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Identification of Quantitative Trait Loci (QTL) for Resistance to Stripe Rust in Wheat Variety TERRAL LA841

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IDENTIFICATION OF QUANTITATIVE TRAIT LOCI (QTL) FOR
RESISTANCE TO STRIPE RUST IN WHEAT VARIETY TERRAL LA841

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Plant, Environmental & Soil Sciences

by

Alejandro Castro

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Abstract

Wheat stripe rust, caused by *Puccinia striiformis*, is a common disease that was found primarily in the Pacific Northwest prior to 1990, with occasional minor outbreaks in the eastern wheat regions. A physiological adaptation to higher temperatures that occurred around 1990 led to stripe rust becoming a major disease problem in the Gulf Coast. Race (pathotype) changes have occurred several times since 2000, resulting in cultivars with previously effective resistance genes becoming susceptible. The cultivar LA 841, developed by the LSU AgCenter, has remained resistant to all races of stripe rust in the area for over 15 years. It contains the gene cluster $Lr_{37}/Yr_{17}/Sr_{38}$ with Yr_{17} , conferring broad-spectrum stripe rust resistance until recent years, when new races of stripe rust became virulent on Yr_{17} . LA841 has remained resistant after Yr_{17} has lost its effectiveness, which indicates that LA 841 contains alternate/additional stripe rust resistance genes. Our objective was to determine the genetics of the resistance in LA 841. A mapping population consisting of 192 F_2 progeny derived from a cross between the resistant line LA 841 and susceptible line NC06BGTAG12, was genotyped with 216 polymorphic SSR and AFLP markers. The $F_{2:3}$ progeny was phenotyped for their reaction to stripe rust in replicated trials at three locations: Winnsboro, Louisiana; Plains, Georgia; and Fayetteville, Arkansas. Three QTL were identified - two on the short arm of chromosome 2A and one on chromosome 3B which together explained 43.2% of the total variation for stripe rust resistance. The QTL on chromosome 3B was flanked by SSR markers Barc164 and Barc1044. One QTL on the 2A chromosome was flanked by SSR markers gwm 636 and gwm 359 while the other QTL on the same chromosome was flanked by gwm 359 and AFLP marker gcg 800-2. Yr_{17} , located on the short arm of chromosome 2A, is most likely the QTL closest to marker gwm 636, which is linked to the gene. This QTL explained 8.6% of the variation but has a LOD value of 11.2, which is the highest of the three. The QTL with the largest effect was found in the short arm of chromosome 2A, which accounted for 22% of the variation and had an LOD value of 9.8. The QTL on chromosome 3B explained 13% of the variation and has a LOD value of 2.7, just over the threshold of 2.5. Identification of new QTL linked to resistance genes is important in breeding programs for disease resistance due to the constant adaptation of pathogens which overcome previously effective resistance genes.

Chapter I

1.1 Wheat History and Genetics

Wheat (*Triticum spp.*) is an edible cereal crop consumed throughout the world. The harvest grain has been consumed by man for centuries and is also used as animal feed. Roughly 95% of the wheat crop is common wheat (*T. aestivum*), used for making bread, cookies, and pastries. The origins of wheat trace back to the fertile half crescent located in Western Asia (Belderk *et al.*, 2000). Some of the earliest remains of the crop have been found in Syria, Jordan, and Turkey and date back almost 9,000 years. Common wheat originated from two independent polyploid crossing events. The first event occurred through the expansion of agriculture where an ancestor of wheat known as domesticated einkorn (*T. urartu* $2n = 2x = 14$, genome AA) was spread across Asia, Europe and Africa and crossed with an unconfirmed species related to *Aegilops speltoides* ($2n = 2x = 14$, genome BB). This cross resulted in the cultivated species emmer wheat (*T. turgidum* ssp. *dicoccum*, $2n = 4x = 28$, genomes AABB) (Yong *et al.*, 2006). Later the domesticated emmer grown in northeast Turkey was crossed with another grass species known as *Aegilops tauschii* (genomes DD) giving way to emergence of the hexaploid common wheat (*T. aestivum*, $2n = 6x = 42$ genomes AABBDD) with three similar genomes (Dubcovsky and Dvorak, 2007).

