Identification and Mapping of Adult Plant Stripe Rust Resistance in Soft Red Winter Wheat

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ABSTRACT

Since 2000, stripe rust, caused by the fungal pathogen (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.) has resulted in yield losses of wheat (*Triticum aestivium* L.) in the United States, that exceeded the combined losses of leaf rust (*Puccinia triticina* Eriks.) and stem rust (*Puccinia gramins* Pers.:Pers f. sp. *Tritici* Eriks. E. Henn.). The objectives of this study are to identify and map adult plant stripe rust resistance quantitative trait loci (QTL) in soft red winter (SRW) wheat that are effective against race PST-100, which is the predominant race of the pathogen in the eastern U.S. Adult plant resistance (APR) was characterized in the resistant wheat lines 'USG 3555', VA00W-38, and 'Coker 9553'. Resistance in each of the lines was evaluated in populations derived from crosses with susceptible lines 'Neuse', Pioneer Brand '26R46', and VA01W-21, respectively. On chromosomes 1AS, 4BL, and 7D of USG 3555, QTL were identified that explain on average 12.8, 73.0, and 13.6 percent of the variation for stripe rust infection type (IT), and 13.5, 72.3, and 10.5 percent of the variation for stripe rust severity. A QTL from Neuse was identified on 3A that on average explains 10.9 percent of the variation for IT and 13.0 percent of the variation for severity. On chromosomes 2AS and 4BL of VA00W-38, QTL were identified that on average explain 58.9 and 19.3 percent of the variation for stripe rust IT, and 51.9 and 12.1 percent of the variation for severity. On chromosomes 6BL and 3BL of Pioneer 26R46, QTL were identified that on average explain 8.9 and 2.1 percent of

the variation for IT and 11.7 and 3.9 percent of the variation for severity. Coker 9553 possesses the QTL on 4BL that is also present in USG 3555 and VA00W-38. The SSR markers, *Xgwm296*, *Xbarc163*, and *Xwmc756* were tightly linked to QTL on chromosomes 2AS, 4BL, and 6BL, respectively, and their use and development of additional diagnostic markers will facilitate the incorporation and pyramiding of stripe rust resistance QTL into SRW wheat lines via marker-assisted selection.

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STRIPE RUST OF WHEAT

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ABSTRACT

Wheat (*Triticum aestivium* L.) is one of the most important crops in the world, supplying the majority of carbohydrates consumed worldwide. Wheat farmers in the United States produce a wide range of wheat classes. Since 2000, stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) has become a major concern to growers in the United States. Prior to 2000, stripe rust had only been a concern to growers in the states of Texas, Washington, Oregon, and California. In 2000, a new population of stripe rust became prevalent that was more virulent and aggressive at higher temperatures. These new races resulted in an expanded geographical impact of stripe rust in the United States. Since 2000, stripe rust has been identified in each year in Virginia, resulting in significant yield losses in 2003, 2005, and 2010. Stripe rust has become the most devastating of the rust diseases in the United States since 2000. Forty-nine stripe rust resistance genes have been identified and catalogued including both hypersensitive and adult plant resistance genes. Many more genes have also been putatively designated. A major goal for wheat breeders in the southeastern U.S. is to identify and characterize the sources of stripe rust resistance that are available in the soft red winter wheat germplasm.

INTRODUCTION

Wheat (*Triticum aestivium* L.) is the main host of the fungal pathogen causing stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.), also known as yellow rust, though it may also infect barley (*Hordeum vulgare* L.) (Chen et al., 1995; Maloy and Inglis, 1993). Wheat farmers in the United States produce the world's widest range of wheat classes; providing grain with diverse end use qualities. Approximately 23.62 million metric tons (MMT) of the United States wheat crop was exported in the 2010/2011 market year, with the largest importers being Sub-

Saharan Africa (3.41 MMT), Egypt (2.62 MMT), Japan (2.37 MMT), Mexico (2.06 MMT), and the Philippines (1.27 MMT) (Vocke, 2011). Wheat is a principal food crop for approximately 35% of the world and consequently is the most widely cultivated crop in the world (Huang and Röder, 2004) supplying nearly 55% of all the carbohydrates consumed worldwide (Gupta et al., 1999). Wheat is grown in a wide range of environments but soft wheat is produced in two distinct regions in the United States: the eastern part of the country with production mainly east of the Mississippi River and the Pacific northwest including the states of Washington, Oregon and Idaho (Morris et al., 2005). The greatest concerns to growers and end-users are yield losses and reduction in grain quality. Consequently a primary research priority of breeders focuses on the development of cultivars expressing more durable resistance for the purposes of limiting yield losses and maintaining high grain quality (Dubcovsky, 2006).

Although fungicides limit the damage due to stripe rust and attempts have been made to control the spread of the pathogen by targeting inoculum sources, genetic resistance is still the most economical and sustainable control measure available (Chen and Line, 1992). Because major hypersensitive resistance genes work on a gene for gene basis, seedling resistance to stripe rust is usually ephemeral due to the ease with which the pathogen evolves virulence to a single resistance gene. Conversely, adult plant resistance (APR) tends to be more durable and effective against multiple races of a pathogen. Therefore, a continual search for genetic material containing novel forms of resistance is necessary. Introgression of multiple hypersensitive resistance genes plus APR genes into a single cultivar will result in a more broadly and durably resistant cultivar (Griffey and Allan, 1988).

Life Cycle and Spread of the Stripe Rust Fungus

Generally, stripe rust is an important disease of wheat grown in cooler climates (Bariana et al., 2002) and where moist weather is common (Wagoire et al., 1998). Stripe rust is a basidiomycete, which spreads in the form of clonally produced dikaryotic urediospores (Hovmøller et al., 2002). Stripe rust is autoecious, owing to the lack of evidence that the fungus has an alternate host upon which it completes a sexual reproductive cycle. Wind-blown spores can be spread over thousands of kilometers from initial infection sites and perpetuate on living host tissue (Kolmer, 2005) where the fungus exploits water and nutrient stores thereby abating plant growth (Chen, 2005). Of all the rusts, stripe rust is the most environmentally sensitive and resistance genes also appear to be sensitive to environmental factors, leading to problems when evaluating host plant resistance (Wagoire et al., 1998). Urediospores are most viable between 5 and 15°C and disease development is most rapid at temperatures between 10 and 15°C. Favorable conditions for stripe rust include; cool, wet falls; mild winters (temperatures above freezing); and long, cool, wet springs. Multiple disease cycles take place in one growing season owing to the brevity of cycles between infection and sporulation. Under optimal conditions, a single cycle may occur in eight days (Maloy and Inglis, 1993).

The three key factors contributing to stripe rust epidemics are: moisture, temperature, and wind (Chen, 2005). Moisture is necessary for urediospores to germinate and require three hours or more of constant moisture (Chen, 2005; Rapilly, 1979). Temperature is an important factor affecting spore germination, latent period and spore survival. Because stripe rust is most pervasive in cooler climates, the disease takes hold early in the growing season and consequently can cause more damage under optimal environmental conditions than either leaf rust (*Puccinia triticina* Eriks.) or stem rust (Chen, 2005). Wind contributes mostly to the spread of stripe rust (Chen, 2005; Kolmer, 2005).

It has long been the desire of crop scientists and growers to control the spread of pathogens to prevent yield losses and financial misfortune. In the 1920s, a barberry (*Berberis vulgaris* L.) eradication program was instituted in attempts to control the inoculum source of stem rust (*Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. E. Henn.) in Minnesota (Roelfs, 1989; Stakman, 1919). Though the program was effective, it also revealed that locally produced aeciospores and windborne urediospores blown from many hundreds of miles from neighboring states contributed to the spread of stem rust. Yet another source of inoculum was identified as urediospores produced from overwintering mycelium released once environmental conditions become conducive to their survival. There is evidence that certain farming practices such as furrow planting and no-tillage cultivation increase the number of overwintering mycelia because of the supply of organic material serving as a substrate of nourishment and protection for the fungus throughout the cold winter months (Roelfs and Long, 1987).

The main concern in controlling stripe rust is the spread of windborne urediospores and the overwintering of mycelium. Large areas of wheat infected by windborne urediospores show a uniform disease incidence as inoculum is spread evenly throughout the field (Roelfs, 1989). Disease incidence resulting from overwintering mycelia results in the development of disease foci or "hot spots" and is characterized by variable disease frequencies throughout the field with highest frequencies found closest to the initial foci. Wheat stripe rust epidemics spread through large fields with increasing velocity over space and time (Cowger et al., 2005). A mechanism for slowing the spread of a stripe rust epidemic is to decrease the infection rate, which can be accomplished by utilizing multiline varieties or incorporating diverse resistant sources in pure stands.

Detection and Evolution of Stripe Rust and Losses in the United States

Wheat stripe rust has been detected on all continents, excluding Antarctica, and in more than 60 countries (Chen, 2005). The first detection of stripe rust in the United States was reported in the western states in 1915 by F. Kolpin Ravn (Carleton, 1915; Line, 2002) though study of herbarium specimens indicates that stripe rust was collected in the 1890's in the United States (Humphrey et al., 1924). Historically, stripe rust in the United States is most frequently found in the western states but has recently (since 2000) become more problematic in central and southeastern states, and the Great Plains (Figure 1.1) (Chen et al., 2002).

According to Griffey (C.A. Griffey, personal communication, 2011), wheat stripe rust is an emerging disease in the eastern U.S. that threatens wheat production in this region. Epidemics of stripe rust have occurred in one or more eastern states since 2000 and were widespread and severe in 2003, 2005, ad 2010. Trace to moderate levels of stripe rust also were observed in Virginia in 2000 and resulted in significant losses in 2005. Stripe rust was present late in the growing season in Virginia in 2011 and did not result in significant yield losses, but was observed in many experimental plots of 'Shirley', which is one of the highest yielding soft red winter (SRW) wheat cultivars for the region. Conditions for stripe rust development were very good in Virginia in 2011 with a long, cool, wet spring, but because stripe rust development in Texas was low, the inoculum load in Virginia was low and late to arrive (Hughes, 2011).

It has been observed that new races of stripe rust have evolved which have a shorter latent period and are more tolerant to higher temperatures (Milus et al., 2006). Before 2000, 59 races of stripe rust had been identified in the United States historically. In the year 2000, 21 new races of stripe rust and 42 races in total were identified (Chen et al., 2002). By 2010, there have been a total of 146 identified races of stripe rust in the United States (X.M. Chen, personal Communication, 2011).

Figure 1.2 was taken from the final Cereal Rust Bulletin in 2010 and shows the incidence of stripe rust for that year across the United States (Long, 2010). Stripe rust can cause major reductions in yield and in the year 2000, a combined loss of over nine million bushels (mbu) of wheat was observed in the states of Arkansas (4,519,600), Washington (1,667,100), Oklahoma (1,472,200), California (757,700), Texas (335,000), Kansas (179,200), Oregon (88,200) and Louisiana (49,500). Consequently it was year 2000 that marked the most widespread occurrence of stripe rust in the United States, with 25 states reporting its presence (Chen et al., 2002). Between 2000 and 2005, an estimated 6.5, 31.8, 3.5, 75.5, 4.9 and 63.1 mbu of wheat were lost due to stripe rust in states east of the Rocky Mountains. In the ten years previous, the highest annual yield loss attributed to stripe rust was an estimated 0.24 mbu in 1993. In 2010, national yield losses due to stripe rust were estimated at over 86 mbu and a 5.8% yield loss (Long, 2011).

Due to the lack of a sexual stage in the life cycle of stripe rust, genetic recombination does not occur. It is believed that new variation in P. *striiformis* is created through mutation from avirulence to virulence and is considered to occur fairly quickly as races of the fungus have been shown to evolve virulence to a single resistance gene in a short period of time (Hovmøller et al., 2002; Steele et al., 2001). Compared to other pathogenic fungi of agricultural crops, genetic diversity within stripe rust at the molecular level is very low, though as mentioned, this does not prevent the quick evolution of new strains causing epidemics on previously resistant cultivars (Hovmøller et al., 2002).

Markell and Milus (2008) determined that two clonal populations have existed in the eastern U.S.; an ‘old’ population (before 2000) and a ‘new’ population (since 2000). Using AFLP (amplified fragment length polymorphism) markers to molecularly characterize and distinguish the different isolates, it was determined that the new introduction was most likely due

to an exotic arrival as opposed to a mutation event. They also observed that all isolates collected after 2000 in the United States were virulent to resistance genes *Yr*8 and *Yr*9 and all isolates collected before 2000 were avirulent to these genes. The new races were the first to show virulence to these genes in the United States. While the resistance gene *Yr*8 has not been deployed in SRW wheat cultivars east of the Rocky Mountains, gene *Yr*9 has been deployed widely due to its linkage with stem rust resistance gene *Sr31* and leaf rust resistance gene *Lr26* on the 1B/1R translocation and is present in several SRW wheat cultivars including: Sisson, SS 550, AGS 2000, USG 3209, Shirley, and Pioneer Brand 26R61. All of these cultivars, except for Pioneer Brand 26R61, are at least moderately susceptible to the new race of stripe rust (PST-100) that is prevalent in the southeastern U.S. The race PST-100 is virulent against resistance genes: *Yr2, Yr3a, Yr4a, Yr6, Yr7, Yr8, Yr9, Yr19, Yr20, Yr21, Yr22, Yr23, YrCle, YrSte, YrYam, YrPr1, YrPr2* and *YrHVII* (Chen, 2007). Virulence to *Yr*9 is of major concern as few other known resistance genes were previously incorporated into SRW wheat (Markell and Milus, 2008).

Table 1.1 is modified from data collected by Xianming Chen (X.M. Chen, personal communication, 2011) and highlights the effectiveness of host plant resistance to stripe rust in Pullman, WA. The most resistant cultivar, Madsen, is shown at the top of the table and the most susceptible line, PS 279, is at the bottom. The data indicate that if a grower chose a highly resistant cultivar, such as Madsen, yield loss due to stripe rust without the application of a fungicide would have been only 1.06%. Conversely, a farmer growing a highly susceptible line, such as PS 279, would have incurred a yield loss of 55.44%.

Under environmental conditions resulting in the worst-case scenario for a grower, stripe rust may cause yield losses up to 85% (Table 1.2) (French-Monar, 2010). This table shows the main reason why stripe is more destructive than either leaf or stem rust, as it can cause infection

earlier in the growing season and subsequently cause greater reductions in yield potential. If the pathogen overwinters in fields where wheat is planted, epidemics can start earlier in the growing season as the inoculum source is present in the field earlier.

Emergence of New Races and Expansion in Area of Adaptation

Since 2000, the new races of stripe rust have evolved to survive in warmer climates and the area in which the fungus overwinters has expanded. As mycelium overwinter in these areas, it serves as an inoculum source for the surrounding regions where the fungus cannot overwinter allowing it to spread further north by windborne urediospores. This development is significant because now the fungus is present in fields earlier than it was previously found and can consequently cause more damage. This also decreases the time it takes for the pathogen to move further north as temperatures become conducive for its survival.

Milus et al. (2006) postulated that the emergence of these new races, which were seemingly completely replacing the old races, was due to an increase in aggressiveness. Beginning in 2000, stripe rust was found to be developing at higher temperatures and thriving in areas where it had not been found previously. The more aggressive races are characterized by significantly shorter latent periods and increased spore germination at 18°C. It is believed that races with a short latent period potentially can cause up to 2.5 times more disease in a given season. It also appears that the new races contain many unnecessary virulence factors for attacking cultivars that are grown east of the Rocky Mountains. Stabilizing selection or negative selection does not appear to be functioning in this population of stripe rust. Generally a race having many unnecessary virulence factors would have a lower fitness value and thus be selected against, narrowing the race genetically to having only virulence factors that are necessary. It has been hypothesized that the reason stabilizing selection does not appear to be acting on this stripe

rust population is that some unnecessary virulence genes may be associated with genes for high aggressiveness and thus they are retained (Milus et al., 2006)

Chen (2005) states that race frequency is dependent on two opposing forces: virulence and the cost of unnecessary virulence. The situation in the case of the 'new' races of stripe rust is that those containing a multitude of virulence factors are able to infect more wheat cultivars. This, coupled with their increased aggressiveness at higher temperatures, has allowed these new races to become more prevalent.

Recent epidemics of stripe rust in the Ethiopian highlands of Bale due to virulence changes of the pathogen have resulted in extreme stripe rust development on tetraploid wheat and barley for the first time (Hailu and Fininsa, 2007). This is an area of the world where immediate attention is necessary because this region produces approximately 11% of the wheat for all of Ethiopia and little research has been done in this region.

Stripe rust was first detected in eastern Australia in 1979 (Steele et al., 2001). The detection of stripe rust in eastern Australia marked the loss of the last major wheat-producing region of the world that was free from stripe rust (Wellings et al., 2003).

As a result of the relatively recent emergence of stripe rust in the eastern U.S., little is known about the existence or diversity of stripe rust resistance in SRW wheat and few cultivars or advanced breeding lines have been selected or evaluated for resistance to the disease. Therefore, immediate and significant effort is needed to identify and genetically characterize stripe rust resistance to elucidate the prevalence and genetic diversity of resistance in SRW wheat.

Host Resistance Genes and Evolution of Pathogen Virulence

Currently, there are 49 designated stripe rust resistance genes catalogued (Table 1.3) though many are no longer effective due to the evolution of virulence factors in the pathogen population (Yahyaoui et al., 2002) and many more have been temporarily designated. Resistance genes *Yr*3 and *Yr*4 have multiple alleles of the respective genes.

