range against PSTv-37 indicating more HRSW genotypes are resistant to PSTv-52. Based on 18 single *Yr* gene differentials, these two *Pst* races have only one gene difference, *YrTr1*, in virulence factors.

The seven MTAs identified for ASR to PSTv-37 were IWB7806, IWB57806, IWB36415, IWB4009, IWB43416, IWB35977, and IWB9373. Interestingly, none of the MTAs to PSTv-37 was not located in vicinity of previously reported *Yr* resistance genes or flanking markers suggesting that these MTAs are highly likely to be associated with novel resistance loci. On the other hand, a total of four MTAs was detected all on chromosome 7A in response to PSTv-52. IWA7755, IWA4845, IWB55310, and IWA4109 were mapped to a QTL with a length of 2.1 Mb. These MTAs were located approximately 6.5 to 8.7 Mb away from a marker (Xbarc49)

linked with *Yrxy1*. *Yrxy1* is an all-stage, recessive resistance gene that was first identified in a wheat cultivar Xiaoyan 54, and is also expressed at high temperature between 18℃ and 28℃ (Zhou et al. 2011). If *Yrxy1* is present in ND HRSW germplasm, it will be a good source of resistance as it is expressed at high temperature.

The APR association analysis revealed significant MTAs only from Mt. Vernon field trial. In response to IT, four MTAs were identified, IWB6668, IWB49537, IWB44837, and IWB6465 on chromosome 1D, 5A, and 6A. None of the MTAs were mapped to loci that have been associated with previously reported *Yr* resistant genes or flanking markers. In addition, two MTAs, IWB72466 and IWB49537, were identified on chromosome 1B and 1D for DS, respectively. IWB49537 was also associated with IT on chromosome 1D and its location did not overlap with previously known resistance gene loci. IWB72466 on chromosome 1B was located in vicinity of a marker Xbarc8 flanking *Yr15* with being 5.2 Mb apart. *Yr15* is originally from wild emmer wheat, but was introgressed into hexaploid wheat (Murphy et al. 2009). Although *Yr15* is known to provide broad-spectrum resistance to many *Pst* races in the U.S., it is still considered all-stage resistance gene (Murphy et al. 2009; Klymiuk et al. 2018). The majority of ASR genes encode proteins with NLR domains, but a recent study revealed *Yr15* encodes a protein with putative kinase and pseudokinase domains in tandem, named as WTK1 (wheat tandem kinase 1) (Klymiuk et al. 2018). *Yr15* may not be the first priority for APR gene, but it is still a very important resistance gene that can be used in ND HRSW germplasm.

In conclusion, 380 NDSU HRSW germplasm were evaluated for disease responses to stripe rust at seedling and adult plant stages. GWAS identified seven and four MTAs for ASR to PSTv-37 and PSTv-52, respectively. Also, there were six MTAs involved in the identification of APR genes. This research provides insight for fine-mapping and genomic selection tools to

deploy novel stripe rust resistance genes in spring wheat varieties by the NDSU HRSW breeding program.

4.6. References

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APPENDIX A. PHENOTYPIC DATA FOR WHEAT STEM AND STRIPE RUST DISEASE ON 380 ND HRSW GERMPLASM

Table A1. Disease evaluation of *Puccinia graminis* f. sp. *tritici* in 380 HRSW genotypes.

Genotypes	QCCJ[a]	TTKSK[b]	TTKTT+[b]	TTRTF[b]	TRTTF[b]	TKTTF[b]	TKKTF+[b]
PARSHALL	0.0	9.0	9.0	9.0	9.0	3.0	9.0
REEDER	1.0	9.0	9.0	8.2	9.0	0.0	4.0
ALSEN	1.0	9.0	9.0	9.0	4.7	9.0	9.0
KNUDSON	2.0	8.7	9.0	9.0	8.3	5.3	9.0
ND740	4.0	9.0	9.0	8.7	9.0	2.3	9.0
ND756	0.0	9.0	9.0	8.3	9.0	0.0	2.7
DAPPS	4.0	9.0	9.0	9.0	5.3	3.8	9.0
ND739	1.0	9.0	9.0	2.7	4.7	2.7	4.7
ND749	7.0	8.7	9.0	8.3	9.0	3.0	8.3
ND750	2.0	8.7	9.0	8.3	9.0	3.7	8.3
ND3085	2.0	9.0	9.0	8.2	9.0	3.0	9.0
ND3077	2.0	9.0	8.7	8.2	9.0	1.0	9.0
ND3084	0.0	8.7	9.0	9.0	4.7	1.2	9.0
BRIGGS	5.0	9.0	9.0	4.0	4.3	4.0	4.3
GRANITE	3.0	9.0	9.0	8.3	9.0	2.5	8.7
ND801	2.0	9.0	9.0	8.5	9.0	3.8	6.8
ND802	1.0	8.7	9.0	9.0	9.0	4.5	8.7
ND803	0.0	8.7	9.0	8.3	9.0	0.0	4.3
ND819	2.0	8.3	9.0	9.0	9.0	5.3	9.0
ND805	2.0	8.7	9.0	9.0	9.0	3.7	9.0
ND806	1.0	9.0	9.0	8.5	9.0	0.0	4.3
ND807	2.0	9.0	9.0	9.0	9.0	4.7	9.0
PROSPER	3.0	9.0	9.0	9.0	7.0	7.2	8.7
2375	7.0	9.0	9.0	9.0	9.0	3.0	9.0
NORPRO	4.0	9.0	9.0	9.0	4.8	2.3	8.7
OKLEE	2.0	9.0	9.0	2.7	4.0	0.0	4.7
FREYR	0.0	9.0	9.0	9.0	9.0	1.2	9.0
ND804	0.0	9.0	9.0	9.0	9.0	1.3	4.7
EXPRESS	7.0	9.0	9.0	8.3	9.0	7.8	8.7
TRAVERSE	2.0	9.0	9.0	9.0	7.3	0.7	8.5
KUNTZ	0.0	9.0	9.0	9.0	9.0	4.5	8.2
ND810	0.0	9.0	9.0	9.0	9.0	0.0	4.7
ND811	5.0	9.0	9.0	8.3	9.0	5.3	8.2

Table A1. Disease evaluation of *Puccinia graminis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	QCCJ[a]	TTKSK[b]	TTKTT+[b]	TTRTF[b]	TRTTF[b]	TKTTF[b]	TKKTF+[b]
SD3635	0.0	9.0	9.0	9.0	9.0	0.0	3.0
ND816	0.0	8.7	8.7	9.0	4.7	5.5	8.5
BUCKPRONTO	1.0	9.0	9.0	7.8	9.0	3.7	8.5
BRICK	5.0	9.0	9.0	9.0	9.0	4.5	8.7
RB07	0.0	8.7	8.7	9.0	9.0	0.7	NA
KELBY	2.0	4.0	9.0	4.0	4.7	1.3	4.7
KEENE	0.0	3.3	3.0	2.0	4.3	0.0	0.5
ERNEST	0.0	7.8	9.0	9.0	7.8	0.0	4.7
ND706	0.0	9.0	9.0	8.3	9.0	2.3	8.7
ND812	0.0	8.7	9.0	9.0	9.0	5.3	9.0
ND813	0.0	9.0	9.0	8.5	9.0	0.0	2.7
ND814	0.0	9.0	9.0	8.7	9.0	8.7	8.7
ALPINE	0.0	9.0	9.0	9.0	9.0	5.7	9.0
00S0219-10W	7.0	4.0	6.8	8.5	4.7	9.0	9.0
S/WCOMPW	0.0	4.0	8.7	3.7	4.7	0.3	3.7
MOTT	1.0	9.0	9.0	9.0	9.0	9.0	9.0
CHOTEAU	0.0	9.0	9.0	8.7	9.0	1.0	8.5
SD3942	1.0	3.3	9.0	3.7	4.0	3.7	4.0
ALBANY	1.0	8.7	9.0	9.0	8.5	2.3	9.0
ND817	1.0	9.0	9.0	9.0	9.0	0.0	4.7
SABIN	0.0	7.7	8.3	9.0	4.7	3.7	8.7
SELECT	0.0	9.0	9.0	7.0	8.3	1.0	8.5
SD4011	0.0	9.0	9.0	9.0	9.0	0.7	3.7
ND822	0.0	9.0	9.0	8.7	9.0	4.5	9.0
ND823	0.0	8.7	8.3	9.0	9.0	0.3	4.7
BRENNAN	0.0	6.5	9.0	9.0	4.3	0.7	8.2
SYSOREN	2.0	9.0	9.0	9.0	4.8	3.0	9.0
BROGAN	0.0	9.0	9.0	9.0	4.7	2.7	2.3
PIVOT	4.5	9.0	9.0	9.0	9.0	3.0	8.7
WBDIGGER	4.0	9.0	9.0	9.0	9.0	9.0	9.0
WBMAYVILLE	1.0	9.0	9.0	3.3	3.3	0.0	3.3
RWG10	8.0	9.0	9.0	9.0	9.0	9.0	9.0
PI277012	6.0	9.0	9.0	8.7	9.0	4.5	9.0
RWG20	4.0	9.0	9.0	9.0	9.0	3.0	9.0
RWG21	7.0	9.0	9.0	9.0	9.0	9.0	9.0
RWG22	7.0	9.0	9.0	8.7	9.0	2.3	9.0