Wheat is one of the world's most important food crops and a staple in dozens of country's diets, especially in temperate zones. Wheat provides nearly 20% of calories consumed by people worldwide (Wiese 1987). The yearly world production for 2009 was around 685,614,399 metric tons, for all crops after maize. In total area wheat was grown on more than any other crop in the world with over 225 million hectares (FAO stat 2010). One of the reasons

that wheat is grown on such a large acreage is its ability to thrive in adverse conditions and environments that are adverse to other major crops. Wheat is often cultivated in wind-swept environments with low rainfall and cold temperatures that tropical crops like rice and corn cannot withstand (Gibson and Benson, 2002).

Bread wheat can be divided into two ecotypes; spring and winter. The ecotype and its growth habit mainly depend on the vernalization requirement. Vernalization is a period of low temperatures necessary to induce flowering in crops. Exposing winter wheat to temperatures near 40° F for several weeks is required for vernalization, although the exact temperature and duration varies according to the different genotypes. Wheat has genetic variability for genes that control flowering time, which imparts adaptability to a wide range of environments (McMaster, *et al.*, 2008). Vernalization is considered to be an adaptation to ensure seed is produced after the harsh winter climate. Spring wheat has little or no vernalization requirements so it can be grown over the spring and summer. It is usually planted in the spring in areas where the winter is too cold and severe for winter wheat, or as a fall planted crop where temperatures are very mild. Spring wheat is grown in the northwestern states of the country such as North Dakota, South Dakota, Montana, Idaho, Oregon and Washington (Baker 1968). Winter wheat is planted in the fall and requires exposure to certain periods of cold, depending on the variety, to initiate flowering (Malla, *et al.*, 2011). It is grown in areas where the summer is too hot and/or dry for wheat to grow but the winter is suitable. Southeastern states primarily grow soft red winter wheat (Baker 1968). Studies have shown that vernalization is controlled by 4 major genes; *VrnA1*, *VrnB1*, *VrnD1*, and *VrnB3* located at the 5A, 5B, 5D, and 7B chromosomes respectively (Dubcovsky, *et al.*, 1998).

Wheat used for baking is generally classified into two groups, hard or soft, depending on its texture. In the USA and most of the world, hard wheat is used for bread baking. Hard wheat flour has stronger gluten and higher protein content. These traits along with the higher amounts of fractured starch granules in the milling process give hard wheat flour a high value for yeast-leavened products like bread (Campbell, *et al.*, 1999). Soft wheat, on the other hand, is mostly used for cookies, crackers and pastry baking. Soft wheat flour is characterized by lower protein content, fine particles, and low water absorption. Soft wheat flour has a low gluten content which allows the products to crumble (Hoseney, *et al.*, 1998). Breeders normally do not cross hard and soft wheat genotypes because of the difficulty in recovering specific desirable end-use traits (Campbell, *et al.*, 1999).

Wheat can also be categorized into groups according to the seed color. The two basic groups are red and white wheat. Each group has certain traits that differ from one another. One major difference is the occurrence of pre-harvest sprouting of the seed types. White wheat is far more susceptible to pre-harvest sprouting than red wheat (Fofana, *et al.*, 2008). Pre-harvest sprouting is a condition where the seed, while still in the head, starts to germinate. Differences have also been found in the taste of products derived from the different types of wheat, although these differences have not been very consistent throughout tests. One test indicates that white wheat is less bitter compared to red and it was found to be sweeter, enabling bakers to use less sweeteners in products with white wheat (Symns, Cogswell 1991). However other tests that evaluated several sensory properties concluded otherwise. Differences in the sensory evaluation did not indicate significant differences in red and white wheat varieties (Armbrister, 1995).