Though the new, more aggressive stripe rust races are virulent on *Yr8* and *Yr9*, other resistance genes, including the qualitative genes *Yr5, Yr15 and Yr17* and the quantitative genes *Yr18* and *Yr29,* have also been incorporated to a lesser extent into the SRW wheat germplasm in the United States. Genes *Yr5* and *Yr15* have just recently been utilized in SRW wheat breeding programs, but *Yr17* may be present in a number of cultivars due to its linkage with the leaf rust resistance gene *Lr37*. Genes *Yr18* and *Yr29* are adult plant resistance (APR) genes present in some SRW wheat cultivars due to linkage with leaf rust resistance genes *Lr34* and *Lr46,* respectively (C.A. Griffey, personal communication, 2011). During the 2010 growing season it was observed that the previously stripe rust resistant cultivar Jagger, known to possess *Yr17*, sustained heavy stripe rust infection. This indicates the presence of a new race(s) of the pathogen that is/are virulent against *Yr17* (Duncan et al., 2010). Currently, genes *Yr5* and *Yr15* are resistant to all known races of stripe rust in the United States (X.M. Chen, personal communication, 2011).

The introduction and movement of stripe rust in eastern Australia in 1979 provides an example of the ability of stripe rust to quickly evolve virulence by a single step mutation (Steele et al., 2001). Within ten years of the initial stripe rust isolate introduction, which was virulent to resistance gene *Yr*2, fifteen different races had evolved by single stepwise mutations and were virulent against resistance genes *Yr*A*, Yr*5*, Yr*6*, Yr*7 and *Yr*8 (Kolmer, 2005). A second introduction occurred in Western Australia in 2002 having virulence for genes *Yr*6*, Yr*7*, Yr*8*, Yr*9

and *Yr*A (Wellings et al., 2003). By 2004 this new race spread throughout eastern Australia and became the predominant race identified throughout the country (Kolmer, 2005). Through AFLP and RAPD (random amplified polymorphic DNA) analyses, it was determined that the Australian *Puccinia striiformis* population showed more similarity to a European isolate compared to a Columbian isolate which supports the hypothesis that stripe rust was brought to Australia through an accidental introduction from Europe (Kolmer, 2005; Steele et al., 2001).

Gene *Yr*18 is a durable slow rusting gene but has been determined to be ineffective under conditions highly favorable for the rapid spread of stripe rust (Imtiaz et al., 2005). Virulence to genes *Yr*2*, Yr*3*, Yr*6 and YrA is very common in the Mexican highlands from which initial stripe rust inoculum may originate each year and enter the United States as spores carried by prevailing winds (Kolmer, 2005). Races of stripe rust virulent to *Yr*8 and *Yr*9 were discovered in 2000 in regions east of the Rocky Mountains (Kolmer, 2005; Milus et al., 2006). Virulence to gene *Yr*17, which is widely used in Europe and was recently deployed in SRW wheat breeding programs in the United States, has been detected in Southern Australia (Wellings et al., 2007) and more recently, in the United States (Duncan et al., 2010). A Louisiana SRW wheat cultivar Terral LA841 was recently released and is known to possess *Yr*17. This cultivar is still resistant to stripe rust, despite the fact that it has been exposed to races known to be virulent against *Yr17*. This indicates that Terral LA841 possesses resistance in addition to *Yr17* (Hunger et al., 2010).

Imtiaz et al. (2005) tested the New Zealand wheat cultivar Karamu for the presence of the high temperature adult plant resistance gene *Yr*18. It was initially believed that Karamu contained *Yr*18 along with the seedling resistance gene *Yr*A. In 1995 a new race of stripe rust virulent to *Yr*A emerged and Karamu suffered significant damage during a severe stripe rust epidemic. This meant one of three things: a race of stripe rust evolved with virulence to *Yr*18,

*Yr*18 was ineffective under high disease pressure, or Karamu did not contain the gene *Yr*18. A genetic study involved crossing Karamu with the cultivar Otane (which carries *Yr*18) and 140 double haploid (DH) lines obtained from the F_1 progeny were evaluated for seedling and adult plant resistance. Segregation ratios indicated that resistance was based on three genes, one from Karamu and two from Otane. It also was determined that the resistance gene from Karamu acted additively with genes from Otane to provide increased adult plant resistance but did not confer resistance on its own, and that gene *Yr*18 by itself does not provide sufficient resistance under high disease pressure. This study also concluded that Karamu does not possess *Yr*18.

In South Africa, stripe rust was first observed in 1996 in the Western Cape and has since spread to all of the important wheat producing areas surrounding it. Two pathotypes were detected; 6E16A- with virulence to *Yr*2, *Yr*6, *Yr*7, *Yr*8, *Yr*11, *Yr*14, *Yr*17 and *Yr*19 and 6E22A-, which appears to be a single step mutation event with added virulence to *Yr*25 (Boshoff et al., 2002).

Recently much attention has been paid to the importance of developing cultivars containing durable resistance based on multiple genes (William et al., 2003). Single hypersensitive major resistance genes may easily be overcome by the pathogen and so methods of pyramiding resistance genes and incorporating race non-specific resistance genes are critical to the development of resistant cultivars that are more durable. Often resistance genes are located within gene clusters or are tightly linked to other resistance genes showing pleiotropic effects. In a recent study by Khlestkina et al. (2007) the *Yrns-B1* stripe rust resistance gene was fine mapped on chromosome 3BS. Closely linked genes included the stripe rust resistance gene *Yr*30 and the stem rust adult-plant resistance gene *Sr*2. It was also determined that another cluster of disease resistance genes resided nearby including the leaf rust resistance gene *Lr*27, the

*Fhb*1 QTL for *Fusarium* head blight (FHB or scab) resistance, and a QTL for leaf rust resistance. The adult plant leaf rust resistance gene *Lr*34 and stripe rust resistance gene *Yr*18 confer slow rusting resistance and have been effective for over 50 years (Suenaga et al., 2003; William et al., 2003). As stated previously, *Yr*18 by itself does not provide sufficient protection from severe stripe rust epidemics, but it may be worth the effort to pyramid *Yr*18 with other sources of resistance as the *Yr18* locus provides durable resistance to many pathogens. Schnurnusch et al. (2004) conducted mapping studies using the winter wheat cultivar Forno to tag and validate a major QTL composed of the gene cluster *Ltn*/*Lr*34/*Yr*18 which has subsequently been found to contain genes governing resistance to stem rust, tolerance against *Barley yellow dwarf virus* (*Bdv*1), inactivation of a stem rust–resistance gene suppressor, and adult plant resistance to powdery mildew (Spielmeyer et al., 2005). This QTL comprised of a cluster of tightly linked genes has provided durable and effective resistance for combating these diseases for many years (Schnurbusch et al., 2004; Suenaga et al., 2003). Recently, much work has been done by the Keller lab in utilizing map-based isolation to clone resistance genes (Keller et al., 2005) and developing SSR (simple sequence repeat) markers for the *Lr*34 resistance region (Bossolini et al., 2006). This QTL has subsequently been cloned and identified as being an ABC (adenosine triphosphate-binding cassette) transporter. It has also been discovered that a single gene confers durable resistance against leaf rust, stripe rust, and powdery mildew and also causes leaf-tip necrosis (LTN), which serves as a phenotypic marker (Krattinger et al., 2009).

Another chromosomal segment with linked resistance genes *Lr*46/*Yr*29 is located at the distal end of the long arm of chromosome 1B (William et al., 2003). The 1B region has also been found to contain a powdery mildew (*Blumeria graminis* f. sp. *tritici*) QTL (Tucker et al., 2006). Inheritance studies revealed that resistance genes *Lr*37/*Yr*17/*Sr*38 for leaf rust, stripe rust,

and stem rust respectively were all closely linked and located distally on the short arm of chromosome 2A (Bariana and McIntosh, 1993). The powdery mildew resistance gene *Pm*4b and an APR QTL are also located in this chromosomal region (Yi et al., 2008). This cluster has been studied and deployed extensively because of its long-lived effectiveness and resistance to a myriad of rust diseases and powdery mildew. PCR marker assays have been developed for this chromosomal region with the hopes of utilizing the molecular markers for marker-assisted breeding (Helguera et al., 2003; Seah et al., 2001; Yi et al., 2008). The prevalence of such gene clusters proves that multi-gene complexes conferring durable resistance to multiple diseases exist in wheat and there are probably many more that have yet to be discovered.

Stripe rust resistance in SRW wheat

Markell et al. (2009) conducted a genetic study to determine the inheritance of resistance in the three SRW wheat lines 'McCormick', VA96W-270, and VA96W-270V. Two to three recessive, race-specific adult plant resistance genes conferred resistance in McCormick. Two recessive, race-specific adult plant genes conferred resistance in VA96W-270. There is at least one gene in common between McCormick and VA96W-270. Resistance in VA96W-270V, which is a variant of VA96W-270, is conferred by one recessive, all stage gene and one dominant, adult plant gene.

Based on evaluations of entries in the Uniform Eastern and Uniform Southern SRW wheat nurseries (http://www.ars.usda.gov/Main/docs.htm?docid=21894) and evaluations in the stripe rust nursery at Laurel Springs, NC, the genotypes 'Branson', 'AGS 2031', Coker 9553, USG 3555, VA00W-38, Pioneer 26R61, VA05W-139, and VA07W-415 have at least moderate levels of adult plant resistance. Little is known about the genes conferring resistance in these lines/cultivars. Susceptible and moderately susceptible lines/cultivars include: Sisson, SS 550,

AGS 2000, USG 3209, VA05W-251, VA05W-250, VA05W-258, VA05W-78, Pioneer Brand 26R46, Chesapeake, and Shirley (Bockelman, 2011).

CONCLUSIONS

Importance of stripe rust for Virginia and growers in the southeastern U.S.

As the area of adaptation of stripe rust expands and inoculum sources move closer to Virginia, stripe rust potentially will become a major problem for Virginia wheat growers in the near future. Currently, there are few highly resistant cultivars specifically adapted to this growing region. Kim and Ward (1997) reported that the genetic base of SRW wheat is fairly broad on the basis of pedigree and marker polymorphism analysis. Genetic improvement of traits and the development of cultivars with more durable resistance is dependent on the availability of genetic diversity (Griffey and Allan, 1988). It is of utmost importance to identify resistance genes within the SRW wheat gene pool and target those genes for introgression and pyramiding into wheat lines adapted to Virginia and the surrounding states. Also, because the germplasm has not been screened extensively for stripe rust resistance, it is possible that novel resistance genes will be discovered.

Based on disease assessments of entries evaluated in the 2005 Virginia state wheat tests at Warsaw and Painter, Virginia (Table 1.4), most of the top yielding SRW wheat cultivars are susceptible to stripe rust. A disease control experiment was also conducted by Stromberg and Kenley (2005) at Warsaw in which stripe rust severity and its effect on grain yield, test weight, and 1000 kernel weight (KWT) in the susceptible cultivar Sisson in fungicide-treated and non-treated plots was evaluated (Table 1.5). Non-treated plots of Sisson had 10% to 16% lower grain yields, 6% to 8% lower kernel weights and 1.1 to 1.7 lb/bu lower test weights than those of the

fungicide treated plots. Upon determining the effects of the pathogen on a susceptible, widely-grown cultivar, the importance of identifying wheat cultivars and lines having resistance to stripe rust and genetically characterizing the resistance genes for mode of inheritance, diversity and effectiveness to prevalent races becomes apparent.

Due to the recent emergence of stripe rust in the eastern U.S., little is known about the existence or diversity of stripe rust resistance in SRW wheat and few cultivars or advanced breeding lines have been selected or evaluated for resistance to the disease. This, coupled with the increase in virulence and expanded area of adaptation of the new races of stripe rust, has led to the need for an effort to identify and genetically characterize stripe rust resistance to elucidate the prevalence and genetic diversity of resistance in SRW wheat.

Proposed Research

This research was proposed to facilitate the development of wheat cultivars having effective and durable stripe rust resistance, as lines having only single genes are vulnerable to the rapidly evolving pathogen resulting in significant yield losses. The identification and mapping of novel genes will expand the toolbox available to breeders to combat this devastating disease. The long-term goals of this project are to identify novel resistance genes and pyramid genes into adapted, high yielding cultivars. Also, by the subsequent fine mapping of resistance genes, marker-assisted selection (MAS) can be implemented to facilitate rapid and efficient selection of new cultivars possessing multiple stripe rust resistance genes along with other important traits.

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Table 1.1. Yield losses caused by stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) and yield increase resulting from fungicide application on winter wheat (*Triticum aestivium* L.) cultivars in experimental plots under natural infection near Pullman, WA in 2010 (X.M. Chen, personal communication, 2011)

Cultivar	Yield (bu/ac)			Yield loss (%) by stripe rust	Yield increase (%) by fungicide treatment	Test Weight (lb/bu)	
	Untreated control	Fungicide treatment	Difference			Untreated control	Fungicide treatment
Madsen	110.12	111.3	1.18	1.06	1.07	59.59	59.59
Chuckar	106.62	114.81	8.19	7.14	7.68	59.21	59.56
Rod	104.87	103.19	-1.69	-1.63	-1.61	57.52	57.41
Stephens	102.07	116.44	14.37	12.34	14.08	58.15	58.61
Brundage 96	98.37	110.98	12.61	11.36	12.82	57.83	58.61
Westbred 528	97.44	108.21	10.77	9.95	11.05	59.35	59.95
Bruehl	95.27	102.05	6.78	6.65	7.12	57.13	56.78
Buchanan	93.32	89.06	-4.25	-4.77	-4.56	57.87	58.19
ORCF-102	90.04	102.57	12.53	12.22	13.92	57.48	58.19
Masami	86.1	100.64	14.53	14.44	16.88	56.21	56.81
Eltan	85.99	96.05	10.07	10.48	11.71	56.6	56.74
ORCF-103	83.07	97.35	14.28	14.67	17.19	56.81	56.78
Cashup	78.78	88.11	9.33	10.59	11.84	59.17	59.14
Tubbs 06	78.75	102.32	23.57	23.03	29.93	55.65	56.32
Eddy	71.37	92.53	21.16	22.87	29.65	60.69	61.92
Xerpha	70.49	95.44	24.95	26.15	35.4	57.83	58.43
Bauermeister	67.28	84.75	17.47	20.61	25.96	57.97	58.43
Farnum	66.43	67.93	1.51	2.22	2.27	59.49	59.77
Lambert	64.43	96.65	32.22	33.34	50.01	56.56	58.04
Paladin	63.72	80.03	16.31	20.38	25.6	58.29	59.56
Finley	61.7	64.98	3.28	5.04	5.31	62.17	62.73
Declo	42.15	75.27	33.12	44	78.58	54.56	56.67
PS 279	29.88	65.19	35.31	54.17	118.19	55.44	58.08
Mean	80.40	94.17	13.81	15.49	22.61	57.89	58.54

Table 1.2. Potential loss of yield from stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) based on growth stage of wheat (*Triticum aestivium* L.) and host susceptibility

Start of epidemic (Epiphytotic)	Percentage loss in crop based on host susceptibility			
	S	MS	MR	R
First node (Z31; F6)	85	75	55	25
Flag leaf (Z39; F9)	75	45	15	5
Mid-boot (Z45; F10)	65	25	7	2
First awns visible; first spikelet of inflorescence visible (Z49; between F10-10.1)	50	10	3	1
Mid-heading, half of inflorescence emerged (Z55; F10.3)	40	5	2	0
Mid-flowering; anthesis half way (Z65; F10.52)	12	2	1	0

S = Susceptible
MS = Moderately susceptible
MR = Moderately resistant
R = Resistant
Z = Zadoks decimal growth stage
F = Feekes growth stage

Table 1.3. Catalogued stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) genes, source, location, linked genes/remarks and citations[†]

Yr Gene	Original Source	Genome Location	Linked Genes, Molecular Markers and other Remarks	Reference(s)
Yr1	Chinese 166	2A		(Lupton and Macer, 1962)
Yr2	Heines VII	7B		(Lupton and Macer, 1962)
Yr3a	Cappelle-Desprez	1B		(Lupton and Macer, 1962)
Yr3b	Hybrid 46	1B		(Lupton and Macer, 1962)
Yr3c	Minister	1B		(Lupton and Macer, 1962)
Yr4a	Cappelle-Desprez	6B		(Lupton and Macer, 1962)
Yr4b	Hybrid 46	6B		(Lupton and Macer, 1962)
Yr5	*T. spelta album*	2BL	Xwgp-17-2B, Xwgp19-2B, YrSTS7/8	(Macer, 1966), (Chen et al., 2003)
Yr6	Heines Kolben	7BS		(Macer, 1966)
Yr7	Lee	2BL	Sr9g	(Macer, 1966)
Yr8	Compair	2D	Sr34	(Riley et al., 1968), (McIntosh et al., 1998)
Yr9	Clement	1BL	Sr31, Lr26, Xwgp4, Xwgp7, Xwgp8, Xwgp9	(Macer, 1975), (Shi et al., 2001)
Yr10	Moro	1BS	RgaYr10a, S26-M47, S13-M63	(Macer, 1975), (Smith et al., 2002)
Yr11	Joss Chambier		APR[‡]	(Priestley, 1978)
Yr12	Mega		APR	(Priestley, 1978)
Yr13	Maris Huntsman		APR	(Priestley, 1978)
Yr14	Hobbit		APR	(Priestley, 1978)
Yr15	*T. dicoccoides* G-25	1BL	Nor1, UBC212a, Xgwm413	(Gerechter-Amitai et al., 1989), (Peng et al., 2000)
Yr16	Cappelle-Desprez	2DS	APR	(Worland and Law, 1986)
Yr17	*T. ventricosa*	2AS	Lr37, Sr38, Vrga1	(Bariana and McIntosh, 1993), (Seah et al., 2001)
Yr18	Frontana	7D	Lr34, Pm38, LTN, Xgwm295, Xgwm1220, Xswm10, HTAP[§] Cloned	(Singh, 1992), (Suenaga et al., 2003), (Spielmeyer et al., 2005), (Bossolini et al., 2006), (Krattinger et al., 2009)
Yr19	Compair	5B		(Chen et al., 1995)
Yr20	Fielder	6D		(Chen et al., 1995)
Yr21	Lemhi	1B		(Chen et al., 1995)
Yr22	Lee	4D		(Chen et al., 1995)
Yr23	Lee	6D		(Chen et al., 1995)
Yr24	*T. turgidum* (K733)	1BS	Xgwm11-1B	(McIntosh et al., 1995), (Zakari et al., 2003)
Yr25	Strubes Dickkopf	1D		(McIntosh et al., 1998)
Yr26	R55	1BS	Xgwm11, Xgwm18	(McIntosh et al., 1998), (Ma et al., 2001)
Yr27	Selkirk	2BS	Lr13, Lr23,	(McDonald et al., 2004)
Yr28	*Ae. Tauschii* W-219	4DS	Xmwg634-4DS	(Singh et al., 2000)
Yr29	Pavon F76	1BL	Lr46, APR	(McIntosh et al., 1998), (McIntosh et al., 2001)