Table A1. Disease evaluation of *Puccinia graminis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	QCCJ[a]	TTKSK[b]	TTKTT+[b]	TTRTF[b]	TRTTF[b]	TKTTF[b]	TKKTF+[b]
RWG24	8.0	9.0	9.0	9.0	8.5	4.5	9.0
RWG25	5.0	9.0	9.0	8.2	8.5	3.7	9.0
RWG28	2.0	9.0	9.0	9.0	8.5	9.0	9.0
ND824	0.0	9.0	9.0	9.0	9.0	2.3	8.7
BW931	0.0	9.0	9.0	2.7	4.7	0.0	3.7
BW932	1.0	9.0	9.0	9.0	9.0	0.7	6.5
NORDEN	0.0	9.0	9.0	8.0	9.0	0.8	8.7
FOREFRONT	4.0	9.0	9.0	8.3	9.0	1.2	8.7
LINKERT	0.0	9.0	9.0	8.3	9.0	0.3	4.0
MN07098-6	4.0	9.0	9.0	8.7	5.0	3.0	9.0
SYROWYN	5.0	9.0	9.0	7.0	8.2	0.7	9.0
SD4189	2.0	9.0	9.0	7.7	9.0	0.7	9.0
SD4215	1.0	9.0	9.0	9.0	9.0	5.0	9.0
SD4299	0.0	9.0	9.0	9.0	9.0	0.7	8.5
BW483	0.0	8.7	9.0	9.0	9.0	3.7	8.7
05S0157-4	0.0	8.7	9.0	9.0	9.0	9.0	8.7
05S0242-6	6.5	9.0	9.0	9.0	9.0	7.7	9.0
06S0157-1	8.0	9.0	8.3	9.0	8.2	2.8	9.0
LCSPRO	1.0	9.0	9.0	9.0	9.0	0.7	9.0
ND826	0.0	9.0	9.0	9.0	9.0	3.7	9.0
FA9S10-0008R	4.0	9.0	9.0	8.8	9.0	2.8	8.7
F9N12-0153	1.0	9.0	9.0	8.7	9.0	0.2	4.0
F9N12-0168	0.0	9.0	9.0	9.0	9.0	1.0	3.0
BOLLES	8.0	9.0	9.0	9.0	4.0	NA	9.0
LCSNITRO	0.0	9.0	8.7	8.7	9.0	3.7	9.0
11YUYR1	0.0	9.0	9.0	9.0	9.0	8.3	9.0
11YUYR5	1.0	9.0	9.0	9.0	4.7	9.0	9.0
WB9507	0.0	8.3	7.8	8.2	5.0	0.7	4.3
ND829	4.0	9.0	9.0	9.0	5.2	1.3	4.3
FA9S10-022R	6.0	9.0	9.0	9.0	9.0	7.3	9.0
FA9S10-0038R	5.0	9.0	9.0	7.7	9.0	0.0	4.7
FA9S10-0048R	5.0	4.0	8.2	3.3	4.3	0.3	4.7
F9N12-0151	2.0	9.0	9.0	9.0	4.7	8.0	9.0
F9N12-0152	0.0	9.0	9.0	8.2	4.7	8.8	9.0
F9N12-0162	0.0	9.0	9.0	9.0	9.0	9.0	9.0
F9N12-0172	1.0	9.0	9.0	9.0	9.0	8.3	8.7

Table A1. Disease evaluation of *Puccinia graminis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	QCCJ[a]	TTKSK[b]	TTKTT+[b]	TTRTF[b]	TRTTF[b]	TKTTF[b]	TKKTF+[b]
SHELLY	0.0	8.7	8.2	9.0	4.8	8.7	9.0
ND643	4.0	4.0	8.2	4.0	4.7	0.7	4.7
SD4393	5.0	9.0	9.0	9.0	9.0	9.0	9.0
MN11394-6	1.0	4.5	4.0	7.8	9.0	8.7	9.0
BW499	0.0	8.7	9.0	9.0	4.0	8.7	9.0
R16109	2.0	9.0	9.0	8.3	9.0	0.8	9.0
R16110	5.0	9.0	9.0	9.0	9.0	2.3	9.0
R16111	5.0	9.0	8.7	8.7	4.7	3.0	9.0
R16112	0.0	9.0	9.0	9.0	9.0	0.5	4.7
ND830	0.0	8.7	9.0	8.7	9.0	0.0	4.3
ND831	1.0	9.0	9.0	8.7	9.0	0.0	6.5
ND832	0.0	4.5	4.7	7.5	9.0	5.0	4.7
ND833	1.0	9.0	9.0	8.3	9.0	5.7	8.7
ND834	1.0	9.0	9.0	6.7	9.0	5.7	8.7
SYVALDA	4.0	9.0	9.0	9.0	9.0	0.0	3.3
NDSW10004	5.0	9.0	9.0	9.0	9.0	0.0	4.0
NDHRS16-12-16	5.0	9.0	9.0	9.0	9.0	0.0	3.7
NDHRS16-12-18	2.0	9.0	9.0	7.8	9.0	4.0	9.0
NDHRS16-12-25	1.0	9.0	9.0	6.8	9.0	2.2	9.0
NDHRS16-12-28	0.0	9.0	9.0	9.0	9.0	3.0	9.0
NDHRS16-12-33	0.0	9.0	9.0	8.7	9.0	1.0	5.0
NDHRS16-12-34	0.0	9.0	9.0	8.3	9.0	2.3	9.0
NDHRS16-12-37	1.0	9.0	9.0	8.7	9.0	1.7	9.0
NDHRS16-12-44	0.0	9.0	9.0	8.5	9.0	0.0	3.0
NDHRS16-13-11	8.0	9.0	9.0	3.3	4.0	0.0	3.3
NDHRS16-13-18	8.0	9.0	9.0	9.0	9.0	3.7	9.0
NDHRS16-13-20	0.0	9.0	9.0	8.7	9.0	0.0	4.5
NDHRS16-13-32	1.0	9.0	9.0	9.0	9.0	7.3	8.7
NDHRS16-13-51	0.0	9.0	9.0	8.7	9.0	4.0	9.0
NDHRS16-13-67	4.0	6.7	8.7	9.0	9.0	1.0	8.8
NDHRS16-13-80	2.0	9.0	9.0	9.0	9.0	5.2	9.0
ND FROHBERG	1.0	9.0	9.0	8.3	9.0	1.0	4.0
MN09157	0.0	9.0	9.0	8.7	9.0	5.3	8.7
MN10055	5.0	6.8	2.7	9.0	9.0	3.7	6.7
MN10285	5.0	8.7	9.0	9.0	4.8	2.3	9.0
MN10362	5.0	9.0	9.0	7.3	9.0	0.0	4.5