In Louisiana and across the southeastern United States soft red winter wheat is a major crop, but total acreage fluctuates constantly due to market and weather conditions. It was

estimated that in 2008 the total area planted with wheat in Louisiana reached 162,000 hectares (400,000 acres) while in 2010 it was only 50,600 hectares (125,000 acres) (USDA-NASS 2010). The value of wheat in Louisiana for 2008 was estimated to reach over \$120,000,000 and in 2010 it dropped to \$26,400,000 (USDA-NASS 2010). In Louisiana wheat is commonly used as a winter cover crop and in a double cropping system with soybean and cotton (Boquet and Paxton, 2005).

1.2 Wheat Diseases

The high rainfall combined with the warm and moist climate of Louisiana provides an environment that is optimum for the development of fungal and bacterial diseases. Wheat is susceptible to more diseases than most grains (Gramene, 2006). Disease incidence and severity in wheat is greater in wet climates like the one in Louisiana, so breeding for resistance in the LSU program is of great importance. Some of the small grain diseases that thrive in warm, humid environments are Fusarium head blight (*Fusarium graminearum*), stripe rust (*Puccinia striiformis*), leaf rust (*Puccinia triticina*), stem rust (*Puccinia graminis*), Septoria glume blotch (*Stagnospora nodorum*), Septoria leaf blotch (*Septoria tritici*), tan spot (*Pryenophora tritici-repentis*), barley yellow dwarf virus (*Luteoviridae luteovirus*) and bacterial streak (*Xanthomonas campestris*) (Schafer, 1987).

The causal agent of Fusarium head blight (FHB), also known as scab, is the parasitic fungus *Fusarium graminearum*. This fungus is known to infect and cause damage on several small grain crops including wheat, durum wheat, triticale, and barley (McMullen *et al.*, 2008). Typical symptoms included a white-bleach colored head and small pink-orange spore masses on the kernels. Fusarium head blight can cause severe reduction in yield and quality on susceptible cultivars (McMullen *et al.*, 2008). In heavily infected fields yield losses may reach 80%. The

highest incidence and severity of the disease in the country occurred between 1993 and 2001 where the total economic loss in wheat and barley due to yield loss and quality reduction was estimated to be around \$7.67 billion (McMullen, *et al.*, 2012). Apart from yield loss this disease can also produce toxins harmful to humans and animals. In Louisiana isolates of *Fusarium* that produce nivalenol (NIV) rather than deoxynivalenol (DON) predominate and occur at moderate frequency in Arkansas and other states (Sarver, 2011). Populations of *Fusarium* in the U.S., specifically the Midwest, that infect wheat and barley produce the mycotoxin DON. Recent surveys found populations of *Fusarium* which produce the mycotoxin nivalenol in regions of southwest Louisiana. This population is found to be genetically different than the DON producing populations in the Midwest (Gale, *et al.*, 2011). These genetically distinct, NIV producing populations made up a majority of the strains found in southern Louisiana. In an evaluation, 150 strains from four parishes in Louisiana were collected in 2002, 2003, and 2005. The strains consisted of 83% NIV producing strains, 13% 3ADON strains, and the remaining are 15ADO strains (Gale, *et al.*, 2005).

Over the past 20 years FHB has become a significant threat to wheat in all major production areas around the world. To help deal with this issue the US Wheat and Barley Scab Initiative (USWBSI) was created. The purpose of the initiative is produce effective control measures and resistant varieties that reduce the threat of FHB as quickly as possible. This includes the reduction of mycotoxin exposure to producers, processors and consumers of wheat and barley. There are currently 86 scientists in 24 universities and the USDA working on a wide range of research projects aimed at solving this issue (USWBSI 2013). The LSU AgCenter wheat breeding program conducts annual trials to screen varieties and breeding lines for reaction to FHB.