Table 1.3. Continued				
Yr30	Opata 85	3BS	Sr2, Lr27, APR	(McIntosh et al., 1998), (McIntosh et al., 2001)
Yr31	Pastor	2BS	Yr27, Yr23, Lr23	(McIntosh et al., 1998)
Yr32	Carstens V	2AS	Xwmc198, M62/P19-156, M59/P37-375	(Eriksen et al., 2004)
Yr33	Batavia	7DL		(McIntosh et al., 2004)
Yr34	WAWHT2046	5AL	Xgwm6-5A, B1	(Bariana et al., 2006)
Yr35	98M71	6BS		(Marais et al., 2005b)
Yr36	*T. dicoccoides* FA-15	6BS	*Gpc-B1*, Xucw74-6B, Xucw77-6B, HTAP	(Uauy et al., 2005), (Chicaiza et al., 2006), (Chen, 2005)
Yr37	Line S14	2DL		(Marais et al., 2005a)
Yr38	Line 0352-4	6AL	Lr56	(Marais et al., 2006)
Yr39	Alpowa	7BL	Xwgp36, Xwgp45, HTAP	(Lin and Chen, 2007)
Yr40	*Aegilops geniculata*	5DS	Lr57	(Kuraparthy et al., 2007)
Yr41	Chuannong 19	2BS	Xgwm410,	(Luo et al., 2008)
Yr42	Aegilops neglecta	6AS		(Marais et al., 2009)
Yr43	IDO377	2BL	Xwgp110, Xwgp103	(Cheng and Chen, 2009)
Yr44	Zak	2BL	XSTS7/8, Xwgp100, Yr5	(Sui et al., 2009), (Cheng and Chen, 2009)
Yr45	PI181434	3DL	Xwp118, Xwp115	(Li et al., 2011)
Yr46	PI250413	4D	Xcfd71, Xbarc98, Lr67, APR	(Herrera-Foessel et al., 2011)
Yr47	V336	5BS	Lr52	(Bariana et al., 2010)
Yr48	PI610750	5AL	Xwmc727, Xwms291, APR	(Lowe et al., 2011)
Yr49	Avocet S*3	3DS	Xgpw7321, Xgwm161	(McIntosh et al., 2011)

† Table does not include the multitude of temporarily designated genes for stripe rust resistance
‡ Adult Plant Resistance
§ High Temperature Adult Plant Resistance

Table 1.4. Stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) reaction of top yielding wheat (*Triticum aestivium* L.) cultivars evaluated in 2005 state wheat test (C.A. Griffey, personal communication, 2006)

Cultivar	2004 / 2005 2-Year yield bu/ac	2004 - 2005 Painter, VA Stripe rust reaction	2004 - 2005 Warsaw, VA Stripe rust reaction
USG 3209	78	I	I
SS MPV 57	77	S	S
Featherstone 176	76	MR	R
Renwood 3260	74	S	S
SS 560	74	S	S
V9412	73	I	I
Pioneer 26R24	73	S	S
Pioneer 26R15	72	MR	R
Sisson	71	VS	VS
Pioneer 26R31	71	S	S
SS 550	71	S	VS
Tribute	70	S	S
Chesapeake	70	VS	S
Crawford	70	MR	MR
SS 520	70	VS	VS

R=Resistant
MR=Moderately Resistant
I=Intermediate
MS=Moderately Susceptible
S=Susceptible
VS=Very Susceptible

Table 1.5. Yield and grain quality losses due to stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) in Sisson wheat (*Triticum aestivium* L.), Warsaw, VA, 2005 (adapted from Stromberg and Kenley, 2005)

Fungicide Treatment	Percentage leaf area diseased	Yield bu/ac	Percentage yield increase by fungicide treatment	Test Weight lb/bu	1000 KWT grams
Non-Treated	24.8	84.8	-	59.7	35.5
Folicur (4.0 fl.oz)	0	98.9	16.6	61.3	38.6
Prosaro (6.5 fl.oz)	0	98.2	15.8	61.4	37.9
Headline (13.5 fl.oz)	0	101.2	19.3	61.3	38.4
Headline (10.0 fl.oz)	1.6	94.8	11.8	60.8	40.0

KWT = Thousand kernel weight

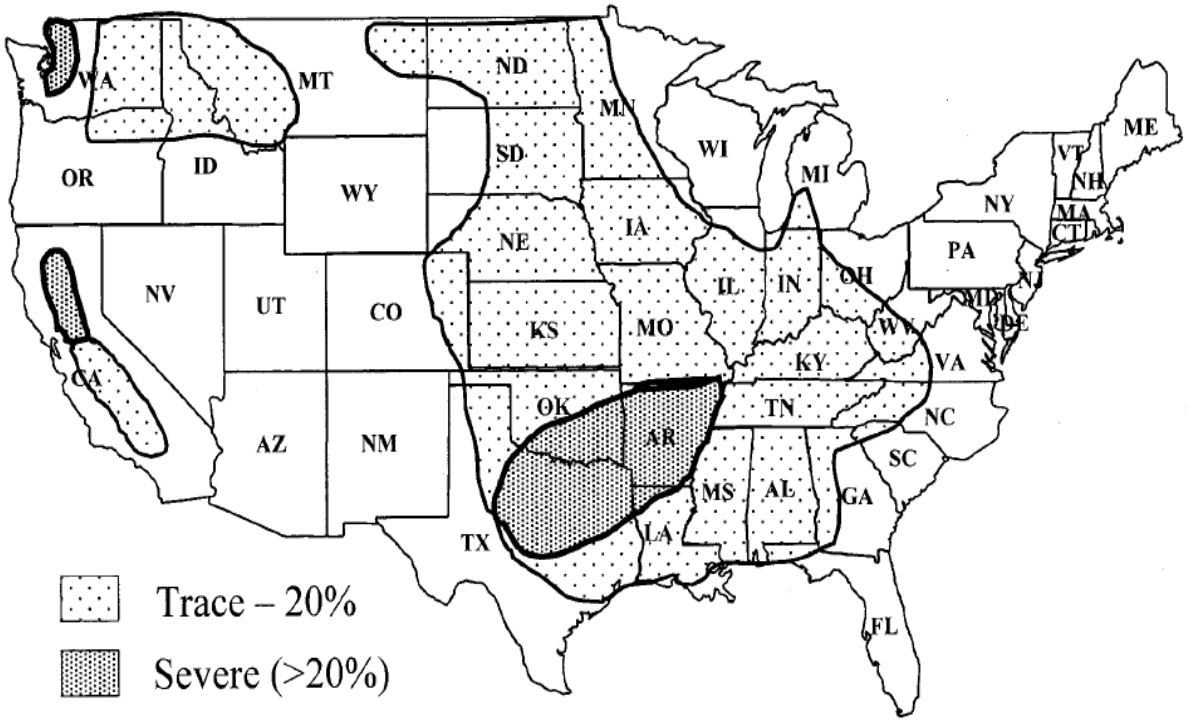

Figure 1.1. Occurrence and disease severity of wheat (*Triticum aestivium* L.) stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) in the United States in 2000 (Chen, et al., 2002 Used under fair use guidelines).

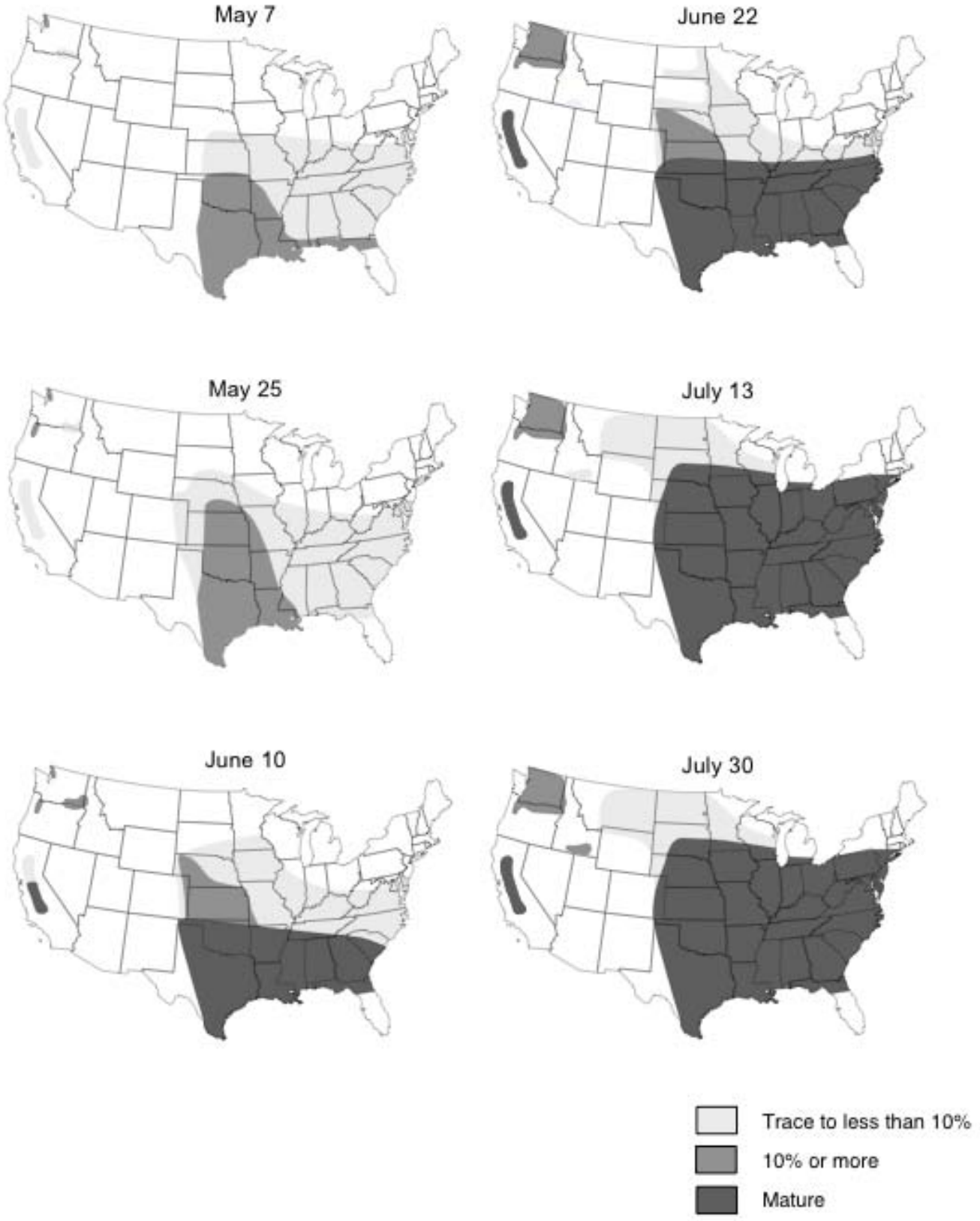

Figure 1.2. Stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici* Eriks.) severities in wheat (*Triticum aestivium* L.) fields and plots in the United States in 2010 (Long, 2010 Used under fair use guidelines).

IDENTIFICATION AND MAPPING OF ADULT PLANT STRIPE RUST RESISTANCE IN SOFT RED WINTER WHEAT CULTIVAR USG 3555

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ABSTRACT

Little is known about the extent or diversity of resistance in soft red winter wheat (*Triticum aestivum* L.) to stripe rust, caused by the fungal pathogen *Puccinia striiformis* Westend. f.sp. *tritici* Eriks. Prior to the spring of 2000, stripe rust had not been identified in Virginia, yet it has been found in each of the subsequent years and was widespread and severe in the commonwealth in 2005 and 2010. The 2010 growing season brought evidence that new races are present in the United States with virulence to the previously effective and frequently deployed gene *Yr17*. The breakdown of *Yr17* highlights the need to identify and characterize novel sources of durable resistance to employ in breeding programs. The recently released soft red winter (SRW) wheat cultivar USG 3555 has effective adult plant resistance to stripe rust. This resistance was characterized in a population derived from a cross between USG 3555 and 'Neuse', which is susceptible to stripe rust. The mapping population consists of 99 recombinant inbred lines, which were evaluated for reaction to stripe rust in field trials in North Carolina in 2010 and 2011 for infection type (IT) and severity. Fields were inoculated with race PST-100, which is the predominant race in the southeastern U.S. Genome wide molecular marker screens using 119 simple sequence repeats and 560 Diversity Arrays Technology markers were employed to identify quantitative trait loci (QTL) for stripe rust resistance. On chromosomes 1AS, 4BL, and 7D of USG 3555, QTL were identified that explain on average 12.8, 73.0, and 13.6 percent of the variation for stripe rust IT, and 13.5, 72.3, and 10.5 percent of the variation for stripe rust severity. A QTL from Neuse was identified on 3A that explained on average 10.9 percent of the variation for IT and 13.0 percent of the variation for severity. Flanking markers *Xbarc163* and *Xwmc692* linked to the QTL on chromosome 4BL, and development of additional

tightly linked markers, will facilitate the incorporation and pyramiding of stripe rust resistance into SRW wheat lines via marker-assisted selection.

INTRODUCTION

Wheat (*Triticum aestivium* L.) is the main host of the fungal pathogen stripe rust, caused by *Puccinia striiformis* Westend. f.sp. *tritici* Eriks., though it also infects barley (*Hordeum vulgare* L.) (Chen et al., 1995b; Maloy and Inglis, 1993). Wheat is grown in a wide range of environments but soft wheat is produced in two distinct regions in the United States (U.S.): the eastern part of the country with production mainly east of the Mississippi River and the Pacific northwest including the states of Washington, Oregon and Idaho (Morris et al., 2005). The greatest concerns of growers and end-users are yield losses and reduction in grain quality. Consequently a primary research priority of breeders is the development of cultivars expressing durable resistance to limit yield losses and maintain high grain quality (Dubcovsky, 2006).

Generally, stripe rust is an important disease of wheat grown in cooler climates (Bariana et al., 2002) and where moist conditions are common (Wagoire et al., 1998). Because stripe rust is pervasive in cooler climates, the disease becomes established early in the growing season and consequently can cause much more damage under optimal environmental conditions for the pathogen than either leaf rust, caused by *Puccinia triticina* Eriks., or stem rust, caused by *Puccinia graminis* Pers.:Pers f. sp. *Tritici* Eriks. E. Henn. (Chen, 2005). Historically, stripe rust is found most often in the Pacific regions of the U.S. but since 2000 it has become problematic in central and southeastern states and the Great Plains (Chen et al., 2002).

In 2000, 21 new races of stripe rust, and 42 races in total, were identified in the U.S. (Chen et al., 2002). Since 2000, additional new races have been identified with a wider range of

virulence factors than the races identified before 2000 (Chen, 2007). Isolates collected after 2000 are more aggressive than those collected previously. The more aggressive isolates are characterized by significantly shorter latent periods and increased spore germination at 18°C (Milus et al., 2006). Isolates with a short latent period may cause up to 2.5 times more disease in a given season.

Markell and Milus (2008) observed that all isolates collected after 2000 were virulent to resistance genes *Yr*8 and *Yr*9, while isolates collected previously were avirulent to these genes. These new races were the first to possess virulence to these genes in the U.S. While the resistance gene *Yr*8 has not been deployed in soft red winter (SRW) wheat cultivars east of the Rocky Mountains, gene *Yr*9 has been deployed widely due to its linkage with leaf rust resistance gene *Lr26* and stem rust resistance gene *Sr31* on the 1B/1R translocation. Gene *Yr*9 is present in several SRW wheat cultivars including: Sisson, SS 550, AGS 2000, USG 3209, Shirley, USG 3555, and Pioneer Brand 26R61 (C.A. Griffey, personal communication, 2010). All of these cultivars except for USG 3555 and Pioneer Brand 26R61 are at least moderately susceptible to the new race of stripe rust (PST-100) that is prevalent in the southeastern U.S. PST-100 is virulent against resistance genes: *Yr2, Yr3a, Yr4a, Yr6, Yr7, Yr8, Yr9, Yr19, Yr20, Yr21, Yr22, Yr23, YrCle, YrSte, YrYam, YrPr1, YrPr2* and *YrHVII* (Chen, 2007). Virulence to *Yr*9 is of major concern as few other known resistance genes were previously incorporated into SRW wheat (Markell and Milus, 2008).

Other resistance genes, including the qualitative genes *Yr5, Yr15 and Yr17* and the quantitative genes *Yr18* and *Yr29,* have also been incorporated recently into SRW wheat germplasm in North America. While genes *Yr5* and *Yr15* have only recently been used in SRW wheat breeding programs, *Yr17* may be present in a number of cultivars because of its linkage

with the leaf rust resistance gene *Lr37*. Genes *Yr18* and *Yr29* are adult plant resistance (APR) genes and also may be present in some SRW wheat cultivars due to their linkage with leaf rust resistance genes *Lr34* and *Lr46* respectively (C.A. Griffey, personal communication, 2011). During the 2010 growing season the previously stripe rust resistant cultivar Jagger, known to possess *Yr17*, sustained heavy stripe rust infection. This indicates the presence of a new race or races of the pathogen that are virulent against *Yr17* (Duncan et al., 2010). Genes *Yr5* and *Yr15* are currently resistant to all known races of stripe rust in the U.S. (X.M. Chen, personal communication, 2011).