Table A1. Disease evaluation of *Puccinia graminis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	QCCJ[a]	TTKSK[b]	TTKTT+[b]	TTRTF[b]	TRTTF[b]	TKTTF[b]	TKKTF+[b]
MN10368	0.0	9.0	9.0	9.0	9.0	3.7	8.7
SD4536	0.0	9.0	9.0	9.0	9.0	9.0	9.0
MN10021	1.0	9.0	9.0	9.0	9.0	4.0	9.0
MN10388	0.0	8.2	8.7	9.0	9.0	3.0	9.0
MN11397-1	0.0	8.7	9.0	7.7	9.0	1.8	4.3
SD4510	0.0	9.0	9.0	7.2	9.0	0.0	2.7
SD4514	5.0	9.0	9.0	9.0	9.0	5.7	9.0
08S0036-19	5.0	9.0	9.0	6.0	9.0	0.2	4.3
LNR10-0177RS6	5.0	9.0	9.0	8.0	9.0	NA	8.7
SD4559	0.0	9.0	9.0	9.0	9.0	3.7	9.0
SD4607	1.0	9.0	9.0	9.0	9.0	0.0	4.0
MN12307-3	0.0	9.0	9.0	8.3	8.8	3.3	9.0
BW485	1.0	8.7	9.0	8.3	9.0	0.0	4.7
BW961	0.0	9.0	9.0	9.0	9.0	8.0	9.0
07S0068-11	0.0	2.7	8.7	2.0	3.3	0.0	4.7
08S0303-16	5.0	9.0	9.0	4.0	4.0	0.7	4.7
08S0339-23	5.0	9.0	9.0	9.0	9.0	0.0	4.8
MN13056-7	8.0	9.0	9.0	5.0	4.5	4.3	5.7
MN13515-8	0.0	9.0	9.0	9.0	9.0	0.0	6.0
SD4587	8.0	4.0	9.0	4.5	4.0	0.7	4.7
SD4675	0.0	9.0	9.0	8.7	9.0	0.7	4.8
09S0018-2	1.0	9.0	9.0	8.5	9.0	0.0	5.0
09S0084-14	5.0	9.0	9.0	9.0	9.0	7.7	9.0
LNR14-0677	0.0	9.0	9.0	9.0	9.0	0.0	4.7
LNR14-0747	0.0	9.0	9.0	9.0	5.0	1.5	9.0
LNR14-1868	0.0	9.0	9.0	8.7	9.0	3.7	9.0
FURANO	5.0	9.0	9.0	9.0	9.0	2.3	9.0
MAJOR	1.0	9.0	9.0	9.0	9.0	4.8	9.0
ORLEANS	1.0	9.0	9.0	6.8	4.7	2.3	9.0
TOPAZE	0.0	9.0	9.0	8.3	8.7	2.3	7.5
HRS3419	0.0	9.0	9.0	9.0	9.0	2.3	6.8
LNR150026	0.0	9.0	9.0	9.0	9.0	0.3	4.7
LNR15-1990	1.0	8.3	8.7	8.0	4.3	0.0	2.7
MN13304-5	2.0	8.7	8.7	9.0	4.3	0.3	1.7
MN14470-5	4.0	9.0	9.0	9.0	4.7	0.7	8.8
NDHRS16-12-19	5.0	9.0	9.0	9.0	4.3	0.7	9.0

Table A1. Disease evaluation of *Puccinia graminis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	QCCJ[a]	TTKSK[b]	TTKTT+[b]	TTRTF[b]	TRTTF[b]	TKTTF[b]	TKKTF+[b]
NDHRS16-12-51	0.0	9.0	9.0	9.0	8.8	0.0	4.7
NDHRS16-13-13	1.0	9.0	9.0	9.0	9.0	3.7	9.0
NDHRS16-13-29	0.0	9.0	9.0	8.8	5.2	0.7	8.7
NDHRS16-13-69	0.0	9.0	9.0	9.0	9.0	7.7	9.0
NDHRS16-13-86	0.0	8.7	9.0	9.0	9.0	1.3	5.2
NDHRS16-13-98	0.0	9.0	9.0	8.8	8.7	3.7	9.0
NDSW1312	0.0	9.0	9.0	9.0	9.0	8.0	8.8
SD4724	1.0	9.0	9.0	8.7	9.0	9.0	9.0
LANNING	0.0	9.0	9.0	9.0	9.0	9.0	9.0
CROMWELL	1.0	9.0	9.0	9.0	9.0	9.0	9.0
NDSW0987	1.0	9.0	9.0	8.0	9.0	8.7	9.0
LCSREBEL	5.0	9.0	8.7	7.7	4.7	1.5	7.8
SYLONGMIRE	0.0	9.0	9.0	6.2	5.0	0.2	4.7
SYMCCLOUD	0.0	9.0	9.0	9.0	9.0	9.0	9.0
NDHRS11-0612-0001	1.0	3.3	8.5	1.3	0.7	0.0	0.0
NDHRS16-12-31	2.0	7.5	9.0	9.0	9.0	1.8	8.7
NDHRS16-13-63	5.0	9.0	8.7	9.0	2.7	1.0	9.0
KWSCOCHISE	8.0	9.0	9.0	8.7	9.0	0.3	9.0
KWSCHILHAM	8.0	9.0	9.0	8.5	9.0	4.7	9.0
KWSWILLOW	1.0	9.0	9.0	8.2	9.0	7.3	9.0
KWSALDERON	0.0	9.0	9.0	8.5	9.0	0.0	3.3
MN15029-8	0.0	9.0	9.0	8.3	9.0	2.3	8.5
MN15219-2	1.0	8.2	8.7	8.8	8.7	0.0	2.0
MN15501-4	0.0	9.0	9.0	8.7	9.0	0.3	6.8
NDHRS16-14-36	2.0	9.0	9.0	9.0	9.0	3.5	5.0
NDHRS16-14-41	0.0	9.0	9.0	9.0	8.5	2.2	9.0
NDHRS16-14-168	8.0	9.0	9.0	8.7	9.0	1.7	9.0
NDHRS16-14-12	0.0	8.7	9.0	9.0	9.0	3.0	7.0
AGAWAM	6.5	9.0	9.0	9.0	9.0	0.0	3.7
WAIKEA	1.0	8.5	9.0	9.0	9.0	5.3	9.0
NDSW0450	0.0	9.0	9.0	9.0	9.0	0.0	4.7
NDSW0601	0.0	9.0	8.8	9.0	4.7	0.7	8.7
NDSW0612	1.0	9.0	9.0	9.0	4.7	2.2	8.7
NDSW0703	5.0	9.0	8.7	0.7	4.0	0.0	0.3
NDSW0802	5.0	8.5	9.0	9.0	9.0	2.2	9.0
NDSW0803	0.0	9.0	9.0	9.0	9.0	6.3	9.0