The fungal disease septoria glume blotch *caused by Stagnospora nodorum*, can be found in many wheat growing areas around the world and thrives in wet conditions with temperatures up to 28°C. This fungus affects all above ground parts of the plant and overwinters/oversummers on infected stubble. Typical symptoms include red-brown lesions with a yellow halo. These lesions later develop into grayish-brown necrotic spots. The pathogen can be spread by windborne spores as well as infected seeds. Yield loss depends on a combination of the susceptibility of the cultivar, climatic conditions, and source of inoculum and can reach up to 30% (CIMMYT, 2009). The disease reduces yield by causing shriveled, light weight grains. If infected seeds are planted, low germination and seeding rate also reduce yield potential. According to Cowger and Murphy (2007) septoria occurs commonly, but with varying degrees of severity, in the southeastern U.S. soft red winter wheat region. Acceptable levels of partial resistance are available in soft red winter wheat lines, however many widely grown varieties are susceptible. Septoria leaf blotch is another fungal disease caused by a different species of Septoria (*Septoria tritici*).

Bacterial streak caused by *Xanthomonas campestris* is one of the major bacterial diseases found in wheat. For infections to occur an opening or wound as well as free water are required. Infection usually occurs after wind-blown particles or freeze damage injures leaf tissue which allows the bacteria to enter the plant. Aphid feeding can also result in elevated levels of bacterial streak. Symptoms start off as translucent olive-green streaks on the leaves and develop into yellow-brown streaks of various lengths. This disease is primarily transmitted through infected seeds in drier regions with cold climate (Hershman and Bachi 2010). Water splash and aphids are other means for dispersing inoculum (Rashid, *et al.*, 2013). Under ideal conditions yield loss can reach up to 40% (Tillman, *et al.*, 1996). Currently there are no effective or practical bactericides

for managing this disease as attempts to control the disease with chemicals have been unsuccessful. Host plant disease resistance offers the best protection against yield loss so developing resistant varieties is important. A problem in breeding for resistance to bacterial streak is the relatively low heritability of the trait. A test using soft red winter wheat in three locations in Louisiana concluded that heritability ranged from 0.12 to 0.70 (average 0.31) (Tillman and Harrison, 1996). Only a limited number of resistant genotypes have been identified that can be used in regional breeding programs so efforts were made to find additional sources of resistance. In a test conducted in 1992, (Tillman, *et al.*, 1996) 5,000 bread wheat ascensions were inoculated and evaluated for resistance to bacterial streak. Results from the test suggest 26 resistant ascensions for use in breeding programs in the southern US.

Of all the wheat diseases, the different rusts are probably the most damaging due to their incidence, severity, genetic variability and frequent changes in virulence combination that allows them to overcome resistance. Rusts are parasitic fungi that belong to the basidiomycete phylum and can infect several different types of grasses. The term, rust fungus, refers to the yellow- or rust-colored urediospores, which are the main dispersal units of the pathogen (Watkins, 2009). Rusts are usually spread by wind-borne spores, making them hard to control. A single infected susceptible plant can produce enormous numbers of spores that can be spread through the same host plant or for greater distances. Rust spores can travel for thousands of miles in the atmosphere because their spores have pigmentation to protect against UV radiation and are relatively robust with thick walls which allows the spores to remain viable while transported over long distance (Viljanen-Rollinson, *et al.*, 2007). The distance the spores can travel is also related to the altitude they are carried to. Spores have been collected at altitudes over 3,000 meters (10,000 feet). The physical traits of the spores such as their shape, size, density and surface area

allow the energy in the turbulent atmosphere to lift large numbers of spores from the fruiting structure in the plant canopy and keep them airborne for long periods of time (Isard and Russo 2012).

Leaf rust caused by *Puccinia triticina* infects the leaves of wheat and triticale, causing small pustules on the leaves and when severe epidemics occur they can be found on the leaf sheaths and glumes. The pustules can be orange to brown and circular in shape. Leaf rust can be found worldwide wherever wheat is grown (Watkins, 2009). Temperatures between 20-25°C during the day and 15° C during the night along with the presence of dew on the plant tissue offer favorable conditions for disease development. Yield loss over large regions is normally light to moderate with losses normally ranging from 1-20%. However, individual fields can be completely destroyed if the disease is severe before flowering (USDA 2012). Having an accurate way of predicting disease severity can be useful. An experiment conducted in Argentina (Moschini and Perez 1999) used data collected from leaf rust epidemics between 1972 to 1990 to create a model that could predict severity ($R^2 = 0.88$). The model uses heat accumulation, days with relative humidity >70% without precipitation, and a cultivar resistance index as prediction factors. Expected and observed severity levels were the same in trials conducted in 1994 and 1996 in Pergamino Experiment Station, Argentina (Moschini and Perez 1999).