There are 49 designated stripe rust resistance genes though many are no longer effective and more have been temporarily designated. Of the 49 designated genes, 13 are categorized as APR genes: *Yr11-Yr14* (chromosome unknown), *Yr16* (2DS), *Yr18* (7DS), *Yr29* (1BL), *Yr30* (3BS), *Yr36* (6BS), *Yr39* (7BL), *Yr46* (4D), *Yr48* (5AL) and *Yr49* (3DS). Markell et al. (2009) conducted a genetic study to determine the inheritance of stripe rust resistance in the three SRW wheat lines 'McCormick', VA96W-270, and VA96W-270V. Two to three recessive race-specific APR genes conferred resistance in McCormick. Two recessive race-specific adult plant genes conferred resistance in VA96W-270. There is at least one gene in common between McCormick and VA96W-270. Resistance in VA96W-270V, which is a variant of VA96W-270, is conferred by one recessive all-stage gene and one dominant adult plant gene.

Although fungicides limit the damage caused by stripe rust and attempts have been made to control the spread of the pathogen by targeting inoculum sources, genetic resistance is still the most economical and sustainable control method (Chen and Line, 1992). Because qualitative hypersensitive resistance genes work on a gene for gene basis, seedling resistance to stripe rust is usually ephemeral due to the ease with which the pathogen evolves virulence to a single

resistance gene. APR tends to be much more durable and effective against multiple races of a pathogen. Therefore, a continual search for genetic material containing novel forms of resistance is necessary. Introgression of multiple hypersensitive resistance genes plus APR genes into a single cultivar will result in a more broadly and durably resistant cultivar (Griffey and Allan, 1988).

The first objective of this study was to map APR to stripe rust in U.S. SRW cultivar USG 3555. After QTL locations were identified in the mapping population, the second objective was to compare these QTL to other known QTL or genes that had previously been mapped to determine if the sources of resistance identified in this study are novel. The third objective was to develop breeder friendly markers suitable for marker-assisted selection.

MATERIALS AND METHODS

Germplasm

The population consists of 288 RILs derived from a cross between USG 3555 (resistant) and Neuse (susceptible). A random sample of 99 RILs was selected from the 288 RILs for marker analysis. USG 3555 was derived from the cross VA94-52-60 / Pioneer Brand '2643' // 'USG 3209' (Griffey et al., 2009). Neuse was derived from the cross 'Coker 86-29' // 'Stella' / CHD 756-80 /3/ 'Coker 9907' (Murphy et al., 2004). The resulting USG 3555 / Neuse F_1 seed was planted in a single 1.2 m headrow in 2005 and harvested and threshed in bulk. The population was then advanced from the F_2 to F_4 generation in 20.9 m^2 blocks at Warsaw, Virginia using a modified bulk breeding method wherein selection was based solely on plant and spike characteristics at maturity. Stripe rust was not observed during the advancement of the

population, so direct selection for stripe rust resistance which would have skewed the population distribution was considered negligible.

Single spikes were harvested from the F_4 population during the summer of 2009 and subsequently maintained as RILs. Each spike was threshed individually and planted as a single 1.2 m $F_{4:5}$ headrow during the fall of 2009 at Laurel Springs, North Carolina, and evaluated in the spring of 2010. Approximately 8 to10 spikes were harvested from each row. The following season, three replications of $F_{5:6}$ RILs were planted in North Carolina in 2010 and evaluated in the spring of 2011. Each individual row was planted from an individual head harvested the previous year.

Field Evaluation

A single replication of RILs of generation $F_{4:5}$ (2010) and three replications of $F_{5:6}$ (2011) RILs were evaluated in 1.2 m headrows with a 0.3 m spacing between rows in a randomized complete block design. Artificial inoculum was generated by transplanting clumps of young plants colonized with *P. striiformis* race PST-100 into susceptible spreader rows. Data were collected when the susceptible parent Neuse, susceptible spreader 'Amigo', and susceptible controls VA00W-21 ('Chesapeake' sib) and Pioneer Brand '26R46' showed high levels of disease. In North Carolina in 2010 and 2011, data were collected visually for infection type (IT), based on a 0 to 9 scale, and severity (percent leaf area diseased), based on a 0 to 100 percent scale (Line and Qayoum, 1992).

DNA Isolation and Amplification

Leaf tissue samples for DNA isolation were collected from laboratory grown seedlings from the 99 randomly selected $F_{5:6}$ RIL plants, the two parents, and two controls. Seedlings were grown on moistened cotton balls in 96-well flats. Tissue was collected in 2 mL test tubes, each

containing a stainless steel bead required for the tissue-grinding step, when seedlings reached the two-leaf stage. Tissue samples were then frozen in an ultra-low (-80°C) freezer. Frozen samples were then ground in a Spex CertiPrep 2000 Geno-Grinder (Metuchen, NJ) for 15 seconds, or until finely ground. DNA extraction was then performed by the CTAB method as described by Saghai Maroof et al. (1994) and Yu et al. (1994) for SSR marker analysis, or by a protocol from Diversity Arrays Technology (DArT) for DArT marker analysis (http://www.diversityarrays.com/genotypingserv.html).

SSR primer pairs were synthesized by Applied Biosystems (ABI) (Carlsbad, CA, USA) and DNA Integrated Technologies (Coralville, IA, USA). Primers were either directly labeled with a fluorescent dye or were indirectly labeled with an M13 fluorescent tail. Fluorescent dye labels used were FAM (blue), NED (yellow/black), VIC (green), HEX (green) and PET (RED).

A single PCR reaction with four direct labeled fluorescent SSR primers was performed in a volume of 13 µl and contained: 2.5 µl of DNA (50 ng), 1.2 µl of 10× buffer, 0.36 µl of $MgCl_2$ (50 mM/µl), 0.96 µl of dNTP (25mM/µl), 0.30 µl of each pair (forward and reverse) of direct labeled primers (10 µM/µl), 0.18 µl of Taq polymerase (5 units/µl), and 6.4 µl of molecular grade water.

A single PCR reaction for each M13-labeled fluorescent primer was performed in a volume of 13 µl and contained: 2.5 µl of DNA (50ng), 1.2 µl of 10x buffer, 0.36 µl of MgCl2 (50 mM/µl), 0.96 µl of dNTP (25mM/µl), 0.96 µl of M13-tailed forward primer (1 µM/µl), 0.72 µl of reverse primer (10 µM/µl), 0.72 µl of M13 dye labeled primer (10 µM/µl), 0.18 µl of Taq polymerase (5 units/µl), and 5.40 µl of molecular grade water. The PCR products of four separate M13 PCR reactions were then combined for analysis in an Applied Biosystems 3130xl Genetic Analyzer (Foster City, CA).

A touchdown PCR program was used and included four stages: Stage 1 was 3 min at 94 ºC; stage 2 was 10 cycles of 30 s at 94 ºC, 45 s at 65 ºC, 1 min at 72 ºC with 1 ºC decrease in each cycle; stage 3 was 25 cycles of 30 s at 94 ºC, 45 s at 50 ºC, 1 min at 72 ºC and; stage 4 was 5 min at 72 ºC then storage at - 4 ºC until needed for electrophoresis.

A portion of the PCR product(s) (3.5 µl for direct labeled and 1.0 ul of each M13 labeled, for a total of 4 ul), was then transferred to a 96-well PCR plate with each well containing 9.8 µl of Hi-Di formamide and 0.08 µl of size standard. Samples were then denatured at 95°C for 5 minutes and cooled at 4°C until needed.

Capillary Electrophoresis and Molecular Marker Analysis

PCR products were visualized on an Applied Biosystems 3130xl Genetic Analyzer and the generated data were analyzed using the genotyping software GeneMarker version 1.70, designed by SoftGenetics (State College, PA, USA). DNA samples of the mapping population were sent to Australia for DArT analysis to increase the genome wide coverage.

Linkage Map Construction

JoinMap 4 (Van Ooijen, 2006) was used for construction of the linkage groups at an LOD threshold of 3.0 by regression interval mapping based on the Kosambi mapping function. MapChart 2.2 (Voorrips, 2006) was used as a tool to combine linkage maps and QTL data for a more seamless visual representation of the chromosomal locations of QTL.

Statistical Analysis

The SAS procedure PROC GLM (SAS, 2009) was used to conduct an ANOVA for the 2011 data. Both line and replication were considered fixed effects. JMP 8.0 (JMP, 2010) was used for preliminary identification of marker/trait associations with one-way ANOVA. Markers

were identified as having high r^2 values and low *p*-values. JMP 8.0 was also used for comparison of means using a Student's t-test.

MapQTL 5 (Van Ooijen, 2004) was used to generate LOD values to identify significant (LOD < 2.85) marker/trait associations and genomic regions harboring QTL for stripe rust resistance. A minimum LOD threshold of 2.85 was used for identifying QTL regions based on a permutation test at 1000 iterations.

RESULTS

Linkage Maps

A total of 560 DArT markers and 119 SSR markers with 126 marker loci were used to construct the genetic map. Of the 364 SSR markers that were screened, 170 (47%) were polymorphic between the two parents. Of the 170 polymorphic markers, 119 were mapped and used to genotype the population. Of the 119 mapped markers, seven mapped to multiple loci giving a total of 126 mapped loci with 80 (63%) being co-dominant and 46 (37%) being dominant. Approximately 560 DArT markers were polymorphic between the two parents and were mapped in the population. Markers were placed on all 21 chromosomes, though coverage on chromosome 5D was incomplete with only two markers mapping there (data not shown for all chromosomes). The chromosome with the most complete coverage was chromosome 3B, the only wheat chromosome for which a physical map is available (Paux et al., 2008). Chromosome distances ranged from 2.6 cM on chromosome 7D to 115.7 cM on 3B, while they vary from 59 cM on 4B to 173 cM on 5B on the 2004 wheat microsatellite consensus map (Somers, 2004). Of the 126 mapped SSR loci, 104 were used in the construction of the genetic maps and 22 were unlinked to a chromosome. Of the 560 mapped DArT loci, including twelve from rye (*Secale*

cereale), 33 from triticale (× *Triticosecale*), and 515 from wheat, 513 were used in the construction of the genetic maps and 47 were unlinked to a chromosome.

Phenotypic Evaluation

The population was evaluated in a single replication in 2010. In 2011, poor field conditions, including flooding and high weed pressure, caused missing data points in the second and third replications. Analysis of variance for disease reaction phenotypes from North Carolina in 2011 is presented in Table 2.1. Statistically significant ($p < 0.05$) variation among RILs was observed for both IT and severity. Replication was not a significant source of variation for the severity phenotype, but was statistically significant for the IT phenotype. Missing data points in the second and third replications caused IT phenotypes to differ significantly among replications; therefore, only data from the first replication were used for QTL and statistical analysis. Parental means and ranges among RILs for each trait by environment and for each trait across environments are presented in Table 2.2

QTL Analysis

Four QTL were identified with three positioned on chromosomes 1AS, 4BL, and 7D of USG 3555 and one positioned on chromosome 3A of Neuse. Markers linked to QTL with the highest LOD values in each environment and for each trait were identified (Table 2.3). The QTL with the largest effect on stripe rust resistance was located on chromosome 4BL and was identified in each environment associated with IT and severity phenotypes.

Phenotypic Distribution

The disease ratings for RILs were averaged across environments for IT and severity. The distribution among RIL phenotypes for IT (Figure 2.1a) and severity (Figure 2.1b) indicates that transgressive segregation is occurring, which confirms the polygenic nature of resistance and the

presence of different resistant genes in each parent. The resistant parent, USG 3555, had a mean IT rating over all environments of 1.2 and a mean severity of 1.8%. The susceptible parent, Neuse, had a mean IT of 7.3 and a mean severity of 67.2%. Of the 288 RILs in the population, 24 had a mean IT rating lower than USG 3555 and 52 had a mean severity rating lower than USG 3555. There were 64 RILs identified with IT ratings higher than Neuse and eighteen with severity ratings higher than Neuse. None of the lines were significantly more resistant than USG 3555 ($p < 0.05$) or more susceptible than Neuse, though this can be attributed to the small population size and the few replications used in this study.

Chromosome 4BL

The LOD curve peak of the QTL of 4BL, designated *QYrus.vt-4BL*, (Figure 2.2a) is located at DArT marker locus *Xwpt8796* and flanked by *Xgwm251* (3.2 cM from QTL), *Xbarc163* (0.9 cM), and *Xwmc692* (1.3 cM). The marker locus *Xwpt8796* explains 62.1% and 82.5% of the phenotypic variation for stripe rust infection type and severity with LOD values ranging from 19.4 to 33.5. The SSR marker loci *Xgwm251*, *Xbarc163*, and *Xwmc692* explain 61.0 to 74.0%, 61.6 to 78.0%, and 56.6 to 77.6% of the phenotypic variation, respectively with LOD values ranging from 18.8 to 27.4, 19.1 to 30.0, and 16.8 to 30.5, respectively. The LOD peak for *QYrus.vt-4BL* reached the threshold in both years and for both IT and severity.

Chromosome 3A

The LOD curve peak of the QTL of 3A, designated *QYrne.vt-3A*, (Figure 2.2b) is located at DArT marker locus *Xwpt7756* and closely linked with *Xwpt2755* (0.2 cM), and *Xwpt1681* (0.4 cM). These marker loci explain 10.1 to 14.3%, 9.9 to 14.5%, and 9.3 to 14.8% of the phenotypic variation, respectively with LOD values ranging from 2.0 to 3.0, 2.0 to 3.0, and 1.9 to 3.1, respectively. The LOD peak for *QYrne.vt-3A* reached the threshold in 2011 for severity.

Chromosome 7D

The LOD curve peak of the QTL of 7D, designated *QYrus.vt-7D*, (Figure 2.2c) is located at DArT marker locus *Xwpt743269* and flanked by *Xwpt744388* (0.2 cM) and *Xwpt663992* (<0.1 cM). The marker locus *Xwpt743269* explains 9.7 to 17.4% of the phenotypic variation with LOD values ranging from 2.0 to 3.6. The marker loci *Xwpt744388* and *Xwpt663992* explain 9.1 to 16.2% and 9.7 to 17.4% of the phenotypic variation, respectively with LOD values ranging from 1.9 to 3.3 and 2.0 to 3.6, respectively. The LOD peak for *QYrus.vt-7D* reached the threshold in 2010 for IT.

Chromosome 1AS

The LOD curve peak of the QTL of 1AS, designated *QYrus.vt-1AS,* (Figure 2.2d) is located at DArT marker locus *Xwpt671596* and flanked by *Xwpt3870* (0.1 cM), *Xgwm33* (1.8 cM), and *Xwpt734107* (0.7 cM) and *Xcfa2153* (3.2 cM). The marker locus *Xwpt671596* explains 11.4 to 15.5% of the phenotypic variation with LOD values ranging from 2.6 to 3.6. The marker loci *Xwpt3870*, *Xgwm33*, *Xwpt734107*, and *Xcfa2153*, respectively, explain 11.4 to 12.7%, 9.4 to 14.2%, 10.8 to 17.0%, and 8.4 to 12.8% of the phenotypic variation with LOD values ranging from 2.6 to 3.6, 2.1 to 3.2, 2.4 to 4.0, and 1.9 to 2.9, respectively. The LOD peak for *QYrus.vt-1AS* reached the threshold for stripe rust IT in both years and for severity in 2010, but not for severity in 2011.

Genotypic Effects of Marker Loci on Stripe Rust

To determine the effect of combining multiple QTL, individuals in the mapping population were identified that have different combinations of marker loci (Table 2.4). The means of the genotypic classes possessing different QTL combinations for both IT and severity were compared using a Student's t-test in which the means of each possible pair of genotypic

classes were compared. The means of genotypic classes sharing a common letter are not significantly ($p < 0.05$) different. There were no lines identified that possessed the QTL combinations 1A + 3A, 1A + 7D, and 1A + 3A + 7D.

For severity and IT, all lines that possess the 4BL QTL fall into the resistant group and all lines lacking the 4BL QTL fall into the susceptible group. For severity, there were no genotypic classes that were statistically ($p < 0.05$) more resistant or susceptible than the genotypic classes of the parents. For IT, there were no genotypic classes more resistant than the genotypic class of the parent USG 3555, but the genotypic classes that include lines only possessing the 7D QTL or no QTL were statistically ($p < 0.05$) more susceptible than the genotypic class of the parent Neuse. The IT data indicates that the QTL on 7D from USG 3555 is not effective by itself and also lines possessing only the QTL on 3A from Neuse are more resistant than lines possessing none of the QTL. Both the IT and severity data indicate that lines with the highest level of resistance possess the 4BL QTL from USG 3555.

DISCUSSION

Novel QTL

QTL associated with adult plant resistance to stripe rust were detected on chromosomes 1AS, 4BL, and 7D of USG 3555 and on 3A of Neuse. Chen et al. (1995a) identified seedling stripe rust resistance genes in the winter wheat cultivars Daws (*YrDa1*) on chromosome 1A, Tres (*YrTr2*) on chromosome 3A, and Clement (*YrCle*) and Moro (*YrMor*) on chromosome 4B. The winter wheat cultivar Yamhill also possesses a seedling resistance gene (*YrYam*) on chromosome 4B (Chen et al., 1994). The Australian wheat cultivar Batavia possesses *Yr33*, which is a seedling resistance gene on the long arm of chromosome 7D (McIntosh et al., 2004). The

previously identified genes are all race-specific, seedling resistance genes and neither USG 3555 nor Neuse possesses seedling resistance to *Puccinia striiformis* race PST-100 (C.A. Griffey, personal communication, 2006). It is also known that Clement (*YrCle*) and Yamhill (*YrYam*) are susceptible to race PST-100 in both the seedling and adult plant stages (Chen, 2007). The QTL identified in the current study differ from the resistance genes previously identified on chromosomes 1A, 3A, and 4B.