Table A1. Disease evaluation of *Puccinia graminis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	QCCJ[a]	TTKSK[b]	TTKTT+[b]	TTRTF[b]	TRTTF[b]	TKTTF[b]	TKKTF+[b]
03S0182-4-5WL	1.0	9.0	9.0	9.0	9.0	7.7	9.0
99S0155-14	1.0	9.0	9.0	8.7	9.0	5.3	9.0
00S0219-10	5.0	9.0	9.0	9.0	9.0	6.7	9.0
ARGENT	8.0	4.0	9.0	4.0	4.7	0.0	4.7
WL711	0.0	9.0	8.0	8.5	9.0	2.2	8.7
HY682	0.0	2.7	2.7	0.7	3.0	0.0	3.7
ND899	2.0	9.0	9.0	8.8	9.0	0.0	3.3
NDSW14087&8	5.0	9.0	9.0	9.0	9.0	0.0	3.3
DUCLAIR	0.0	9.0	9.0	9.0	9.0	8.3	9.0
SX600	0.0	9.0	9.0	9.0	9.0	0.0	3.7
NDSW0932	0.0	9.0	9.0	7.7	9.0	0.8	9.0
NDSW10044	1.0	9.0	9.0	9.0	5.2	3.7	9.0
NDSW0701	1.0	9.0	9.0	9.0	4.3	9.0	9.0
NDSW0849	2.0	4.0	9.0	4.0	4.7	3.3	4.3
NDSW0805'S'	8.0	9.0	7.8	9.0	4.7	0.0	4.7
NDSW0914	0.0	9.0	9.0	9.0	9.0	0.7	4.2
NDSW1370	0.0	5.3	9.0	5.8	4.7	6.3	8.3
EXPLORER	0.0	9.0	9.0	9.0	9.0	3.0	9.0
NDSW10043	1.0	9.0	9.0	8.7	9.0	9.0	9.0
NDSW1309	2.0	9.0	9.0	9.0	4.7	9.0	9.0
NDSW10084	5.0	9.0	9.0	9.0	9.0	0.0	4.8
NDSW10118	6.0	9.0	9.0	9.0	9.0	3.7	9.0
B110-125	4.0	9.0	9.0	8.3	9.0	3.7	9.0
NDSW10090	0.0	9.0	9.0	9.0	9.0	9.0	9.0
NDSW1356	0.0	7.2	9.0	7.0	4.7	7.7	6.7
NDSW1379	5.0	6.5	9.0	0.7	3.7	0.0	4.0
NDSW14056	2.0	9.0	9.0	9.0	9.0	4.7	9.0
NDSW15128	0.0	9.0	9.0	9.0	9.0	3.7	9.0
NDSW16-12W-8	1.0	3.3	4.3	9.0	0.0	0.0	0.0
NDHWS13-0012-0028	2.0	9.0	8.7	9.0	6.5	2.3	9.0
NDHWS13-0023-0006	4.0	3.3	8.7	1.7	4.0	4.0	3.7
NDHWS13-0041-0008	0.0	4.3	8.7	3.7	4.0	4.0	4.8
NDHRS16-14-126	0.0	9.0	9.0	9.0	4.3	8.5	9.0
NDHRS16-15-166	4.0	9.0	9.0	4.0	4.3	0.3	4.7
NDHRS16-15-225	4.0	9.0	9.0	9.0	4.0	9.0	9.0
NDHRS16-15-228	6.0	9.0	9.0	9.0	9.0	1.7	9.0

Table A1. Disease evaluation of *Puccinia graminis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	QCCJ[a]	TTKSK[b]	TTKTT+[b]	TTRTF[b]	TRTTF[b]	TKTTF[b]	TKKTF+[b]
NDHRS16-15-252	6.0	9.0	9.0	8.0	5.3	8.8	8.5
NDHRS16-15-320	6.0	9.0	9.0	9.0	9.0	4.7	9.0
NDHRS16-15-329	4.0	4.0	9.0	2.0	4.3	0.0	4.3
NDHRS16-15-362	5.0	9.0	8.2	5.8	4.7	0.2	5.0
NDHRS16-15-378	5.0	1.3	9.0	9.0	6.5	9.0	6.5
NDHRS16-15-66	4.0	9.0	8.2	5.2	9.0	4.0	6.5
NDHRS16-16-429	6.0	9.0	9.0	8.7	9.0	0.2	4.8
NDHRS16-16-482	0.0	1.0	8.7	1.7	3.3	0.0	4.7
NDHRS16-16-487	1.0	1.3	8.3	3.3	2.0	0.7	4.7
NDHRS16-16-488	4.0	1.3	9.0	1.7	3.3	0.0	4.7
NDHRS16-16-499	0.0	9.0	9.0	8.3	8.8	3.0	9.0
NDHRS16-16-507	0.0	9.0	9.0	8.5	9.0	0.0	3.7
NDHRS16-16-550	2.0	8.5	9.0	9.0	4.7	1.5	4.8
NDHRS16-16-615	1.0	9.0	9.0	9.0	9.0	NA	9.0
NDHRS16-16-654	5.0	9.0	9.0	9.0	8.8	NA	8.8
NDHRS16-16-678	0.0	9.0	9.0	9.0	9.0	0.3	4.8
NDHRS16-16-679	4.0	9.0	9.0	9.0	9.0	0.0	4.7
NDHRS16-16-697	1.0	9.0	9.0	9.0	9.0	4.7	9.0
NDHRS06-14-162	1.0	9.0	9.0	9.0	9.0	3.7	9.0
NDHRS06-14-45	1.0	9.0	9.0	9.0	9.0	1.7	9.0
NDHRS11-0244-0001	2.0	9.0	9.0	8.5	9.0	0.5	4.8
NDHRS11-0765-0002	5.0	8.8	9.0	9.0	4.7	1.5	8.7
NDHRS12-0065-0004	5.0	9.0	9.0	9.0	8.8	8.0	9.0
NDHRS12-0145-0002	1.0	9.0	9.0	9.0	9.0	NA	9.0
NDHRS12-0146-0002	5.0	9.0	9.0	7.7	9.0	0.8	8.8
NDHRS12-0185-0003	0.0	9.0	9.0	9.0	9.0	0.7	8.5
NDHRS12-0195-0006	0.0	9.0	9.0	9.0	9.0	9.0	9.0
NDHRS12-0498-0006	5.0	9.0	9.0	8.0	8.8	6.3	9.0
NDHRS12-0646-0006	5.0	8.7	9.0	9.0	9.0	4.5	9.0
NDHRS12-0788-0001	0.0	8.3	9.0	8.3	9.0	5.3	8.5
NDHRS12-0821-0004	5.0	8.8	8.3	6.5	9.0	0.0	2.0
NDHRS12-0821-0006	8.0	9.0	9.0	8.3	9.0	0.0	3.3
NDHRS12-0822-0003	5.0	9.0	9.0	8.7	8.8	0.0	7.8
NDHRS12-0979-0007	0.0	9.0	9.0	9.0	9.0	5.3	9.0
NDHRS12-1005-0004	5.0	9.0	8.7	8.5	8.8	0.7	8.5
NDHRS12-1031-0007	4.0	9.0	8.7	9.0	9.0	4.5	8.2

Table A1. Disease evaluation of *Puccinia graminis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	QCCJ[a]	TTKSK[b]	TTKTT+[b]	TTRTF[b]	TRTTF[b]	TKTTF[b]	TKKTF+[b]
NDHRS12-1120-0008	4.0	9.0	9.0	9.0	9.0	3.3	9.0
NDHRS12-1148-0002	0.0	8.2	9.0	7.5	8.0	9.0	8.7
NDHRS13-0144-0001	0.0	6.7	9.0	9.0	9.0	2.2	9.0
NDHRS13-0165-0003	8.0	9.0	9.0	9.0	9.0	4.7	9.0
NDHRS13-0177-0001	0.0	9.0	9.0	8.7	9.0	0.0	4.7
NDHRS13-0177-0006	0.0	9.0	8.3	8.7	9.0	0.3	8.7
NDHRS13-0181-0002	0.0	9.0	9.0	9.0	9.0	6.7	9.0
NDHRS13-0205-0009	1.0	9.0	9.0	9.0	9.0	4.7	8.7
NDHRS13-0210-0026	2.0	9.0	9.0	7.7	9.0	3.7	8.7
NDHRS13-0247-0032	5.0	9.0	9.0	9.0	9.0	1.7	8.5
NDHRS13-0248-0009	5.0	9.0	9.0	8.8	9.0	0.0	4.3
NDHRS13-0273-0036	4.0	9.0	9.0	9.0	9.0	3.7	9.0
NDHRS13-0310-0010	0.0	8.7	9.0	7.3	7.8	4.5	8.5
NDHRS13-0314-0027	4.0	9.0	9.0	6.8	9.0	0.0	4.7
NDHRS13-0318-0003	0.0	9.0	9.0	9.0	9.0	0.0	4.8
NDHRS16-13-89	5.0	8.5	9.0	8.5	9.0	0.7	8.5
NDHRS16-14-28	0.0	9.0	9.0	9.0	8.7	3.7	9.0
NDHRS16-14-108	1.0	9.0	9.0	9.0	9.0	0.0	4.3
NDHRS16-14-26	5.0	9.0	9.0	9.0	9.0	6.2	6.8
NDHRS16-14-205	0.0	9.0	9.0	9.0	9.0	3.0	9.0
NDHRS16-14-258	0.0	9.0	9.0	9.0	8.8	4.7	9.0
NDHRS16-14-64	0.0	9.0	9.0	8.0	9.0	0.0	4.3
NDHRS16-14-94	1.0	9.0	9.0	7.7	9.0	0.0	4.3
NDHRS16-14-119	1.0	9.0	9.0	9.0	9.0	5.7	9.0
NDHRS16-14-305	4.0	9.0	9.0	8.8	8.7	6.8	9.0
NDHRS16-15-172	0.0	8.7	9.0	8.5	8.7	0.2	4.0
NDHRS16-15-221	2.0	9.0	9.0	9.0	9.0	7.7	9.0
NDHRS16-15-233	2.0	9.0	9.0	8.0	9.0	4.7	9.0
NDHRS16-15-239	2.0	9.0	9.0	9.0	6.8	3.0	9.0
NDHRS16-15-243	4.0	9.0	9.0	8.3	9.0	1.2	6.5
NDHRS16-15-247	2.0	8.7	9.0	9.0	9.0	5.5	8.5
NDHRS16-15-248	1.0	9.0	8.7	9.0	9.0	1.7	8.7
NDHRS16-15-283	1.0	8.8	9.0	9.0	9.0	0.8	8.5
NDHRS16-15-287	1.0	9.0	9.0	9.0	9.0	0.0	4.3
NDHRS16-15-293	1.0	9.0	9.0	7.2	9.0	0.0	4.3
NDHRS16-15-296	0.0	9.0	9.0	7.2	9.0	0.0	4.3