Puccinia graminis, the causal agent of stem rust, is a fungus that usually infects, but is not confined to the stems of several small grain crops (wheat, barley, rye, triticale, oat). Symptoms include brownish to dark red pustules that rupture the epidermis tissue on plants. Stem rust develops at an optimum temperature higher than other rusts (18-29°C) (Watkins, 2009). Stem rust is generally considered the most damaging of all the rusts on wheat partially because it can affect several parts of the plant including the head. Stem rust infections are likely

to cause lodging of the plants, complicating harvest and increasing yield loss even more. The greatest losses occur when plants are infected in the early stages of their development. In very susceptible lines, loss can range between 50-90% (Wolf, *et al.*, 2011). This disease is responsible for at least 8 major epidemics on wheat across the United States since 1916, which exemplifies the ever present threat of rust on domestic cultivars (Watkins 2009). Stem rust spores travel thousands of miles every growing season. Urediospores are wind blown northward every spring from Mexico into the United States. The traveling ranges from southern Texas, throughout the Great Plains and into southern Canada. In the fall the spores are disseminated back south towards wheat growing regions where winter wheat is beginning to grow (Nagarajan and Singh 1990). Recent studies have found that barberry serves as an alternate host during the sexual stage of the disease cycle (Jin, *et al.*, 2010).

There are currently a number of useful resistance genes for both leaf and stem rust, and the widespread use of resistant varieties helps lower yield loss and economic impact. In trials across six locations in Louisiana during 2005 Terral LA 841 had a leaf rust rating of 0% and the highest grain yield (89.3 bu/acre), while susceptible varieties like McCormick had an average 45% leaf rust rating and yielded only 38.4 bu/acre (Harrison *et al.*, 2005). There are over 100 leaf rust resistance genes identified in wheat, however many of them are race specific. This conveys a vertical resistance which can easily be overcome by new races. In order to obtain effective, durable resistance these race specific genes are being combined with slow rusting genes that offer protection from several races. *Lr*₃₄ and *Lr*₄₆ are two commonly used slow rusting genes for leaf rust (Soria, *et al.*, 2010). The types of markers commonly used to identify the major resistance genes are SSR, SNP and DART (Vida, *et al.*, 2009).

Genes for resistance to stem rust are widely used by breeders. These genes are found in wheat and have been introgressed from related species, such as rye. There have been over 50 stem rust (*Sr*) resistance genes identified (Das, *et al.*, 2006). Genes and quantitative trait loci (QTL) for resistance have been found in chromosomes 1A, 1D, 2B, 2D, 3B, 4A, 4B, 5A, 5B, 5D, 6B, 7A and 7D (Singh, *et al.*, 2013). Some of these are major, specific genes are *Sr*₃₁, *Sr*₃₅, *Sr*₅₇ and some provide general, slow rusting resistance like *Sr*₂. The *Sr*₂ gene was introduced into wheat through an inter-specific cross with tetraploid emmer wheat in the 1920s. Since then this gene has been used throughout many parts of the world. The gene confers adult plant resistance and remains effective to all known races of stem rust, including Ug99. Ug99 is a virulent race of stem rust which was discovered in Uganda in 1999. Ug99 is a cause for concern because no other stem rust race has overcome so many resistance genes, including the widely used gene *Sr*₃₁ (FAO 2010). However under strong disease pressure *Sr*₂ does not provide sufficient protection by itself as it only confers partial resistance (Mago, *et al.*, 2011). To provide a more complete resistance this gene must be combined with other major resistance gene targeted for local stem rust races. *Sr*₃₅ is a major resistance gene that is currently of high importance because of its effectiveness against stem rust race Ug99. *Sr*₃₅ was prioritized because it confers “near-immunity” against Ug99. This gene is located in chromosome 3A and can be identified with EST markers SFGH and AK331487 (Saintenac, *et al.*, 2013).