The QTL on chromosome 4BL was also identified in the Virginia Tech experimental wheat line VA00W-38 and the SRW wheat cultivar Coker 9553, which both possess APR to stripe rust (Christopher et al., unpublished, 2011). The presence of this QTL in multiple SRW lines, which were not bred for resistance to stripe rust, indicates that this QTL likely is linked with another trait of interest and is prevalent in the SRW wheat germplasm in the southeastern U.S.

The adult plant resistance gene *Yr18* was first identified in the South American cultivar Frontana on the short arm of chromosome 7D (Singh, 1992). The QTL *QYrus.vt-7D* identified in the current study was mapped only by DArT markers with no linked SSR markers in the QTL region. It is difficult to determine which chromosome arm the QTL is located on due to the lack of a DArT consensus map for wheat. Nevertheless, available information indicates that *QYrus.vt-7D* is different than *Yr18*. A group of SSR markers, unlinked to the group of DArT markers associated with *QYrus.vt-7D*, mapped to the short arm of chromosome 7D in the same region where *Yr18* is located. The SSR marker loci *Xbarc126* and *Xgwm44* were mapped in the population and did not show any association with stripe rust resistance (data not shown). These loci flank *Xgwm295*, which is an SSR marker commonly used for MAS of *Yr18* (Schnurbusch et al., 2004). It would be expected that *Xbarc126* and *Xgwm44* would show some association with

stripe rust resistance if *Yr18* were present in the population. Also, *Yr18* is linked with the phenotypic marker LTN (leaf tip necrosis), but USG 3555 does not exhibit LTN in the field. Based on marker data obtained from the genotyping center in Raleigh, North Carolina, USG 3555 does not possess the marker allele associated with *Yr18* (G. Brown-Guedira, personal communication, 2011). These observations indicate that *QYrus.vt-7D* and *Yr18* are not the same.

Use of Markers for MAS

The goal of this study was to identify the chromosomal location and number of QTL present in USG 3555 and to identify diagnostic molecular markers to use for the incorporation of APR to stripe rust into breeding populations. Informative microsatellite markers have been identified for *QYrus.vt-4BL* (*Xgwm251*, *Xbarc163*, and *Xwmc692*) and *QYrus.vt-1AS* (*Xcfa2153* and *Xgwm33*) (Table 2.5), which are the two major QTL for stripe rust resistance identified in USG 3555. The marker locus *Xbarc163* is an excellent candidate for use in MAS as it is the most tightly linked SSR marker to *QYrus.vt-4BL* at a distance of 0.9 cM and on average explains 69.2% of the variation in stripe rust phenotype. The marker locus Xgwm33 is also a good candidate for use in MAS as it is the most tightly linked SSR marker to *QYrus.vt-1AS* at a distance of 1.8 cM and on average explains 11.4% of phenotypic variation. Use of these markers will lead to a more feasible and directed effort in the introgression of adult plant stripe rust resistance into adapted wheat lines.

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conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

ATTRIBUTIONS

Preliminary seedling and adult plant screens to multiple stripe rust races were conducted in growth chamber and field experiments by David Marshall, Jerry Johnson, Gene Milus, Kimberly Garland-Campbell, Carl Griffey, and Xianming Chen. A pure collection of race PST-100 was provided by Gene Milus for preliminary growth chamber experiments. Recombinant inbred lines were planted and evaluated at Laurel Springs, NC, by David Marshall and Myron Fountain. Shuyu Liu performed analysis of variance on the phenotypic data and provided technical support. Marla Hall and Carl Griffey provided technical support and guidance.

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Table 2.1. Mean squares for analysis of variance of USG 3555/Neuse recombinant inbred line (RIL) wheat population for reaction to stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.) for 99 RILs grown at Laurel Springs, NC in 2011

Source of variation	df	MS IT[†]	MS % Severity[‡]
Line	98	11.7***	1621***
Replication	2	4.0*	266
Error	129	1.0	155

[†] Mean square of infection type rating from 0-9: 0=resistant to 9=susceptible
[‡] Mean square of severity rating based on % leaf area diseased
* Significant at the 0.05 probability level
** Significant at the 0.01 probability level
*** Significant at the 0.001 probability level

Table 2.2. Mean stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.) reaction over two environments for USG 3555, Neuse, and the 99 recombinant inbred wheat lines (RIL)

Trait	USG 3555 Mean	Neuse Mean	RIL Mean	RIL Min	RIL Max
2010					
Infection Type (0-9)	1.1	6.9	4.4	0.0	9.0
% Severity	1.7	60.4	19.9	0.0	70.0
2011					
Infection Type (0-9)	1.6	7.5	4.4	0.0	9.0
% Severity	1.8	78.2	26.7	0.0	90.0
2010/2011					
Infection Type (0-9)	1.2	7.3	4.4	0.0	9.0
% Severity	1.8	67.2	24.2	0.0	90.0

Table 2.3. Quantitative trait loci associated with reaction to stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.) infection type (IT) and severity identified in USG 3555 and Neuse wheat (*Triticum aestivum* L.) cultivars in 2010 and 2011

Trait	Year	Chromosome	Marker	Position (cM)	LOD	Additive†	% Variation‡	Source	QTL
	2010	4BL	*Xwpt8796*	16.0	21.4	-2.4	66.1	USG 3555	*QYrus.vt-4BL*
		1AS	*Xwpt671596*	8.3	2.9	-1.0	12.6	USG 3555	*QYrus.vt-1AS*
		7D	*Xwpt743269*	0.9	3.6	-1.2	17.4	USG 3555	*QYrus.vt-7D*
	2011	4BL	*Xwpt8796*	16.0	31.5	-2.1	80.0	USG 3555	*QYrus.vt-4BL*
IT (0-9)		1AS	*Xwpt3870*	8.4	3.0	-0.8	12.9	USG 3555	*QYrus.vt-1AS*
	2010	4BL	*Xwpt8796*	16.0	19.4	-18.5	62.1	USG 3555	*QYrus.vt-4BL*
		1AS	*Xwpt734107*	7.6	4.0	-9.6	17.0	USG 3555	*QYrus.vt-1AS*
	2011	4BL	*Xwpt8796*	16.0	33.5	-25.2	82.5	USG 3555	*QYrus.vt-4BL*
Severity (%)		3A	*Xwpt1681*	1.6	3.1	-10.6	14.8	Neuse	*QYrne.vt-3A*

† The additive effect of reducing stripe rust IT and severity associated with the resistant marker allele.
‡ Estimate of the percentage of the phenotypic variation explained by the resistant marker allele.

Table 2.4. Mean infection type (IT) and severity of recombinant inbred line genotypic classes of combinations of resistance quantitative trait loci (QTL)

QTL Combination[†]	Severity (%) [‡]	IT (0-9)
4B, 1A, 7D[§]	2.03[a]	1.22[a]
4B, 7D	2.25[a]	2.25[b]
4B, 1A, 3A, 7D	2.62[a]	2.18[b]
4B, 1A, 3A	2.64[a]	2.72[ab]
4B	3.06[a]	2.33[b]
4B, 3A	3.28[a]	2.83[b]
4B, 1A	4.56[a]	2.71[ab]
4B, 3A, 7D	5.76[a]	2.40[ab]
3A, 7D	40.28[b]	6.22[cd]
1A	43.33[b]	6.37[cd]
3A[¶]	45.21[b]	5.94[c]
NONE	52.89[b]	7.26[d]
7D	52.92[b]	7.67[d]

[†] 4B = *QYrus.vt-4BL*; 1A = *QYrus.vt-1AS*; 7D = *QYrus.vt-7D*; 3A = *QYrne.vt-3A*

[‡] Mean phenotype of different QTL combinations sharing the same letter are not significantly different ($p < 0.05$)

[§] Genotype of resistant parent USG 3555

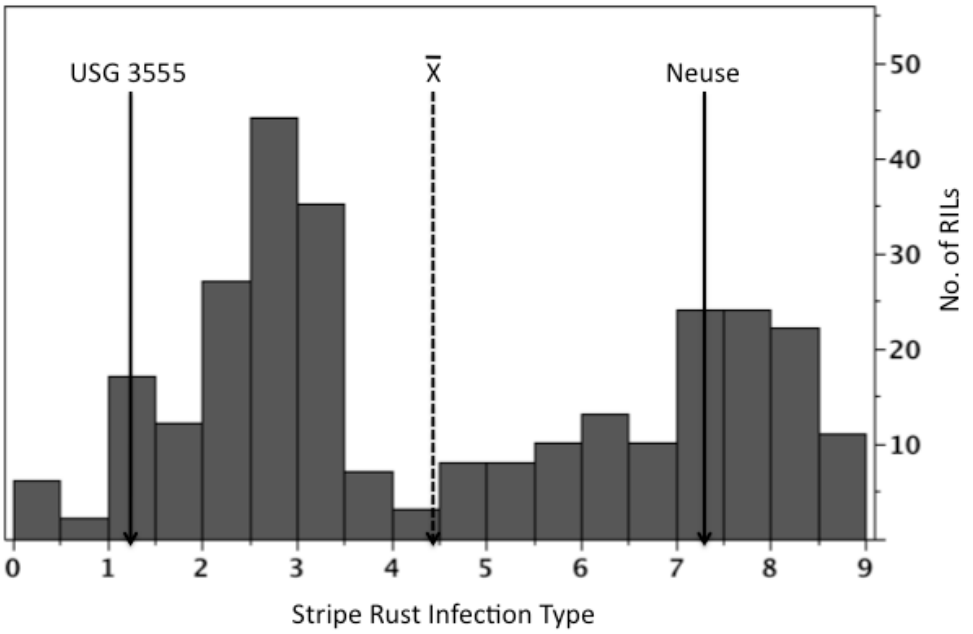
[¶] Genotype of susceptible parent Neuse

Table 2.5. Simple sequence repeat (SSR) markers suitable for marker-assisted selection for adult plant stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.) resistance in wheat (*Triticum aestivum* L.) cultivar USG 3555

QTL	Marker	Chromosome	Distance[†] (cM)	% Variation[‡]	Fragment Size[§]	Source
QYrus.vt-4BL	*Xgwm251*	4BL	3.2	66.9	109	USG 3555
	Xbarc163	**4BL**	**0.9**	**69.5**	**156**	**USG 3555**
	Xwmc692	4BL	1.3	67.2	114	USG 3555
QYrus.vt-1AS	*Xcfa2153*	1AS	3.2	10.1	220	USG 3555
	Xgwm33	**1AS**	**1.8**	**11.4**	**163**	**USG 3555**

[†] Distance in cM from the QTL
[‡] These numbers were obtained by averaging scores from both years and for IT and severity
[§] Fragment size from the resistant source

a



b

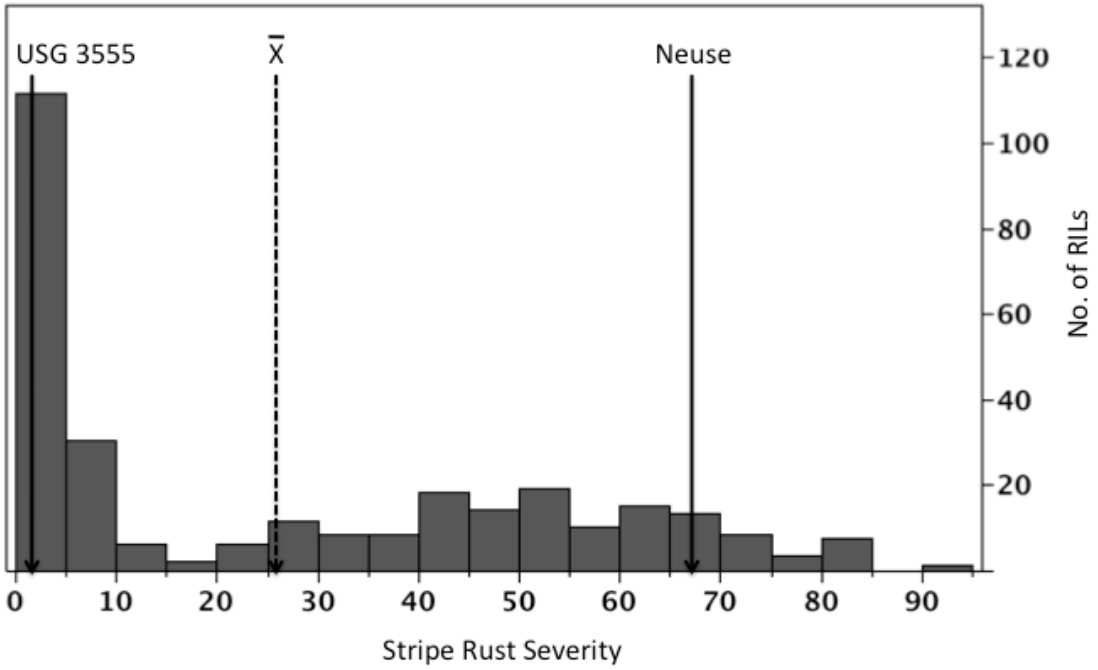


Figure 2.1. Phenotypic distribution of the 288 USG 3555/Neuse recombinant inbred lines and the two parents for (a) stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.) infection type (IT=0-9) and (b) stripe rust severity (%) phenotypes. Disease scores are presented as means over environments. The solid line arrows and the dashed line arrow indicate parental inbred means and RIL population mean, respectively.

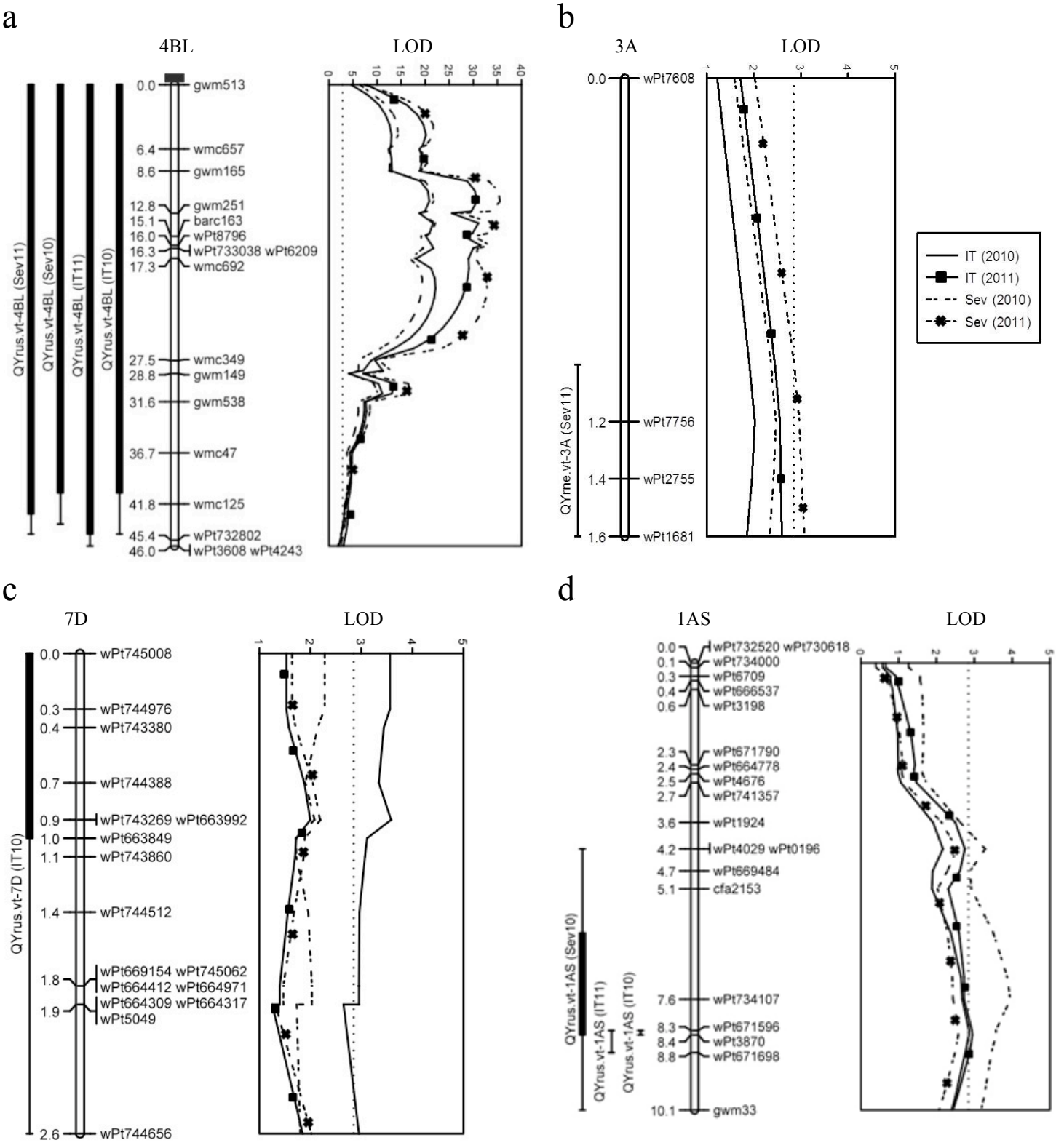


Figure 2.2. Linkage maps of wheat (*Triticum aestivum* L.) chromosomes 4B (a), 1A (b), 7D (c) and 3A (d) with the QTL interval on the left side of the linkage map and the LOD graph on the right side. The thick line on the QTL interval indicates an LOD > 3.5 and the thin line indicates an LOD >2.85. On the linkage maps, marker positions are indicated on the left side in cM and marker names on the right side. The black rectangle in the map of chromosome 4BL refers to the approximate position of the centromere according to the wheat microsatellite consensus map. On the LOD graph, the vertical dotted line indicates the 2.85 LOD threshold.