Table A1. Disease evaluation of *Puccinia graminis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	QCCJ[a]	TTKSK[b]	TTKTT+[b]	TTRTF[b]	TRTTF[b]	TKTTF[b]	TKKTF+[b]
NDHRS16-15-315	0.0	9.0	9.0	7.8	9.0	0.0	4.2
NDHRS16-15-349	0.0	9.0	9.0	9.0	9.0	2.3	9.0
NDHRS16-15-64	0.0	8.7	9.0	9.0	9.0	1.8	9.0
NDHRS16-15-65	1.0	9.0	9.0	8.7	9.0	0.0	4.3
NDHRS16-16-413	1.0	9.0	9.0	8.7	9.0	0.3	4.0
NDHRS16-16-454	4.0	8.5	9.0	8.2	9.0	3.7	8.7
NDHRS16-16-465	1.0	9.0	9.0	8.3	9.0	0.0	4.7
NDHRS16-16-491	1.0	9.0	9.0	8.3	9.0	0.3	4.7
NDHRS16-16-515	0.0	9.0	9.0	9.0	9.0	0.3	4.3
NDHRS16-16-551	5.0	9.0	9.0	7.5	9.0	0.0	4.0
NDHRS16-16-554	4.0	9.0	9.0	9.0	9.0	4.0	9.0
NDHRS16-16-624	1.0	9.0	9.0	8.7	9.0	8.0	9.0
NDHRS16-16-627	2.0	9.0	9.0	7.5	9.0	3.7	9.0
NDHRS16-16-666	0.0	9.0	9.0	9.0	9.0	4.7	9.0
NDHRS16-16-672	5.0	9.0	9.0	9.0	9.0	3.0	9.0
NDHRS16-16-676	0.0	9.0	9.0	9.0	9.0	4.0	9.0
NDHRS16-16-696	1.0	9.0	9.0	8.7	9.0	2.7	5.3
NDHRS16-16-700	0.0	9.0	9.0	9.0	9.0	0.0	3.7
NDHRS16-16-732	5.0	8.7	9.0	9.0	9.0	0.0	4.7
NDHRS16-16-733	2.0	9.0	9.0	8.3	9.0	7.3	8.7
NDHRS16-13-4	4.0	9.0	9.0	9.0	9.0	3.8	8.7
NDHRS16-13-24	4.0	9.0	9.0	9.0	9.0	2.3	9.0
NDHRS16-13-26	0.0	9.0	9.0	8.8	9.0	0.0	3.7
NDHRS16-13-27	4.0	9.0	9.0	9.0	9.0	0.7	4.7
NDHRS16-13-28	2.5	9.0	9.0	9.0	9.0	2.3	8.7
NDHRS16-13-50	1.0	8.7	8.7	8.0	9.0	0.0	3.0
NDHRS16-13-25	4.0	9.0	9.0	9.0	9.0	7.7	9.0
NDHRS16-13-5	0.0	9.0	9.0	8.2	9.0	0.0	9.0
NDHRS16-13-82	4.0	9.0	9.0	8.8	9.0	7.0	9.0
NDHRS16-13-84	1.0	9.0	9.0	8.8	9.0	6.3	9.0
NDHRS16-13-94	0.0	9.0	9.0	9.0	9.0	0.0	3.3
NDHRS16-14-29	4.0	9.0	9.0	9.0	4.8	5.3	9.0
NDHRS16-14-24	2.0	9.0	9.0	8.8	9.0	4.7	8.7
NDHRS16-14-27	4.0	9.0	9.0	8.8	9.0	3.0	9.0
NDHRS16-14-31	4.0	9.0	9.0	9.0	9.0	0.0	3.0
NDHRS16-14-104	4.0	9.0	9.0	9.0	9.0	9.0	8.7

Table A1. Disease evaluation of *Puccinia graminis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	QCCJ[a]	TTKSK[b]	TTKTT+[b]	TTRTF[b]	TRTTF[b]	TKTTF[b]	TKKTF+[b]
NDHRS16-14-110	4.0	9.0	9.0	9.0	9.0	0.7	4.0
NDHRS16-14-113	0.0	9.0	9.0	9.0	9.0	7.5	8.7
NDHRS16-14-116	0.0	8.7	9.0	9.0	9.0	4.7	9.0
NDHRS16-14-189	2.0	9.0	9.0	9.0	9.0	4.5	9.0
NDHRS16-14-204	4.0	9.0	9.0	7.7	9.0	0.0	3.0
NDHRS16-14-217	2.0	9.0	9.0	9.0	9.0	4.7	9.0
NDHRS16-14-252	1.0	9.0	9.0	9.0	9.0	4.7	9.0
NDHRS16-14-257	0.0	9.0	9.0	9.0	9.0	5.7	9.0
NDHRS16-14-260	4.0	9.0	9.0	8.7	9.0	3.7	9.0
NDHRS16-14-265	1.0	9.0	9.0	9.0	9.0	0.0	3.7
NDHRS16-14-58	0.0	9.0	9.0	9.0	9.0	3.3	8.7
NDHRS16-14-59	1.0	9.0	9.0	9.0	9.0	6.3	9.0
NDHRS16-14-71	4.0	9.0	9.0	9.0	9.0	4.7	8.8
NDHRS16-14-73	5.0	9.0	9.0	9.0	9.0	6.7	9.0
NDHRS16-14-90	2.0	9.0	9.0	7.7	9.0	6.3	9.0
NDHRS16-14-98	0.0	9.0	9.0	9.0	9.0	4.7	9.0
NDHRS16-14-124	2.0	9.0	9.0	9.0	9.0	4.5	6.5
NDHRS16-14-127	4.0	9.0	9.0	7.0	9.0	0.0	4.0
NDHRS16-14-141	4.0	9.0	9.0	9.0	9.0	4.7	9.0
NDHRS16-14-143	4.0	9.0	9.0	8.7	9.0	7.8	9.0
NDHRS16-14-149	2.0	9.0	9.0	8.8	9.0	4.7	9.0
NDHRS16-14-153	8.0	9.0	9.0	9.0	9.0	4.7	9.0
NDHRS16-14-156	4.0	9.0	9.0	9.0	8.5	2.2	8.3

a: North American native *P. graminis* f. sp. *tritici* race.
b: Ug99 and foreign *P. graminis* f. sp. *tritici* races from Africa, the Middle East, and Europe.

Table A2. Disease evaluation of Puccinia striiformis f. sp. tritici in 380 HRSW genotypes.