1.3 Stripe Rust

The causal agent for Stripe rust is *Puccinia striiformis*. This fungus, a basidiomycete, is an obligate parasite. It is primarily dispersed by windblown urediniospores. Urediniospores were considered the only source of inoculum, however recent studies have determined that different species of barberry serve as alternate host for this pathogen as well as stem rust (Jin, *et al.*,

2010). Survival between crop seasons is by dormant mycelium and uredinia on infected volunteer wheat in most areas. The disease affects wheat, barley, triticale, and several wild grass species. Usual symptoms include stunting of the plants and parallel rows of yellowish orange colored pustules on the leaves or spikes of adult plants. Yield losses in wheat due to the disease can range from 40 to 100% (Chen, *et al.*, 2004).

Of all the rusts, stripe rust has the lowest optimal temperature, which ranges from 10°-15° C. However new races have adapted to warmer climates. Continual moisture for more than six hours is required for spores to germinate and infect new plants. Dew is more effective than rainfall for promoting spore germination and infection. Urediniospores are a functional form of spore stage which are produced on living hosts and dispersed over long distances to new hosts. Urediniospores that cause the initial infections in the area probably come from the southwest (Milus and Carthwright 2006).

Because stripe rust is considered to be a fungal disease adapted to cooler climates it historically has been the most frequently destructive disease in wheat in the Pacific Northwest U.S. (Chen, *et al.*, 2004). It occurs mostly in the U.S. northwest and was considered a minor and sporadic problem in wheat in the U.S. southeast region. However over the past 15 years stripe rust has become a major threat to soft red winter wheat in the southeast. It has been a yearly problem since 2000 when it was first reported (Chen, *et al.*, 2004). That year the most wide spread and severe epidemic occurred in the United States, with reports of the disease in 20 states east of the Rocky Mountains (Sthapit, *et al.*, 2012). According to Sthapit *et al.*, (2012) “Based on an amplified fragment length polymorphism (AFLP) phenotype, the races causing epidemics east of the Rocky Mountains since 2000 are genetically distinct from races found in the United States before 2000 and are most likely the result of an exotic introduction”. The pathogen seems

to have adapted to our warmer climates through a physiological change and has become more aggressive, making it a serious problem for wheat producers in the southeast (Milus *et al.*, 2009). Before 2000 there were 59 races of stripe rust named in the United States but only 4 were detected east of the Rocky Mountains and in few cases (Chen *et al.*, 2002). In 2000, 21 new races of stripe rust were identified in the United States, several of which were east of the Rocky Mountains (Chen 2007). A total of 40 new races were discovered in the country between 2000 and 2004. “Since then, new and more aggressive races with a wider range of virulence factors than the races identified before 2000 have been identified” (Chen, *et al.*, 2004).

In production areas within the southeast region the total yield loss due to stripe rust ranged from 10-70% depending on the degree of susceptibility and environmental factors (Hao, *et al.*, 2011). The total yield loss in the US in 2000, the year several new races appeared, was 9,685,000 bushels. In Louisiana the total loss was 49,500 bushels for the same year (Chen *et al.*, 2002). The total economic loss due to stripe rust for the US in 2003 was estimated at 88,920,480 bushels (\$267,000,000). In addition to the yield loss, millions of dollars were spent on fungicide applications each year (Chen, *et al.*, 2004). In 2008 the total loss of wheat due to stripe rust in Louisiana was about 338,000 bushels (Long, 2009) indicating an increase in the presence and virulence of the disease in the state.