IDENTIFICATION AND MAPPING OF ADULT PLANT STRIPE RUST RESISTANCE IN SOFT RED WINTER WHEAT VA00W-38, PIONEER BRAND 26R46, AND COKER 9553

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ABSTRACT

Since 2000, many of the previously effective seedling genes conferring resistance to stripe rust, caused by *Puccinia striiformis* Westend. f.sp. *tritici* Eriks, in wheat (*Triticum aestivum* L.) have become ineffective to new, more aggressive races of the pathogen. Because seedling resistance genes work on a gene for gene basis, seedling resistance to stripe rust is generally short-lived due to the ease with which the pathogen evolves virulence to a single resistance gene. Adult plant resistance and high temperature adult plant resistance are more durable and effective against multiple races of the pathogen. The Virginia Tech experimental wheat line VA00W-38 has APR to PST-100, which is the predominant race of stripe rust in the southeastern U.S. This resistance was characterized in a population derived from a cross between VA00W-38 and Pioneer Brand '26R46', which is moderately susceptible to stripe rust. The mapping population consists of 182 recombinant inbred lines (RILs), which were evaluated in field tests for stripe rust infection type (IT) in Georgia in 2008 and 2009, and for IT and severity in North Carolina in 2009 and 2010. Field tests were inoculated with stripe rust race PST-100. Genome wide molecular screens using 143 simple sequence repeats and 405 Diversity Arrays Technology markers were employed to identify quantitative trait loci (QTL) for stripe rust resistance. On chromosomes 2AS and 4BL of VA00W-38, QTL were identified that on average explain 58.9 and 19.3 percent of the variation for stripe rust IT, and 51.9 and 12.1 percent of the variation for severity. On chromosomes 6BL and 3BL of Pioneer 26R46, QTL were identified that on average explain 8.9 and 2.1 percent of the variation for IT and 11.7 and 3.9 percent of the variation for severity. Markers *Xgwm296b*, *Xbarc163*, and *Xwmc756*, were most tightly linked to QTL on chromosomes 2AS, 4BL, and 6BL respectively. Use of these

markers and development of additional diagnostic markers will facilitate the incorporation and pyramiding of stripe rust resistance into soft red winter wheat lines via marker-assisted selection.

INTRODUCTION

Wheat (*Triticum aestivium* L.) is the main host of the fungal pathogen causing stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. (Chen et al., 1995b; Maloy and Inglis, 1993). The fungus is a basidiomycete that spreads in the form of clonally produced dikaryotic urediospores (Hovmøller et al., 2002). Stripe rust is autoecious, owing to the lack of evidence that the fungus has an alternate host upon which it completes a sexual reproductive cycle. Wind-blown spores can be spread over thousands of kilometers from initial infection sites and perpetuate on living host tissue (Kolmer, 2005) where the fungus exploits water and nutrient stores thereby reducing plant growth (Chen, 2005). Urediospores are most viable between 5°C and 15°C and disease development is most rapid at temperatures between 10°C and 15°C (Maloy and Inglis, 1993). The three key factors contributing to stripe rust epidemics are moisture, temperature, and wind (Chen, 2005). Moisture is necessary for urediospores to germinate and require 3 hours or more of free moisture (Chen, 2005; Rapilly, 1979). Temperature is an important factor affecting spore germination, latent period and spore survival. Due to the fact that stripe rust is most pervasive in cooler climates, the disease establishes early in the growing season and consequently can cause much more damage under optimal environmental conditions than either leaf rust, caused by *Puccinia triticina* Eriks., or stem rust, caused by *Puccinia gramins* Pers.:Pers f. sp. *Tritici* Eriks. E. Henn. (Chen, 2005).

The main concern in controlling stripe rust is the spread of windborne urediospores and the overwintering of mycelium (Chen, 2005; Kolmer, 2005). Large areas of wheat infected by

windborne urediospores show a uniform disease incidence as inoculum is spread evenly throughout the field (Roelfs, 1989). Disease incidence resulting from overwintering mycelia results in the development of disease foci or "hot spots" and is characterized by variable disease frequencies throughout the field with highest frequencies found closest to the initial foci. Wheat stripe rust epidemics spread through large fields with increasing velocity over space and time. A mechanism for slowing the spread of a stripe rust epidemic is to decrease the infection rate, which can be accomplished by utilizing multiline varieties or incorporating diverse resistant sources in pure stands (Cowger et al., 2005).

New races of stripe rust have evolved which have a shorter latent period and have increased tolerance to higher temperatures (Milus et al., 2006). Before 2000, 59 races of stripe rust had been identified in the United States (U.S.) historically. In the year 2000, 21 new races of stripe rust and 42 races in total were identified (Chen et al., 2002). By 2010, there were a total of 146 identified races of stripe rust in the U.S.

Stripe rust can cause major reductions in yield and in the year 2000, a combined loss of over 244,000 metric tons (MT) of wheat was observed in the states of Arkansas (123,005 MT), Washington (45,372 MT), Oklahoma (40,067 MT) California (20,622 MT), Texas (9,117 MT) Kansas (4,877 MT), Oregon (2,400 MT) and Louisiana (1,347 MT). Consequently, it was year 2000 that marked the most widespread occurrence of stripe rust in the U.S., with 25 states reporting its presence (Chen et al., 2002). In 2010, national yield losses due to stripe rust were estimated at over 2,340,500 MT and a 5.8% yield loss in the U.S. (Long, 2011).

Of all the rusts, stripe rust is the most environmentally sensitive and some resistance genes also are sensitive to environmental factors (high temperature resistance), leading to problems when evaluating host plant resistance (Wagoire et al., 1998). Currently, there are 49

designated stripe rust resistance genes though many are no longer effective and many more have only been temporarily designated and need to be confirmed as unique. Of the 49 designated genes, 13 are categorized as adult plant resistance genes: *Yr11-Yr14* (chromosomal location unknown), *Yr16* (2DS), *Yr18* (7DS), *Yr29* (1BL), *Yr30* (3BS), *Yr36* (6BS), *Yr39* (7BL), *Yr46* (4D), *Yr48* (5AL) and *Yr49* (3DS). Of the 13 APR genes, three are classified as high temperature adult plant (HTAP) resistance genes: *Yr18*, *Yr36*, and *Yr39*. Expression of HTAP resistance is dependent on the stage of plant growth and temperature. Plants possessing HTAP resistance are susceptible in the seedling stage and when grown under low temperatures but become more resistant as plants become older and temperatures rise (Line and Chen, 1995).

Little is known about the extent or diversity of stripe rust resistance in soft red winter (SRW) wheat germplasm in the eastern U.S. Markell et al. (2009) conducted a genetic study to determine the inheritance of resistance in three SRW wheat lines ('McCormick', VA96W-270, and VA96W-270V). Two to three recessive and race-specific adult plant resistance (APR) genes conferred resistance in McCormick. Two recessive race-specific APR genes conferred resistance in VA96W-270, which has at least one gene in common with McCormick. Resistance in VA96W-270V, which is a variant of VA96W-270, is conferred by one recessive all stage resistance gene and one dominant APR gene.

Based on evaluations of entries in the Uniform Eastern and Uniform Southern SRW wheat nurseries (http://www.ars.usda.gov/Main/docs.htm?docid=21894) and evaluations in a stripe rust nursery at Laurel Springs, NC, wheat genotypes 'Branson', 'AGS 2031', 'Coker 9553', 'USG 3555', VA00W-38, Pioneer Brand '26R61', VA05W-139, and VA07W-415 were identified as having at least moderate levels of APR (Bockelman, 2011). However, the genes conferring resistance in these lines/cultivars have not been studied. Genetic tests and mapping

projects on SRW wheat genotypes expressing high levels of APR to stripe rust are needed as only limited data and genetics information are available concerning the identity and diversity of stripe rust resistance in SRW wheat in the eastern U.S. Such knowledge is critically needed by breeders and molecular biologists to facilitate the incorporation of more durable and effective stripe rust resistance into elite lines and cultivars. The development of diagnostic markers will facilitate the use of marker-assisted selection (MAS) for the rapid and efficient selection of new cultivars possessing durable stripe rust resistance. The first objective of this research was to identify QTL for stripe rust resistance in the VA00W-38/Pioneer Brand 26R46 recombinant inbred line (RIL) population. The second objective was to compare the identified QTL with known QTL or genes from other sources. The third objective was to test if there are any major QTL shared between the VA00W-38/Pioneer Brand 26R46 population and the resistant cultivar Coker 9553. The fourth objective was to develop breeder friendly markers for MAS of stripe rust resistant lines in breeding populations.

MATERIALS AND METHODS

Germplasm

The mapping population consists of 182 RILs derived from a cross between Virginia Tech experimental line VA00W-38 (resistant) and Pioneer Brand 26R46 (susceptible). This population will be referred to as the 'VP' population. VA00W-38 was derived from the cross VA91-54-343 (IN71761A4-31-5-48 // VA71-54-147 / 'McNair 1813') / 'Roane' sib (VA91-54-222 = VA71-54-147 / 'Coker 68-15' // IN65309C7-18-2-3-2). Pioneer 26R46 was derived from the cross FL7927-G14 // Pioneer Brand '2555'*3 / 'Coker 80-28'. The resulting VA00W-38 / Pioneer Brand 26R46 F_1 seed was planted in a single 1.2 m headrow in 2003 and harvested and

threshed in bulk. The population was then advanced from the F_2 to the F_4 generation in 20.9 m^2 blocks at Warsaw, Virginia using a modified bulk breeding method wherein selection was based solely on plant and spike characteristics at maturity. Stripe rust was not observed during the advancement of the population, so direct selection for resistance, which would have skewed the population distribution, was considered negligible.

Single spikes were harvested from the F_4 population during the summer of 2007 and subsequently maintained as RILs. Each spike was threshed individually and planted as a single 1.2 m $F_{4:5}$ headrow during the fall of 2007 at Griffin, GA and evaluated in the spring of 2008. Approximately 8 to10 spikes were harvested from each row of the population grown at Warsaw, Virginia. The following season, two replications of $F_{5:6}$ RILs were planted in Georgia and three replications in Laurel Springs, North Carolina and were evaluated in the spring of 2009. In 2009, a single replication of the $F_{6:7}$ RILs was planted in North Carolina and evaluated in the spring of 2010. Each individual row was planted from an individual head harvested the previous year.

Molecular markers that were found to be linked to QTL in the VP population were selected to screen 94 RILs derived from the cross ‘Coker 9553’ (resistant) and VA01W-21 (susceptible) to determine if the lines possess common QTL. This population will be referred to as the ‘CV’ population. Coker 9553 was derived from the cross 89M-4035A / Pioneer Brand '2580'. VA01W-21 is a ‘Chesapeake’ sister line and was derived from the cross VA91-54-222 (Roane sib) / 'FFR555W' // VA93-52-55 ('Massey'*3/'Balkan'//'Saluda'). The resulting Coker 9553 / VA01W-21 F_1 seed was planted in individual six-inch greenhouse pots in the summer of 2006 and advanced in the greenhouse. Seed was harvested from three individual F_1 plants and 150 F_2 seed were planted into individual six-inch greenhouse pots and advanced in the

greenhouse from the F_2 to F_4 generation. Approximately 3 to 5 spikes were harvested from each of the 150 plants and planted in the fall of 2009 as single 1.2 m F_4 headrows in Warsaw, Virginia for advancement. Approximately 8 to 10 spikes were harvested from each row and planted in the fall of 2010 as single 1.2 m $F_{4:5}$ headrows in Laurel Springs, North Carolina to characterize individual RILs for stripe rust reaction.

Field Evaluations

RILs of the VP population of generation $F_{4:5}$ (2008), $F_{5:6}$ (2009), and $F_{6:7}$ (2010) were evaluated in 1.2 m headrows with 0.3 m spacing between rows. Artificial inoculum was generated by transplanting clumps of young plants colonized with *P. striiformis* race PST-100 into susceptible spreader rows. Data were collected when the susceptible parent Pioneer Brand 26R46, susceptible spreader 'Amigo', and susceptible control Roane showed high levels of disease. Data were collected in Griffin, GA in 2008 and 2009 for infection type (IT), based on a 0 to 9 scale, and in Laurel Springs, NC in 2009 and 2010 for both IT and severity (percent leaf area diseased), based on a 0 to100 percent scale (Line and Qayoum, 1992).

RILs of the CV population of generation $F_{4:5}$ were evaluated in 1.2 m headrows with 0.3 m spacing between rows under the same environmental conditions and experimental protocols as the VP population. Data were collected when the susceptible parent VA01W-21, susceptible spreader Amigo, and susceptible controls showed high levels of disease. Data were collected in North Carolina in 2011 for both IT and severity.

DNA Isolation and Amplification

Leaf tissue samples from each of the 182 $F_{6:7}$ VP RILs, two parents, and two checks, and from 94 CV $F_{4:5}$ RILs, two parents, and two checks were collected from laboratory grown seedlings for DNA isolation. Seedlings were grown on moistened cotton balls in 96-well flats.

Tissue was collected in 2-mL test tubes, each containing a stainless steel bead required for tissue grinding, when seedlings reached the two-leaf stage. Tissue samples were then frozen in an ultra-low (-80°C) freezer. Frozen samples were then ground in a Spex CertiPrep 2000 Geno-Grinder (Metuchen, NJ) for 15 seconds or until finely ground. DNA extraction was then performed by the CTAB method as described by Saghai Maroof et al. (1994) and Yu et al. (1994) for SSR marker analysis or by a protocol from Diversity Arrays Technology (DArT) in Australia for DArT marker analysis (VP population only) (http://www.diversityarrays.com/genotypingserv.html).

SSR primer pairs were synthesized by Applied Biosystems (ABI) (Carlsbad, CA, USA) and DNA Integrated Technologies (Coralville, IA, USA). Primers were either directly labeled with a fluorescent dye or were indirectly labeled with an M13 fluorescent tail. Fluorescent dye labels used were: FAM (blue), NED (yellow/black), VIC (green), HEX (green) and PET (RED).

A single PCR reaction with four direct labeled fluorescent SSR primers was performed in a volume of 13 µl and contained: 2.5 µl of DNA (50 ng), 1.2 µl of 10x buffer, 0.36 µl of $MgCl_2$ (50 mM/µl), 0.96 µl of dNTP (25mM/µl), 0.30 µl of each pair (forward and reverse) of direct labeled primers (10 µM/µl), 0.18 µl of Taq polymerase (5 units/µl), and 6.4 µl of molecular grade water.

A single PCR reaction for each M13-labeled fluorescent primer was performed in a volume of 13 µl and contained: 2.5 µl of DNA (50ng), 1.2 µl of 10x buffer, 0.36 µl of MgCl2 (50 mM/µl), 0.96 µl of dNTP (25mM/µl), 0.96 µl of M13-tailed forward primer (1 µM/µl), 0.72 µl of reverse primer (10 µM/µl), 0.72 µl of M13 dye labeled primer (10 µM/µl), 0.18 µl of Taq polymerase (5 units/µl), and 5.40 µl of molecular grade water. The PCR products of four separate M13 PCR reactions were then combined for analysis in the genetic analyzer.

A touchdown PCR program was used and included four stages: Stage 1 was 3 min at 94 ºC; stage 2 was 10 cycles of 30 s at 94 ºC, 45 s at 65 ºC, 1 min at 72 ºC with 1 ºC decrease in each cycle; stage 3 was 25 cycles of 30 s at 94 ºC, 45 s at 50 ºC, 1 min at 72 ºC and; stage 4 was 5 min at 72 ºC then storage at - 4 ºC until needed for electrophoresis.

A portion of the PCR product(s) (3.5 µl for direct labeled and 1.0 ul of each M13 labeled, for a total of 4 ul), was then transferred to a 96-well PCR plate with each well containing 9.8 µl of Hi-Di formamide and 0.08 µl of size standard. Samples were then denatured at 95°C for 5 minutes and cooled at 4°C until needed.

Capillary Electrophoresis and Molecular Marker Analysis

PCR products were visualized on an Applied Biosystems 3130xl Genetic Analyzer (Foster City, CA) and the generated data was analyzed using the genotyping software GeneMarker version 1.70 designed by SoftGenetics (State College, PA, USA). Approximately 800 molecular markers were screened for polymorphism in the VP population, 394 SSR markers and 405 DArT markers. DNA samples from 92 of the 182 RILs and the two parents were sent to Australia for DArT analysis.

Linkage Map Construction

JoinMap 4 (Van Ooijen, 2006) was used for construction of the linkage groups for mapping stripe rust resistance genes at an LOD threshold of 3.0 by regression interval mapping based on the Kosambi mapping function. MapChart 2.2 (Voorrips, 2006) was used as a tool to combine linkage maps and QTL data for a more seamless visual representation of the chromosomal locations of QTL.

Statistical Analysis

The SAS procedure PROC GLM (SAS, 2009) was used to conduct an ANOVA for the 2009 data. Both line and replication were considered fixed effects. JMP 8.0 (JMP, 2010) was used for preliminary identification of marker/trait associations with one-way ANOVA. Markers were identified as having high r^2 values and low *p*-values. JMP 8.0 was also used for comparison of means using a Student's t-test.

MapQTL 5 (Van Ooijen, 2004) was used to generate LOD values to identify significant (LOD < 2.37) marker/trait associations and genomic regions harboring QTL for stripe rust resistance. A minimum LOD threshold of 2.37 was used for identifying QTL regions based on a permutation test at 1000 iterations.

RESULTS

Linkage Maps

Of the 394 SSR markers that were screened for polymorphism between the two parents of the VP population, 225 (57%) were polymorphic. Of the 225 polymorphic markers, 145 were mapped and used to analyze genotypes of the population. Of the 145 mapped markers, 15 mapped to multiple loci giving a total of 161 mapped loci with 83 (52%) being co-dominant and 78 (48%) being dominant. Approximately 405 DArT markers were polymorphic between the two parents and were mapped in the population.