Genotypes	PSTv-37	PSTv-52	FAR21-IT[a]	FAR21-DS[b]	MV21-IT[c]	MV21-DS[d]
PARSHALL	8.5	7	8.5	12.5	9	100
REEDER	8	6.5	3	10	3	30
ALSEN	8.5	8	8.5	70	8	90
KNUDSON	6	6	8.5	80	9	100
ND740	6.5	6.5	8.5	30	8	90
ND756	8	8	9	7.5	8	80
DAPPS	6	6	7.5	30	8	90
ND739	4.5	5	3.5	10	8	90
ND749	7.5	5.5	8.5	20	8	70
ND750	3.5	5	7.5	25	8	80
ND3085	8.5	5	7.5	90	8	70
ND3077	6	6	8	70	8	80
ND3084	5	8	9	50	9	100
BRIGGS	7	9	7.5	40	5	40
GRANITE	7	6	8	60	8	80
ND801	6	6	8.5	50	8	90
ND802	6	5	8.5	80	9	100
ND803	7.5	5	7	40	8	90
ND819	6	4	8.5	60	9	100
ND805	7.5	6	7.5	80	8	90
ND806	8	7	7.5	40	9	100
ND807	5.5	8	9	60	9	100
PROSPER	8	8	8.5	90	5	40
2375	6	6	2.5	7.5	8	40
NORPRO	8	8	8	40	9	100
OKLEE	2.5	2.5	5.5	5	5	40
FREYR	6	6	8.5	35	5	40
ND804	2	5	7	30	5	40
EXPRESS	6.5	4	8.5	17.5	8	90
TRAVERSE	7.5	0	8	10	8	80
KUNTZ	5	5.5	8.5	90	9	100
ND810	6	8	7	60	5	30
ND811	6.5	3	8.5	45	5	40
SD3635	6.5	7	8.5	50	8	80
ND816	3	2	6.5	5	8	80
BUCKPRONTO	1	2	8	2.5	9	100

Table A2. Disease evaluation of *Puccinia striiformis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	PSTv-37	PSTv-52	FAR21-IT[a]	FAR21-DS[b]	MV21-IT[c]	MV21-DS[d]
BRICK	8	6	8.5	20	7	60
RB07	6	5	9	55	7	60
KELBY	7.5	8	7.5	60	5	50
KEENE	7.5	7	9	100	8	80
ERNEST	7	6	5.5	15	7	50
ND706	7.5	5.5	9	70	8	80
ND812	6	7	9	80	8	80
ND813	8	2.5	7	40	3	30
ND814	8	3	7.5	5	3	20
ALPINE	7.5	4	8.5	7.5	3	20
00S0219-10W	8.5	0	8	5	5	30
S/WCOMPW	8	5	8.5	50	8	80
MOTT	5	4	7	15	9	90
CHOTEAU	7.5	4.3	8	7.5	8	90
SD3942	8.5	5.5	0	0	9	90
ALBANY	5	5.3	7	12.5	9	90
ND817	7.5	5.5	6	80	5	30
SABIN	7	6	7.5	22.5	9	100
SELECT	7	7.5	8.5	60	9	100
SD4011	8	7.5	8	15	8	80
ND822	7	6.3	8	40	8	90
ND823	6.5	6.0	8	70	8	80
BRENNAN	6	3.8	8	15	8	80
SYSOREN	7	6.8	8.5	12.5	9	100
BROGAN	3.5	6.0	6.5	20	9	100
PIVOT	8	6	8.5	27.5	8	80
WBDIGGER	8.5	7.8	8	35	8	70
WBMAYVILLE	8.5	5.0	8	17.5	5	50
RWG10	7.5	5.8	9	10	5	30
PI277012	7.5	7.5	9	80	5	50
RWG20	7	7.0	8	40	5	50
RWG21	7.5	5.8	8	50	5	50
RWG22	6	1.0	8	5	3	20
RWG24	7	0.0	8	80	5	40
RWG25	7.5	6.0	6	20	5	40
RWG28	8	7.3	6.5	70	8	0

Table A2. Disease evaluation of *Puccinia striiformis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	PSTv-37	PSTv-52	FAR21-IT[a]	FAR21-DS[b]	MV21-IT[c]	MV21-DS[d]
ND824	7.5	6.0	9	5	5	50
BW931	6	4.5	5	50	5	50
BW932	8.5	4.8	8	15	5	50
NORDEN	7.5	6.8	6	80	9	100
FOREFRONT	5	1.0	8	2.5	8	70
LINKERT	8.5	0.5	7.5	75	9	100
MN07098-6	7	2.0	8	70	9	100
SYROWYN	8	7.0	9	80	8	70
SD4189	7.5	7.0	8.5	75	9	100
SD4215	6	7	7.5	12.5	8	70
SD4299	7.5	5.5	7	70	5	40
BW483	8	8.0	8.5	60	3	30
05S0157-4	3.5	6.0	8	70	2	15
05S0242-6	7	6	6	7.5	5	40
06S0157-1	6	6	7	50	8	70
LCSPRO	8.5	6.3	7.5	60	8	90
ND826	8.5	4	7.5	75	9	100
FA9S10-0008R	6.5	1.5	7	17.5	5	40
F9N12-0153	4.5	1.5	8.5	7.5	5	40
F9N12-0168	7	6.0	0	0	8	70
BOLLES	8	7.0	7	17.5	8	70
LCSNITRO	0.5	1.0	NA	NA	2	2
11YUYR1	2	0.0	0	0	2	2
11YUYR5	7.5	6.5	8.5	75	9	100
WB9507	8.5	6.3	7.5	35	9	100
ND829	7	9	7.5	5	9	100
FA9S10-022R	6	7.5	8	10	5	40
FA9S10-0038R	7	7.0	8	15	8	70
FA9S10-0048R	9	7.5	8	25	5	40
F9N12-0151	6.5	4.0	7.5	17.5	5	40
F9N12-0152	5	6.0	6	5	5	30
F9N12-0162	8.5	8.5	8.5	80	9	100
F9N12-0172	8	5.3	4	10	3	20
SHELLY	8.5	4.0	6.5	17.5	5	40
ND643	8.5	0.0	5.5	12.5	8	70
SD4393	1	3	2	2.5	2	5

Table A2. Disease evaluation of *Puccinia striiformis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	PSTv-37	PSTv-52	FAR21-IT[a]	FAR21-DS[b]	MV21-IT[c]	MV21-DS[d]
MN11394-6	7	3.5	6	12.5	8	70
BW499	6.5	7	6.5	60	3	20
R16109	8	6	7	10	9	100
R16110	8	4.3	8	70	9	90
R16111	8	6.0	8.5	15	8	80
R16112	6.5	1.5	9	90	8	80
ND830	8	6.5	8	90	8	70
ND831	7.5	1.5	8	80	8	80
ND832	8	6.5	7	70	5	40
ND833	6.5	5.8	8	40	3	20
ND834	7.5	8	8.5	70	9	100
SYVALDA	8	7.3	7.5	45	5	30
NDSW10004	6.5	7.0	7	27.5	8	90
NDHRS16-12-16	6.5	7	7.5	55	8	80
NDHRS16-12-18	6.5	8	8	40	8	80
NDHRS16-12-25	7	7	8	60	8	90
NDHRS16-12-28	5.5	4	6	10	5	60
NDHRS16-12-33	7	8	7	45	8	90
NDHRS16-12-34	7.5	7	7.5	30	8	90
NDHRS16-12-37	7.5	6.5	8.5	60	8	90
NDHRS16-12-44	8	4.5	8	35	9	100
NDHRS16-13-11	8	7.5	8	70	9	100
NDHRS16-13-18	6	5.5	8	80	9	100
NDHRS16-13-20	7.5	5.3	8	85	9	100
NDHRS16-13-32	5	4.0	7	10	8	80
NDHRS16-13-51	6.5	2.5	7	5	8	80
NDHRS16-13-67	7	3.8	8.5	75	9	100
NDHRS16-13-80	7	3.0	8	60	8	80
ND FROHBERG	7.5	7	8.5	70	9	80
MN09157	7.5	5.5	7	30	3	20
MN10055	8.5	5.0	7	17.5	3	20
MN10285	8	5.0	6	15	3	20
MN10362	7.5	4.0	8	40	5	40
MN10368	6.5	7.0	7.5	70	3	20
SD4536	5	5	8	5	5	40
MN10021	8.5	7.0	9	80	5	40