There are several commercial fungicides available that help reduce stripe rust incidence and yield loss. An experiment (Reid and Sward 2004) conducted in north Texas tested 6 commercial brands (Tilt[®], Quilt[®], Stratego[®], Headline[®], Quadris[®], and Folicur[®]) labeled for use on stripe rust on a susceptible soft red winter wheat variety known as Agripro Patton. The fungicide treatments were equally effective and all of them helped increase grain production significantly when compared to the untreated check. Yield increases over the untreated plots

ranged from 34 to 41% (Reid and Sward 2004). The approximate cost for triazole fungicides (Bayleton[®], Tilt[®] and Folicur[®]) in a trial ranged from \$3.00/ha to over \$12.00/ha. The greatest economic return in the trial was \$222/ha when wheat was treated with a combination of Jockey[®] and Bayleton[®] compared to the untreated susceptible variety (Duff, Hamblin *et al.*, 2006).

According to Milus (2008) “Resistant varieties are the most cost-effective means of managing stripe rust and leaf rust, but new races of the pathogens can overcome some types of resistance. Little is known about the genes for stripe rust and leaf rust resistance in contemporary soft red winter wheat cultivars.” Some of the genes that have conferred general but not race specific resistance are *Yr₁₇*, *Yr₁₈*, and *Yr₂₉* (Suenaga, *et al.*, 2003). There are also a number of major, specific genes that provide race specific resistance. Durable resistance can be obtained through a combination or pyramiding of major resistance genes and non-specific genes.

Currently there are 65 designated stripe rust resistance genes (*Yr₁*-*Yr₆₅*) and approximately 40 temporarily designated genes (Cheng, *et al.*, 2014). Some of the permanently designated genes that confer adult plant resistance are *Yr₁₁*, *Yr₁₂*, *Yr₁₃*, *Yr₁₄*, *Yr₁₆*, *Yr₁₈*, *Yr₂₉*, *Yr₃₀*, *Yr₃₄*, *Yr₃₆*, *Yr₃₉*, *Yr₄₆*, *Yr₄₈* and *Yr₅₂* (Xu, *et al.*, 2013). These genes are not race specific so they can provide partial protection against several races of stripe rust and are more durable. However most resistance genes are considered all stage or seedling stage resistance. This type of resistance is race specific and controlled by a single or few genes and is usually overcome by new races, providing a short effective life (Hao, *et al.*, 2011). A small number of slow rusting genes are also available for breeders to incorporate into their material and complement with race specific genes. Slow rust genes provide horizontal or partial resistance to a number of races and work by increasing latent period and by decreasing uredinial size, infection frequency and spore production. Two of the commonly used slow rust genes for stripe rust are *Yr₁₈* and *Yr₂₉*

(Rosewarne, Singh *et al.*, 2008). There are still a number of resistance genes that are undesigned and some of these are adult plant resistance (APR) genes which could be used to pyramid with the large number of all stage resistance genes. There is a need to elucidate these novel genes so they can be incorporated into new varieties.

The new races discovered in 2000 in our region were of great concern because they appeared to be virulent to commonly used resistance genes *Yr₈* and *Yr₉*. The new races were the first to be reported virulent on these genes in the United States. According to Chen (2002) “the most important discovery of the 2000 race survey was the detection of 13 new races virulent on *Yr₈*, *Yr₉*, or both, because all the races identified prior to 2000 were avirulent on *Yr₈* and *Yr₉*.” These races were widely distributed east of the Rocky Mountains in 2000 and were found in Louisiana (Chen *et al.*, 2002). The gene *Yr₉* is considered one of the most widely used resistance sources for stripe rust in wheat breeding history (Hao, *et al.*, 2011). *Yr₉* is commonly found in soft red winter wheat varieties east of the Rocky Mountains because of its linkage to stem rust resistance gene *Sr₃₁* and leaf rust resistance gene *Lr₂₆* (Markell and Milus 2008).

The LSU AgCenter developed a variety named Terral LA841 that has recorded high levels of resistance to all stripe rust races in the region since its release in 1995. Initially the resistance was considered to be due to the gene *Yr₁₇*. This gene is not race-specific and provides general resistance. The *Yr₁₇* gene is derived from *Aegilops ventricosa* and is found on chromosome 2A, specifically in the short arm. It is part of the gene cluster *Lr₃₇/Yr₁₇/Sr₃₈* and is commonly found in varieties in Europe, Australia and the US. This cluster has been widely used in breeding programs in the US and has provided durable resistance to stripe rust for years (Hao, *et al.*, 2011). However when new stripe rust races were discovered, lines with the gene *Yr₁₇* were no longer resistant to prevalent races, while Terral LA841 remained resistant. In 2010 the variety

Jagger, which contains *Yr17*, was heavily infected with stripe rust (Duncan, *et al.*, 2010). Therefore other, non-identified, genes were responsible for the resistance in LA841 to the new races of stripe rust. Studies are needed to identify the sources and genes of resistance in this variety.