Markers were placed on 20 of the 21 chromosomes. Linked markers were not obtained for chromosome 5D. The chromosome with the most complete coverage was chromosome 3B, which is the only wheat chromosome for which the physical map is available (Paux et al., 2008). Chromosome distances ranged from 6.4 cM (on chromosome 6D) to 100.1 cM (3B) while they vary from 59 cM (4B) to 173 cM (5B) on the 2004 wheat microsatellite consensus map (Somers,

2004). Of the 161 mapped SSR loci, 131 were used in the construction of the genetic maps and 30 were not linked to a specific chromosome. Of the 405 mapped DArT loci, including one from rye (*Secale cereale*), twelve from triticale (× *Triticosecale*), and 392 from wheat, 330 were used in the construction of the genetic maps and 75 were not linked to a specific chromosome.

Phenotypic Analysis

Analysis of variances were conducted for the data collected from Georgia in 2009 and North Carolina in 2009 (Table 3.1). Variation among RILs was significant ($p < 0.001$) for IT in 2009 at both locations and severity in 2010 at NC. Variation among replications was not significant ($p < 0.05$) for IT or severity. For QTL analysis, replications were averaged and analysis was performed separately for IT and severity for each environment. Parental means and the means and ranges for RILs for each trait and environment are presented in Table 3.2.

QTL Analysis

Four QTL were identified with two from VA00W-38 on chromosomes 2AS and 4BL and two from Pioneer Brand 26R46 on chromosomes 6BL and 3BL. QTL positions were designated by identifying the highest average LOD score for both IT and severity at chromosome positions from all locations and years (Table 3.3). The QTL with the largest effect on stripe rust resistance was located on chromosome 2AS. The QTL from chromosome 2AS and 4BL were identified in each year, location and for both IT and severity.

Phenotypic Distribution

The distribution of VP RILs for both IT (Figure 3.1a) and severity (Figure 3.1b) indicates that transgressive segregation is occurring, which confirms the polygenic nature of resistance and the presence of different resistance factors in each parent. The resistant parent, VA00W-38, had an average IT rating of 1.1 and an average severity of 1.3%. The susceptible parent, Pioneer

Brand 26R46, had an average IT of 6.7 and an average severity of 42.9%. Based on field observations, of the 182 RILs, 21 had an average IT rating lower than VA00W-38 and 18 had an average severity rating lower than VA00W-38. There were 24 RILs with IT ratings higher than Pioneer Brand 26R46 and 39 with severity ratings higher than Pioneer Brand 26R46. None of the lines were significantly ($p < 0.05$) more resistant than VA00W-38; however, 12 lines had significantly higher disease severities than Pioneer Brand 26R46 (Table 3.4). Six of the susceptible transgressive segregants possessed the susceptible marker allele at all four of the QTL positions.

Chromosome 2AS

The QTL of VA00W-38 on 2AS, designated *QYrva.vt-2AS,* (Figure 3.2a) is located at chromosomal position 21.8 cM and flanked by *Xgwm296b* (5.5 cM from the QTL) and by *Xwpt7721* (13.0 cM). The marker loci *Xgwm296b* and *Xwpt7721* explain 22.2 to 36.3% and 5.2 to 14.1% of the phenotypic variation in stripe rust resistance, respectively with LOD values ranging from 8.6 to 17.1 and 1.5 to 4.5, respectively. The LOD peak for *QYrva.vt-2AS* reached the threshold for stripe rust IT and severity in all years and locations.

Chromosome 3BL

The LOD curve peak of the QTL of Pioneer 26R46 on 3BL, designated *QYrpi.vt-3BL*, (Figure 3.2b) is located at marker locus *Xwmc787* (50.8 cM). The marker explains 0.7 to 6.6% of the phenotypic variation with LOD values ranging from 0.3 to 2.7. The LOD peak for *QYrpi.vt-3BL* only reached the threshold for severity in 2010.

Chromosome 4BL

The LOD curve peak of the QTL of VA00W-38 on 4BL, designated *QYrva.vt-4BL*, (Figure 3.2c) is located at SSR marker locus *Xbarc163* and flanked by *Xwmc692* (1.8 cM),

Xgwm149 (6.7 cM) and *Xwmc652* (3.1 cM). The marker locus *Xbarc163* explains 5.0 to 27.3% of the phenotypic variation with LOD values ranging from 2.0 to 12.6. The flanking marker loci *Xwmc692*, *Xgwm149*, and *Xwmc652* explain 5.6 to 24.8%, 7.4 to 20.8%, and 4.2 to 18.5% of the phenotypic variation, respectively with LOD values ranging from 2.2 to 11.1, 3.0 to 9.2, and 1.7 to 8.1, respectively. The LOD peak for *QYrus.vt-4BL* reached the threshold for stripe rust IT and severity in all years and locations.

Chromosome 6BL

The QTL of Pioneer Brand 26R46 on 6BL, designated *QYrpi.vt-6BL*, (Figure 3.2d) is located at chromosomal position 6.2 cM and flanked by *Xwmc105* (1.1 cM), *Xwmc756* (1.0 cM) and *Xwmc397* (2.8 cM). The marker loci *Xwmc105*, *Xwmc756*, and *Xwmc397* explain 6.1 to 13.6%, 5.7 to 13.5%, and 5.2 to 11.1% of the phenotypic variation, respectively with LOD values ranging from 2.0 to 5.0, 2.2 to 5.3, and 2.1 to 4.6, respectively. The LOD peak for *QYrpi.vt-3BL* reached the threshold for IT and severity in all years and locations except for IT in North Carolina in 2010.

Genotypic Effects of Marker Loci on Stripe Rust

To determine the effect of combining multiple QTL, individuals in the mapping population were identified that have different combinations of marker loci (Table 3.5). The means of the genotypic classes possessing different QTL combinations for both IT and severity were compared using a Student's t-test in which the means of each possible pair of genotypic classes were compared. The means of genotypic groups sharing a common letter are not significantly ($p < 0.05$) different.

The most resistant lines are those that possess the combination of QTL on 2A, 4B, and 6B. For both IT and severity, all of the resistant groups are significantly different from the

susceptible groups (susceptible groups not shown except for the group possessing all susceptible marker loci). For IT and severity, all of the resistant genotypic groups fall into a single group having the lowest average ratings, except for genotypic groups 6B3B, 6B, and 3B. This indicates that lines with the highest level of resistance either possess *QYrva.vt*-2AS or *QYrva.vt-4BL*.

Coker 9553 Resistance

RILs from the CV population were screened for the presence of QTL on chromosomes 2AS, 4BL, and 6BL. The markers *Xgwm296b* (2AS), *Xbarc163* (4BL), and *Xwmc756* (6BL) were used to screen the 94 RILs. The marker, *Xbarc163*, on chromosome 4BL, was the only marker that was associated with stripe rust resistance. This indicates that Coker 9553 also possesses the QTL on chromosome 4BL. The marker, *Xbarc163*, explained 40 percent variation for IT and 43.7 percent of the variation for severity on the basis of r^2 values. A comparison of means for IT and severity (Table 3.6) indicates that the means of the susceptible and resistant genotypes are significantly ($p < 0.05$) different, which indicates that the marker allele from Coker 9553 at the *Xbarc163* marker locus is associated with stripe rust resistance.

DISCUSSION

Novel QTL

QTL associated with adult plant resistance to stripe rust were detected on chromosomes 2AS and 4BL of VA00W-38 and on 6BL and 3BL of Pioneer Brand 26R46. The putatively designated adult plant stripe rust resistance gene *QYR 2* (Boukhatem et al., 2002) is located on chromosome 2A, though mapping results place it distally on the long arm while *QYrva.vt-2AS* is located on the short arm, making it highly unlikely that these two QTL are the same. The

seedling resistance genes *Yr1*, *Yr17*, and *Yr32* are also located on chromosome 2A. Genes *Yr1* and *Yr17* are known to confer resistance to race PST-100 (Chen, 2007) indicating that *QYrva.vt-2AS* is not one of these genes as VA00W-38 is susceptible in the seedling stage. The gene *Yr32* has been mapped on the long arm of chromosome 2A (Eriksen et al., 2004) making it unlikely that *QYrva.vt-2AS* and *Yr32* are the same.

Chen et al. (1995a) identified seedling stripe rust resistance genes in the winter wheat cultivars Clement (*YrCle*) and Moro (*YrMor*) on chromosome 4B. The winter wheat cultivar Yamhill also possesses a seedling resistance gene (*YrYam*) on chromosome 4B (Chen et al., 1994). The previously identified genes are all race-specific, seedling resistance genes and neither VA00W-38 nor Pioneer 26R46 possesses seedling resistance to *Puccinia striiformis* race PST-100. It is also known that Clement (*YrCle*) and Yamhill (*YrYam*) having resistance genes on chromosome 4B are susceptible to race PST-100 in both the seedling and adult plant stages (Chen, 2007). Therefore *QYrva.vt-4BL* appears to be different from the resistance genes previously identified on chromosome 4B.

The QTL on chromosome 4BL was also identified in the SRW wheat cultivar USG 3555, which also possesses APR to stripe rust (Christopher et al., unpublished, 2011). The presence of this QTL in multiple SRW lines, which were not bred for resistance to stripe rust, indicates that this QTL likely is linked with another trait of interest and is prevalent in the SRW wheat germplasm in the southeastern U.S.

The high temperature adult plant (HTAP) resistance gene *Yr36* has been mapped proximally on chromosome 6BS in the centromeric region (Uauy et al., 2005). This is approximately the same region where *QYrpi.vt-6BL* was mapped in the current study. Expression of HTAP resistance genes such as *Yr36* is partial and dependent on plant growth stage and

environmental conditions. Therefore, it is possible that *Yr36* and *QYrpi.vt-6BL* are the same gene as the latter conferred only moderate levels of resistance in the current study. *QYrpi.vt-6BL* may not have conferred high levels of resistance in the field because temperatures were too cool for full expression of HTAP resistance. The monthly average of daily maximum air temperatures in April, May, and June were 16.3°C, 20.0°C, and 24.7°C, respectively in 2009, and 19.1°C, 21.6°C, and 27.1°C, respectively in 2010 (Environment and Climate Observing Network, Upper Mountain Research Station, Laurel Springs, North Carolina: http://www.nc-climate.ncsu.edu/cronos/index.php?station=LAUR). Growth chamber experiments to detect the presence of HTAP resistance utilize a diurnal maximum temperature of 35°C (Chen and Line, 1995). Additional research is needed to confirm whether *Yr36* and *QYrpi.vt-6BL* are the same gene.

The adult plant resistance gene *Yr30* is located proximally on the short arm of chromosome 3B and is known to be linked with the stem rust resistance gene *Sr2* and the leaf rust resistance gene *Lr27* (Singh et al., 2005). *QYrpi.vt-3BL* was mapped to the long arm of chromosome 3B in this study and, therefore, likely is different than *Yr30*.

Pyramiding QTL

Identification of 12 susceptible transgressive segregants in the mapping population indicates that selection for stripe rust resistant lines possessing multiple QTL identified in this population will result in lines with higher levels of resistance and also confirms the presence of resistance in the moderately susceptible parent Pioneer Brand 26R46. This has important implications for a breeding program. Although the level of resistance did not differ significantly between lines possessing either *QYrva.vt-2AS* or *QYrus.vt-4BL*, the practical implication is that combining multiple QTL into a single line will result in broader and more durable resistance.

Use of Markers for MAS

The ultimate goal of this study was to identify the chromosomal location and number of QTL present in VA00W-38 and Pioneer Brand 26R46 and to identify diagnostic molecular markers to utilize for the incorporation of adult plant stripe rust resistance into other genetic backgrounds. Informative microsatellite markers have been identified for *QYrva.vt-2AS* (*Xgwm296b*, *Xgwm359*, and *Xwmc382*), *QYrva.vt-4BL* (*Xbarc163*, *Xwmc692*, and *Xwmc652*), and *QYrpi.vt-6BL* (*Xwmc756*, *Xwmc105*, and *Xwmc397*) (Table 3.7).

The marker *Xbarc163* is tightly linked with the QTL *QYrva.vt-4BL* in the VA00W-38 / Pioneer Brand 26R46 mapping population and on average explained 16.9% of the phenotypic variation for stripe rust resistance. It was also observed that Coker 9553 most likely possesses *QYrva.vt-4BL* and the marker *Xbarc163* explained 40 and 43.7 percent of the phenotypic variation for IT and severity, respectively. This QTL was also identified in the SRW wheat cultivar USG 3555. Marker *Xgwm296b* is most tightly linked with the QTL *QYrva.vt-2AS* at a distance of 5.5 cM and on average explains 32.7 percent of the phenotypic variation. Marker *Xwmc756* is the most tightly linked with the QTL *QYrpi.vt-6BL* at a distance of 1.0 cM and on average explains 9.0 percent of the variation. Use of these markers will lead to a more feasible and directed effort in the introgression of adult plant stripe rust resistance into adapted wheat lines.

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ATTRIBUTIONS

Preliminary seedling and adult plant screens to multiple stripe rust races were conducted in growth chamber and field experiments by David Marshall, Jerry Johnson, Gene Milus, Kimberly Garland-Campbell, Carl Griffey, and Xianming Chen. A pure collection of race PST-100 was provided by Gene Milus for preliminary growth chamber experiments. Recombinant inbred lines were planted and evaluated at Griffin, GA, by Jerry Johnson, and at Laurel Springs, NC, by David Marshall and Myron Fountain. Shuyu Liu performed analysis of variance on the phenotypic data and provided technical support. Marla Hall and Carl Griffey provided technical support and guidance.

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Table 3.1. Mean squares for analysis of variance of VA00W-38/Pioneer 26R46 recombinant inbred line (RIL) wheat population for reaction to stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.) infection type (IT) and severity for 92 RILs grown at Griffin, GA and Laurel Springs, NC in 2009[†]

Source of variation	df	MS IT[‡]	MS % Severity[§]
2009 GA			
Line	91	17.3***	-
Replication	1	2.7	-
Error	90	1.2	-
2009 NC			
Line	91	18.0***	1970***
Replication	2	9.9	370
Error	181	3.4	272

[†] ANOVA was performed on the 92 RILs included in DArT analysis
[‡] Mean squares of infection type rating from 0-9: 0=resistant to 9=susceptible
[§] Mean squares of severity rating based on % leaf area diseased. Severity was not rated in Griffin, GA
* Significant at the 0.05 probability level
** Significant at the 0.01 probability level
*** Significant at the 0.001 probability level

Table 3.2. Mean stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.) reaction over four environments for VA00W-38, Pioneer 26R46, and the 182 recombinant inbred lines (RIL)

Trait	VA-38	P 26R46	RIL Mean	Min	Max
2008 GA					
IT=Infection Type (0-9)	0.5	6.5	3.8	0.0	9.0
2009 GA					
IT	0.0	7.5	2.8	0.0	8.5
2009 NC					
IT	0.5	6.8	4.0	0.0	9.0
% Severity	0.9	52.5	25.1	0.0	88.3
2010 NC					
IT	2.4	6.1	3.8	0.0	9.0
% Severity	1.8	34.9	15.5	0.0	80.0
2008/2009/2010					
IT	1.1	6.7	3.6	0.0	9.0
% Severity	1.3	42.9	22.7	0.0	88.3

Table 3.3. Quantitative trait loci associated with reaction to stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.) infection type (IT=0-9) and severity (%) identified in VA00W-38 and Pioneer 26R46 wheat (*Triticum aestivum* L.) cultivars in 2008, 2009, and 2010

Trait	Year	Location	Chromosome	Marker	Position (cM)	LOD	Additive[†]	% Variation[‡]	Source	QTL
	2008	GA	2AS	*Xgwm296*	23.8	8.8	-1.7	31.8	VA-38	*QYrva.vt-2AS*
			4BL	*Xwmc692*	38.4	9.0	-1.5	22.6	VA-38	*QYrva.vt-4BL*
			6BL	*Xwmc756*	7.2	2.5	-0.8	7.3	P26R46	*QYrpi.vt-6BL*
	2009	GA	2AS	*Xgwm296*	19.8	28.6	-2.6	81.8	VA-38	*QYrva.vt-2AS*
			4BL	*Xbarc163*	40.2	11.1	-1.5	24.4	VA-38	*QYrva.vt-4BL*
			6BL	*Xgwm296*	1.7	3.0	-0.9	9.7	P26R46	*QYrpi.vt-6BL*
		NC	2AS	*Xgwm296*	23.8	17.0	-1.7	48.6	VA-38	*QYrva.vt-2AS*
			4BL	*Xgwm149*	33.5	3.3	-0.8	7.9	VA-38	*QYrva.vt-4BL*
			6BL	*Xwmc756*	7.2	5.02	-0.9	12.8	P26R46	*QYrpi.vt-6BL*
	2010	NC	2AS	*Xgwm296*	20.8	19.8	-2.3	65.1	VA-38	*QYrva.vt-2AS*
IT			4BL	*Xbarc163*	40.2	12.6	-1.6	27.3	VA-38	*QYrva.vt-4BL*
	2009	NC	2AS	*Xgwm296*	20.8	17.3	-20.0	66.7	VA-38	*QYrva.vt-2AS*
			4BL	*Xbarc163*	40.2	3.0	-7.3	7.4	VA-38	*QYrva.vt-4BL*
			6BL	*Xwmc756*	7.2	5.3	-8.8	13.5	P26R46	*QYrpi.vt-6BL*
	2010	NC	2AS	*Xgwm296*	27.3	14.1	-11.6	30.8	VA-38	*QYrva.vt-2AS*
			3BL	*Xwmc787*	50.8	2.7	-5.4	6.6	P26R46	*QYrpi.vt-3BL*
			4BL	*Xbarc163*	40.2	7.2	-9.2	16.7	VA-38	*QYrva.vt-4BL*
Severity			6BL	*Xwpt7935*	0.0	3.5	-6.9	11.1	P26R46	*QYrpi.vt-6BL*

[†] The additive effect of reducing stripe rust IT and severity associated with the resistant marker allele.
[‡] Estimate of the percentage of the phenotypic variation explained by the resistant marker allele.