Table A2. Disease evaluation of *Puccinia striiformis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	PSTv-37	PSTv-52	FAR21-IT[a]	FAR21-DS[b]	MV21-IT[c]	MV21-DS[d]
MN10388	6.5	6.5	8	70	3	20
MN11397-1	7	8	8.5	100	5	40
SD4510	7	4.5	8	70	5	40
SD4514	4.5	3.3	7.5	70	9	100
08S0036-19	8	6	9	70	9	100
LNR10-0177RS6	6.5	6.5	8	80	9	100
SD4559	8.5	4.0	6	35	8	90
SD4607	2.5	5.0	6	40	5	50
MN12307-3	6.5	3	7.5	50	9	90
BW485	7	2.5	4.5	2.5	8	80
BW961	8	3.3	7.5	7.5	5	40
07S0068-11	4.5	3.0	8	5	8	80
08S0303-16	7	5	9	2.5	8	90
08S0339-23	6.5	4.5	8.5	80	9	100
MN13056-7	5.5	3.0	8	20	9	100
MN13515-8	6	8.5	8	90	3	20
SD4587	7.5	7.0	7.5	5	5	40
SD4675	8	8.0	9	7.5	8	80
09S0018-2	7	5.5	8.5	70	8	70
09S0084-14	6	4.8	8	30	9	100
LNR14-0677	8	1.5	8.5	35	9	100
LNR14-0747	7	0.3	9	75	9	100
LNR14-1868	8	2.0	7	10	2	10
FURANO	5.5	4	2.5	5	2	15
MAJOR	8	5	9	60	9	100
ORLEANS	4	1.5	NA	NA	2	15
TOPAZE	8	4.0	0	0	2	15
HRS3419	7.5	6.5	8	10	8	80
LNR150026	7.5	7.0	7	17.5	5	40
LNR15-1990	7.5	3.5	7	2.5	3	20
MN13304-5	8	5	7	5	8	80
MN14470-5	8.5	6	8.5	80	9	100
NDHRS16-12-19	8.5	6.5	7.5	60	8	80
NDHRS16-12-51	4	5	7.5	70	8	80
NDHRS16-13-13	8	5.5	7.5	40	9	90
NDHRS16-13-29	8.5	7.0	6	5	3	20

Table A2. Disease evaluation of *Puccinia striiformis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	PSTv-37	PSTv-52	FAR21-IT[a]	FAR21-DS[b]	MV21-IT[c]	MV21-DS[d]
NDHRS16-13-69	7.5	0.0	8.5	60	9	100
NDHRS16-13-86	6.5	4	7	70	5	30
NDHRS16-13-98	8.5	6	9	30	5	30
NDSW1312	7	6	8.5	100	3	20
SD4724	7	7	8.5	70	8	80
LANNING	8	7	8	12.5	8	80
CROMWELL	5.5	8	5	5	8	80
NDSW0987	7	3	7	5	8	70
LCSREBEL	6	4	7	5	5	40
SYLONGMIRE	7	2	8	12.5	8	80
SYMCCLOUD	3	0	NA	NA	2	2
NDHRS11-0612-0001	8.5	0	4.5	2.5	5	40
NDHRS16-12-31	9	7	8	90	9	100
NDHRS16-13-63	3.5	3	0	0	2	10
KWSCOCHISE	1.5	0	NA	NA	2	5
KWSCHILHAM	5.5	6	NA	NA	2	10
KWSWILLOW	8	0	6	5	3	10
KWSALDERON	2.5	2.0	6.5	12.5	9	100
MN15029-8	6.5	3	5.5	7.5	8	70
MN15219-2	6.5	0	3.5	2.5	2	15
MN15501-4	8	3	9	2.5	9	100
NDHRS16-14-36	8	6	7.5	40	8	80
NDHRS16-14-41	8	4	7	17.5	9	100
NDHRS16-14-168	7.5	3	7	12.5	5	40
NDHRS16-14-12	6	3	7.5	5	9	100
AGAWAM	7.5	5	7	80	5	30
WAIKEA	8	7	8	30	8	80
NDSW0450	6.5	6	7	20	3	20
NDSW0601	7	5	8	30	3	20
NDSW0612	4.5	0	8	5	8	80
NDSW0703	8	3	6.5	60	3	20
NDSW0802	7.5	5	9	50	8	80
NDSW0803	8.5	2	9	70	9	100
03S0182-4-5WL	9	3	9	65	9	100
99S0155-14	8.5	7	7	100	9	100
00S0219-10	8	7	8.5	70	3	20

Table A2. Disease evaluation of *Puccinia striiformis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	PSTv-37	PSTv-52	FAR21-IT[a]	FAR21-DS[b]	MV21-IT[c]	MV21-DS[d]
ARGENT	5.5	5	7	40	8	90
WL711	3.5	9	NA	NA	9	100
HY682	8	8	8	55	8	70
ND899	7	9	8.5	80	8	70
NDSW14087&8	7	9	8	100	9	70
DUCLAIR	2.5	0	NA	NA	5	40
SX600	6.5	0	9	35	8	80
NDSW0932	7	0	9	10	7	60
NDSW10044	8.5	0	7.5	100	9	90
NDSW0701	4	3	7	40	9	100
NDSW0849	7.5	5	5.5	80	9	100
NDSW0805'S'	4	4.0	8.5	20	3	20
NDSW0914	7.5	3	6	10	3	20
NDSW1370	3	5	NA	NA	5	40
EXPLORER	7	7	9	75	8	90
NDSW10043	7	6	8.5	100	9	90
NDSW1309	7	5	8.5	90	8	80
NDSW10084	7.5	7	9	100	9	100
NDSW10118	8.5	9	8	80	8	70
B110-125	7.5	7	8	40	9	80
NDSW10090	8	8	6.5	60	5	50
NDSW1356	7.5	6	7	70	5	50
NDSW1379	8	5	8.5	55	5	50
NDSW14056	6	7	7.5	40	9	90
NDSW15128	4.5	2	9	80	3	20
NDSW16-12W-8	6.5	6	7.5	60	8	80
NDHWS13-0012-0028	7.5	4	8	90	8	80
NDHWS13-0023-0006	8	5.5	8.5	70	8	70
NDHWS13-0041-0008	7	6	8.5	100	8	90
NDHRS16-14-126	8	7	8	40	9	100
NDHRS16-15-166	9	7	8	70	9	70
NDHRS16-15-225	7	2	8	80	5	50
NDHRS16-15-228	7.5	6	7	40	9	100
NDHRS16-15-252	8	6	8	42.5	9	100
NDHRS16-15-320	8.5	6	9	65	9	100
NDHRS16-15-329	6.5	5	9	15	8	80

Table A2. Disease evaluation of *Puccinia striiformis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	PSTv-37	PSTv-52	FAR21-IT[a]	FAR21-DS[b]	MV21-IT[c]	MV21-DS[d]
NDHRS16-15-362	8	6	8	90	5	50
NDHRS16-15-378	8.5	6	6.5	7.5	8	90
NDHRS16-15-66	8.5	6.5	8	10	8	80
NDHRS16-16-429	8	7	6.5	5	5	50
NDHRS16-16-482	7.5	8	8.5	80	9	100
NDHRS16-16-487	6.5	3	8.5	80	9	100
NDHRS16-16-488	6.5	5	8.5	17.5	8	80
NDHRS16-16-499	7	4	8	5	3	30
NDHRS16-16-507	7.5	4	7	30	7	50
NDHRS16-16-550	6	6	8	80	8	80
NDHRS16-16-615	7	7	7.5	17.5	9	100
NDHRS16-16-654	8.5	6.5	8	55	9	100
NDHRS16-16-678	8.5	7	8	35	9	100
NDHRS16-16-679	7.5	7	8	20	9	100
NDHRS16-16-697	7.5	3	8	15	3	20
NDHRS06-14-162	8	4	9	5	9	100
NDHRS06-14-45	6.5	5	7	10	9	100
NDHRS11-0244-0001	8	4	7	12.5	8	80
NDHRS11-0765-0002	4.5	7	8	10	9	100
NDHRS12-0065-0004	7	4	8	5	5	40
NDHRS12-0145-0002	5.5	6	8.5	7.5	8	80
NDHRS12-0146-0002	7.5	7	8.5	60	8	80
NDHRS12-0185-0003	8.5	7	8.5	70	9	100
NDHRS12-0195-0006	7	8	8	20	5	40
NDHRS12-0498-0006	6.5	8	8	5	8	70
NDHRS12-0646-0006	8.5	6.5	6.5	7.5	8	80
NDHRS12-0788-0001	8	4.0	7.5	40	8	70
NDHRS12-0821-0004	8	8	6	10	8	80
NDHRS12-0821-0006	6	4	7	12.5	8	70
NDHRS12-0822-0003	7	4.3	5.5	35	8	90
NDHRS12-0979-0007	5.5	3.8	7.5	50	9	100
NDHRS12-1005-0004	8	4.0	7.5	30	9	100
NDHRS12-1031-0007	8.5	7.0	7.5	50	5	40
NDHRS12-1120-0008	5	1.5	7	85	5	50
NDHRS12-1148-0002	6	7.0	6.5	12.5	8	80
NDHRS13-0144-0001	6	5.5	7	30	8	80

Table A2. Disease evaluation of *Puccinia striiformis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	PSTv-37	PSTv-52	FAR21-IT[a]	FAR21-DS[b]	MV21-IT[c]	MV21-DS[d]
NDHRS13-0165-0003	7.5	6.0	6	70	5	50
NDHRS13-0177-0001	7	4	8	70	5	50
NDHRS13-0177-0006	7.5	5	6	70	8	90
NDHRS13-0181-0002	8	7.0	8	80	8	70
NDHRS13-0205-0009	8	7.0	8	90	9	100
NDHRS13-0210-0026	8.5	6.0	7	2.5	5	50
NDHRS13-0247-0032	8.5	5.8	8	7.5	8	80
NDHRS13-0248-0009	8.5	7.0	6.5	70	9	80
NDHRS13-0273-0036	8.5	5	8.5	20	8	80
NDHRS13-0310-0010	8	5.5	8	70	9	80
NDHRS13-0314-0027	8.5	3	5	80	9	10
NDHRS13-0318-0003	8.5	4.0	8	80	9	100
NDHRS16-13-89	8.5	3.0	4	50	8	80
NDHRS16-14-28	7.5	0.0	7	12.5	9	80
NDHRS16-14-108	7.5	8.0	6.5	10	8	90
NDHRS16-14-26	7.5	8.0	7	40	8	90
NDHRS16-14-205	8.5	7.0	8.5	45	8	90
NDHRS16-14-258	8.5	5.0	7	10	8	90
NDHRS16-14-64	8	5.3	8	12.5	8	90
NDHRS16-14-94	8	5.0	NA	NA	9	100
NDHRS16-14-119	8	4	7	7.5	8	70
NDHRS16-14-305	7.5	6.5	7	80	8	90
NDHRS16-15-172	6.5	7	8	80	8	90
NDHRS16-15-221	8	5.3	8.5	17.5	8	90
NDHRS16-15-233	8.5	7	6.5	50	5	40
NDHRS16-15-239	7	7	8.5	50	8	70
NDHRS16-15-243	7.5	8	6.5	15	9	100
NDHRS16-15-247	7.5	4	NA	NA	8	70
NDHRS16-15-248	8.5	5	7	15	8	70
NDHRS16-15-283	8.5	0	8	30	9	100
NDHRS16-15-287	8.5	0	6	7.5	8	70
NDHRS16-15-293	8	4.5	7	80	8	90
NDHRS16-15-296	8.5	5	6	20	5	30
NDHRS16-15-315	8	4.0	7	15	8	70
NDHRS16-15-349	8.5	7.5	7.5	7.5	9	100
NDHRS16-15-64	8	4.5	7	10	5	40

Table A2. Disease evaluation of *Puccinia striiformis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	PSTv-37	PSTv-52	FAR21-IT[a]	FAR21-DS[b]	MV21-IT[c]	MV21-DS[d]
NDHRS16-15-65	7.5	3.0	7	60	8	90
NDHRS16-16-413	8.5	7.5	7.5	20	8	90
NDHRS16-16-454	9	7.0	7.5	25	5	50
NDHRS16-16-465	7.5	7.5	7	35	8	90
NDHRS16-16-491	8.5	4.0	8	12.5	8	70
NDHRS16-16-515	8.5	6.0	7.5	70	8	90
NDHRS16-16-551	9	6	7	35	8	90
NDHRS16-16-554	9	5.0	8	25	3	3
NDHRS16-16-624	8.5	0.0	8	5	5	40
NDHRS16-16-627	9	7	8	40	8	90
NDHRS16-16-666	8.5	6.5	9	40	5	50
NDHRS16-16-672	8.5	6.5	8	60	9	100
NDHRS16-16-676	8	6	5.5	12.5	9	100
NDHRS16-16-696	7	4.5	6	17.5	8	80
NDHRS16-16-700	8.5	8.0	5.5	50	8	70
NDHRS16-16-732	6	7.0	7	10	8	70
NDHRS16-16-733	8.5	3.0	7.5	40	5	40
NDHRS16-13-4	9	8	6	55	8	90
NDHRS16-13-24	8.5	4.5	8.5	80	9	100
NDHRS16-13-26	8	8.0	8	70	8	90
NDHRS16-13-27	7	7	7.5	15	8	80
NDHRS16-13-28	6.5	7.5	7.5	60	5	40
NDHRS16-13-50	3	6.5	6	7.5	5	40
NDHRS16-13-25	6.5	7.5	7.5	45	5	40
NDHRS16-13-5	4.5	5.5	8	35	8	70
NDHRS16-13-82	8.5	6.5	8	30	8	80
NDHRS16-13-84	7.5	7.5	7.5	60	8	90
NDHRS16-13-94	8	6	8.5	30	9	100
NDHRS16-14-29	7.5	8	8.5	30	8	90
NDHRS16-14-24	7	7	7.5	27.5	9	100
NDHRS16-14-27	6	8.0	8	90	8	90
NDHRS16-14-31	7	6.5	7.5	20	8	90
NDHRS16-14-104	8.5	6.5	5	17.5	9	90
NDHRS16-14-110	7	7.0	7	100	8	90
NDHRS16-14-113	5.5	6.0	4.5	10	9	90
NDHRS16-14-116	6	7	7	2.5	5	50

Table A2. Disease evaluation of *Puccinia striiformis* f. sp. *tritici* in 380 HRSW genotypes (continued).

Genotypes	PSTv-37	PSTv-52	FAR21-IT[a]	FAR21-DS[b]	MV21-IT[c]	MV21-DS[d]
NDHRS16-14-189	5.5	4	7.5	17.5	8	80
NDHRS16-14-204	7.5	6.3	7	15	8	80
NDHRS16-14-217	6.5	3.0	9	10	8	80
NDHRS16-14-252	6	6.0	7	17.5	5	50
NDHRS16-14-257	6.5	7.0	8	10	8	80
NDHRS16-14-260	7.5	4.0	8	20	8	80
NDHRS16-14-265	7.5	6.0	8.5	90	5	50
NDHRS16-14-58	4.5	5.5	7	25	5	50
NDHRS16-14-59	5	6.0	5	7.5	5	50
NDHRS16-14-71	3	5.5	7	12.5	8	70
NDHRS16-14-73	8.5	6.5	6.5	25	8	80
NDHRS16-14-90	5.5	0	6	17.5	8	80
NDHRS16-14-98	4	6.5	8	5	8	90
NDHRS16-14-124	7.5	9.0	8.5	70	5	50
NDHRS16-14-127	7.5	7.0	8	50	8	70
NDHRS16-14-141	5.5	0.0	7.5	40	8	90
NDHRS16-14-143	7	7.0	8	50	5	50
NDHRS16-14-149	7	0.0	6	45	5	50
NDHRS16-14-153	5.5	0.0	9	50	9	100
NDHRS16-14-156	6	9.0	9	35	9	100

a: Fargo adult plant resistance infection type in 2021.
b: Fargo adult plant resistance disease severity in 2021.
c: Mt. Vernon adult plant resistance infection type in 2021.
d: Mt. Vernon adult plant resistance disease severity in 2021.

Table A3. Resistant HRSW genotypes to TTKTT+.

Genotypes	Infection response	Classification
Keene	3.0	ND historical line
ND832	4.7	NDSU breeding line
NDSW16-12W-8	4.3	NDSU breeding line
MN11394-6	4.0	UMN breeding line
MN10055	2.7	UMN breeding line
HY682	2.7	Canadian historical line