Table 3.4. Mean stripe rust severity and genotypes at the most tightly linked marker loci of the four quantitative trait loci (QTL) of recombinant inbred wheat lines that are more susceptible than Pioneer Brand 26R46

Line	Mean[†]	*Xgwm296*[‡]	*Xbarc163*[§]	*Xwmc756*[¶]	*Xwmc787*[#]
10	78.75 [a]	S	S	S	S
149	76.25 [a]	S	S	S	S
17	73.75 [a]	S	S	S	S
140	68.75 [a]	S	S	S	S
150	68.75 [a]	S	S	S	S
162	68.75 [a]	S	S	S	S
15	66.25 [a]	S	S	R	S
53	65.00 [a]	S	S	S	R
65	65.00 [a]	H	S	R	S
72	65.00 [a]	S	S	R	S
73	65.00 [a]	H	S	R	S
93	63.75 [a]	S	S	S	H
Pioneer 26R46	42.27 [b]	S	S	R	R

[†] Mean phenotype of different lines sharing the same letter are not significantly different ($p < 0.05$)

[‡] Most tightly linked marker to the QTL on chromosome 2AS from VA00W-38

R=resistant marker allele

H=heterozygous

S=susceptible marker allele

[§] Most tightly linked marker to the QTL on chromosome 4BL from VA00W-38

[¶] Most tightly linked marker to the QTL on chromosome 6BL from Pioneer Brand 26R46

[#] Most tightly linked marker to the QTL on chromosome 3BL from Pioneer Brand 26R46

Table 3.5. Mean stripe rust infection type (IT) and severity of recombinant inbred wheat line genotypic classes for combinations of resistance quantitative trait loci (QTL)

QTL Combination[†]	Infection Type (0-9)[‡]	Severity (%)
2A, 4B, 6B	0.76 [a]	2.42 [a]
2A, 4B, 6B, 3B	0.96 [a]	3.47 [a]
4B, 6B	1.07 [abc]	1.46 [ab]
2A, 6B	1.35 [abc]	2.50 [a]
4B	1.56 [abcd]	7.67 [abc]
2A, 4B, 3B	1.58 [abcd]	5.54 [ab]
2A, 4B[§]	1.67 [abcd]	6.83 [abc]
2A, 6B, 3B	2.12 [bcd]	6.32 [ab]
2A, 3B	2.71 [bcd]	7.42 [abc]
2A	2.86 [bcd]	12.28 [abc]
4B, 6B, 3B	3.00 [d]	16.94 [bc]
4B, 3B	3.00 [bcd]	22.09 [cd]
6B	5.66 [e]	30.77 [de]
3B	6.12 [ef]	42.29 [ef]
6B, 3B[¶]	6.51 [ef]	45.00 [f]
NONE	7.00 [f]	55.58 [g]

[†] 2A = *QYrva.vt-2AS*; 4B = *QYrva.vt-4BL*; 6B = *QYrpi.vt-6BL*; 3BL = *QYrpi.vt-3BL*

[‡] Mean phenotype of different QTL combinations sharing the same letter are not significantly different ($p < 0.05$)

[§] Genotype of resistant parent VA00W-38

[¶] Genotype of susceptible parent Pioneer Brand 26R46

Table 3.6. Comparison of means for stripe rust infection type and severity among 94 Coker 9553/VA01W-21 wheat RILs possessing different marker alleles at the *Xbarc163* locus

Genotype[†]	Infection Type (0-9)[‡]	Severity (%)
B	5.97 [b]	47.97 [b]
H	4.40 [ab]	24.00 [a]
A	2.98 [a]	10.88 [a]

[†] B=allele from the susceptible parent VA01W-21, H=heterozygous genotype, A=allele from the resistant parent Coker 9553

[‡] Mean phenotype of different genotypes sharing the same letter are not significantly different ($p < 0.05$)

Table 3.7. Simple sequence repeat (SSR) markers suitable for marker-assisted selection for adult plant stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.) resistance in VA00W-38 and Pioneer Brand 26R46 wheat (*Triticum aestivum* L.) cultivars

QTL	Marker	Chromosome	Distance[†] (cM)	% Variation[‡]	Fragment Size[§]	Source
QYrva.vt-2AS	*Xgwm359*	2AS	21.8	8.8	235	VA00W-38
	Xgwm296	**2AS**	**5.5**	**32.7**	**137**	**VA00W-38**
	Xwmc382	2AS	16.0	14.0	232	VA00W-38
QYrva.vt-4BL	*Xgwm149*	4BL	6.7	14.3	220	VA00W-38
	Xwmc692	4BL	1.8	16.0	114	VA00W-38
	Xbarc163	**4BL**	**0.0**	**16.9**	**156**	**VA00W-38**
	Xwmc652	4BL	3.1	11.2	164	VA00W-38
QYrpi.vt-6BL	*Xwmc105*	6BL	1.1	9.3	352	Pioneer 26R46
	Xwmc756	**6BL**	**1.0**	**9.0**	**198**	**Pioneer 26R46**
	Xwmc397	6BL	2.8	7.5	163	Pioneer 26R46

[†] Distance in cM from the QTL
[‡] These numbers were obtained by averaging scores for IT and severity in all environments
[§] Fragment size from the resistant source

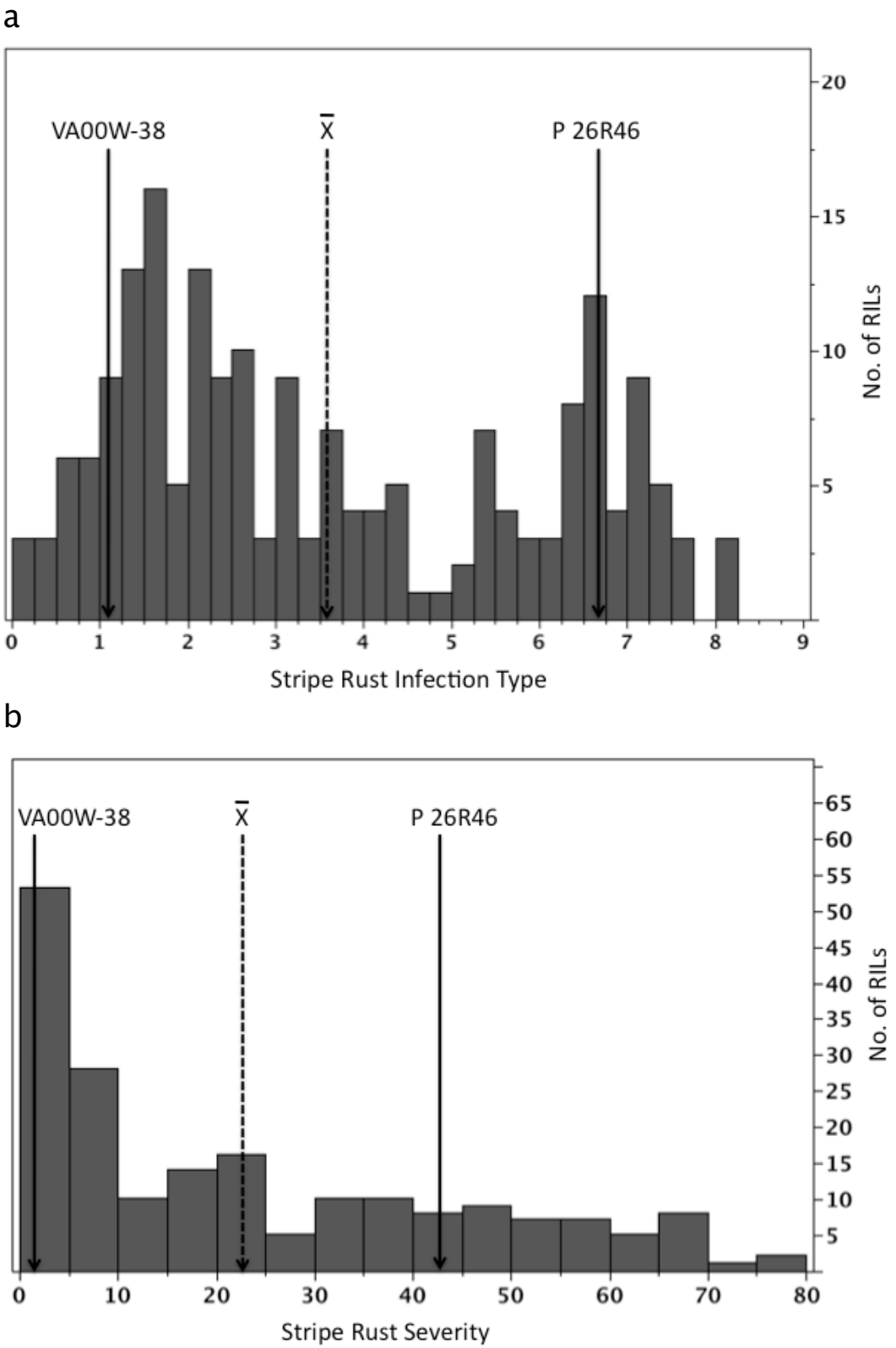

Figure 3.1. Phenotypic distribution of the 182 VA00W-38/Pioneer 26R46 recombinant inbred wheat lines and the two parents for (a) stripe rust (*Puccinia striiformis* Westend. f.sp. *tritici* Eriks.) infection type (IT=0-9) and (b) stripe rust severity(%). Disease scores are presented as means over environments. The solid line arrows and the dashed line arrow indicate parental inbred means and RIL population mean, respectively.

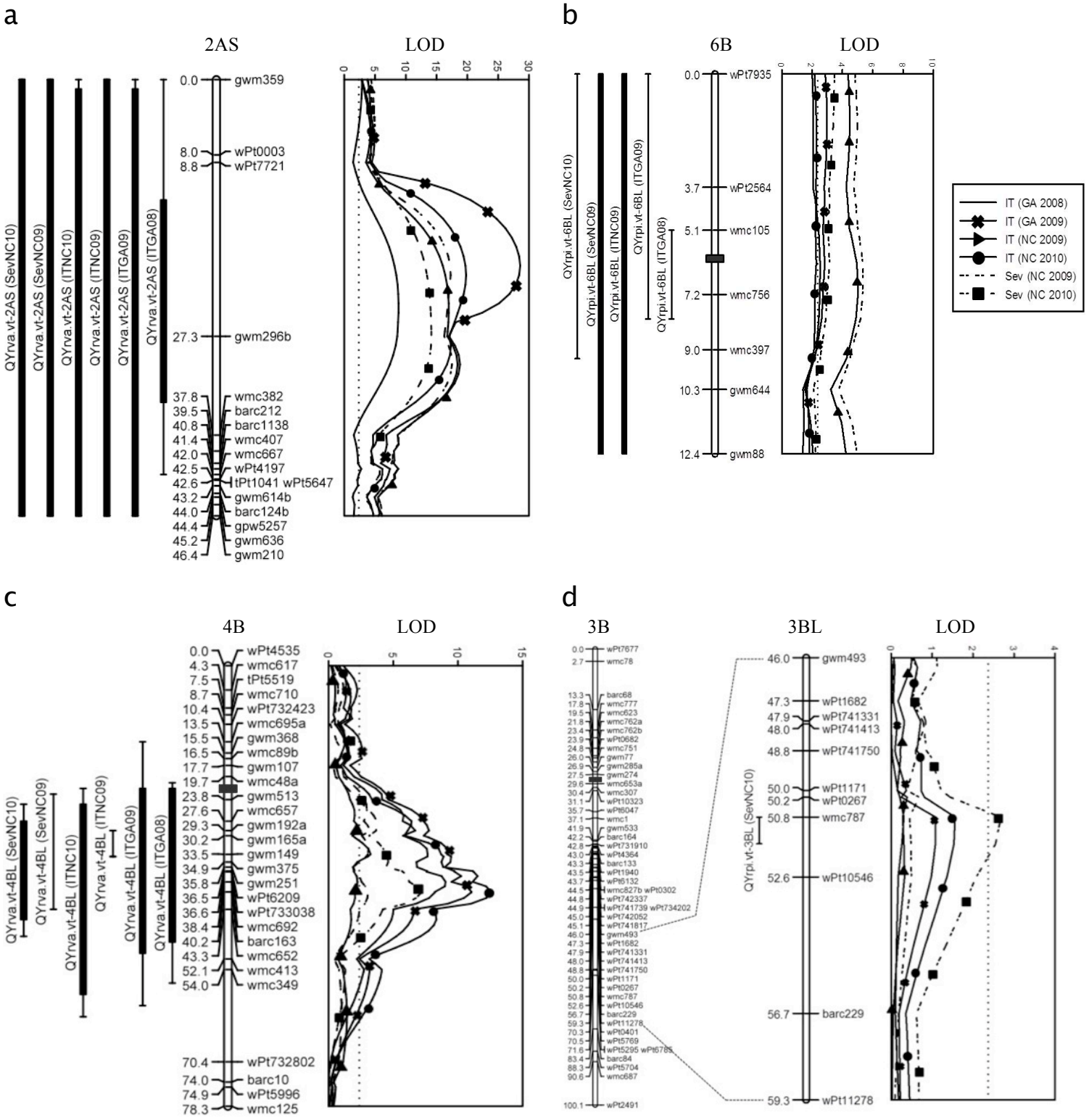

Figure 3.2. Linkage maps of wheat (*Triticum aestivum* L.) chromosomes 2A (a), 3B (b), 4B (c) and 6B (d) with the QTL interval on the left side of the linkage map and the LOD graph on the right side. The thick line on the QTL interval indicates an LOD > 3.5 and the thin line indicates an LOD >2.37. On the linkage maps, marker positions are indicated on the left side in cM and marker names on the right side. The black rectangle in the map refers to the approximate positions of the centromere according to the wheat microsatellite consensus map. On the LOD graph, the vertical dotted line indicates the 2.37 LOD threshold.

SUMMARY AND CONCLUSIONS

In this study, adult plant stripe rust resistance quantitative trait loci (QTL) were detected on chromosomes 1AS (*QYrus.vt-1AS)*, 4BL (*QYrus.vt-4BL)*, and 7D (*QYrus.vt-7D)* of USG 3555, on 2AS (*QYrva.vt-2AS*) and 4BL (*QYrva.vt-4BL)* of VA00W-38, on 6BL (*QYrpi.vt-6BL)* and 3BL (*QYrpi.vt-3BL)* of Pioneer Brand 26R46, and on 3A (*QYrne.vt-3A)* of Neuse. All of these QTL, with the possible exception of *QYrpi.vt-6BL*, which could be the high temperature adult plant (HTAP) resistance gene *Yr36,* appear to be different from all currently designated stripe rust resistance genes.

The QTL with the largest effect on stripe rust resistance is *QYrus.vt-4BL* in the USG 3555 / Neuse mapping population, explaining on average 72.7 percent of the phenotypic variation for stripe rust resistance, and *QYrva.vt-2AS*, explaining 56.6 percent of the variation in the VA00W-38 / Pioneer Brand 26R46 mapping population. The QTL on 4BL was also identified in VA00W-38 and Coker 9553 and on average explained 16.9 percent of the variation in the VA00W-38 / Pioneer 26R46 population and 41.9 percent of the variation in the Coker 9553 / VA01W-21 population.

Informative simple sequence repeat (SSR) markers have been identified for *QYrus.vt-4BL* (*Xbarc163*, *Xgwm251*, and *Xwmc692*), *QYrus.vt-1AS* (*Xcfa2153* and *Xgwm33*)*, QYrva.vt-2AS* (*Xgwm296*, *Xgwm359*, and *Xwmc382*), *QYrva.vt-4BL* (*Xbarc163*, *Xwmc692*, and *Xwmc652*), and *QYrpi.vt-6BL* (*Xwmc756*, *Xwmc105*, and *Xwmc397*). The use of these diagnostic markers and development of additional tightly linked markers for the identified QTL will facilitate the incorporation and pyramiding of stripe rust resistance into adapted wheat lines resulting in more effective and durable resistance.

The QTL and associated markers identified in this study should be validated in other backgrounds to determine their usefulness and practicality for marker-assisted selection (MAS). The development of larger populations to fine map these QTL may identify markers that are more closely linked to the QTL and could ultimately make it possible to clone the QTL, though map-based cloning in wheat is extremely difficult. Additional mapping studies may be useful to identify other useful stripe rust resistance sources in the soft red winter (SRW) wheat germplasm and further mapping in the populations analyzed in this study may identify additional QTL.

Further research should be done in the 'VP' population to determine if the cultivar Pioneer Brand 26R46 possesses HTAP resistance (*Yr36*). Lines possessing only the marker allele associated with *QYrpi.vt-6BL* should be identified and screened for adult plant stripe rust resistance in greenhouse experiments at both normal (10-22°C) and high (10-35°C) temperatures. The screen should include the parents, Pioneer Brand 26R46 and VA00W-38, known susceptible lines, and lines known to possess *Yr36*, to determine if the cultivar Pioneer Brand 26R46 possesses HTAP resistance and to compare the reaction to lines known to possess *Yr36*.

The QTL on chromosome 4BL was identified in all of the resistant lines evaluated in this study. The presence of this QTL in multiple SRW lines, which were not bred for resistance to stripe rust, indicates that this QTL likely is linked with another trait of interest and is prevalent in the SRW wheat germplasm in the southeastern U.S. This source of stripe rust resistance may already be present in many of the currently available SRW wheat cultivars. Incorporation of this resistance into newly developed wheat cultivars should be relatively straightforward for SRW wheat breeders.