Table 1 *Yr* Resistance Genes table found in paper by Maccaferri *et al.*, 2015

Loci	chr.	start (%)	end (%)	Reference
<i>Yr10</i>	1B	0	1.7	Ma, J., R. Zhou, Y. Dong, L. Wang, X. Wang <i>et al.</i> , 2001 Molecular mapping and detection of the yellow rust resistance gene <i>Yr26</i> in wheat transferred from <i>Triticum turgidum</i> L. using microsatellite markers. <i>Euphytica</i> 120: 219–226.
<i>Yr9</i>	1B	1.7	9.3	Lukaszewski, A. J., 2000 Manipulation of the 1RS.1BL translocation in wheat by induced homoeologous recombination. <i>Crop Sci.</i> 40: 216-225.
<i>YrAlp</i>	1B	24.8	34.6	Catalogue of Gene Symbols for Wheat
<i>Yr15</i>	1B	30.1	32.1	Cheng, P., L. S. Xu, M. N. Wang, D. R. See, and X. M. Chen, 2014 Molecular mapping of genes <i>Yr64</i> and <i>Yr65</i> for stripe rust resistance in hexaploid derivatives of durum wheat accessions PI 331260 and PI 480016. <i>Theor. Appl. Genet.</i> 127: 2267–2277.
<i>YrH52</i>	1B	30.9	32.7	Cheng, P., L. S. Xu, M. N. Wang, D. R. See, and X. M. Chen, 2014 Molecular mapping of genes <i>Yr64</i> and <i>Yr65</i> for stripe rust resistance in hexaploid derivatives of durum wheat accessions PI 331260 and PI 480016. <i>Theor. Appl. Genet.</i> 127: 2267–2277.
<i>Yr64</i>	1B	32.7	34.5	Cheng, P., L. S. Xu, M. N. Wang, D. R. See, and X. M. Chen, 2014 Molecular mapping of genes <i>Yr64</i> and <i>Yr65</i> for stripe rust resistance in hexaploid derivatives of durum wheat accessions PI 331260 and PI 480016. <i>Theor. Appl. Genet.</i> 127: 2267–2277.
<i>Yr650</i>	1B	37.4	39	Cheng, P., L. S. Xu, M. N. Wang, D. R. See, and X. M. Chen, 2014 Molecular mapping of genes <i>Yr64</i> and <i>Yr65</i> for stripe rust resistance in hexaploid derivatives of durum wheat accessions PI 331260. <i>Theor. Appl. Genet.</i>

Table 1 *Yr* Resistance genes table found in paper by Maccaferri *et al.*, 2015 *contd.*

<i>Yr24/Yr26</i>	1B	39	41.3	Cheng, P., L. S. Xu, M. N. Wang, D. R. See, and X. M. Chen, 2014 Molecular mapping of genes Yr64 and Yr65 for stripe rust resistance in hexaploid derivatives of durum wheat accessions PI 331260 and PI 480016. <i>Theor. Appl. Genet.</i> 127: 2267–2277.
<i>YrExp1</i>	1B	70.8	75.2	Catalogue of Gene Symbols for Wheat
<i>Yr29/Lr46</i>	1B	90	100	Lan, C., G. M. Rosewarne, R. P. Singh, S. A. Herrera-Foessel, J. Huerta-Espino <i>et al.</i> , 2014 QTL characterization of resistance to leaf rust and stripe rust in the spring wheat line Francolin#1. <i>Mol. Breed.</i> 34: 789–803.
<i>Yr17</i> (2NS -2AS translocation)	2A	0	20	Helguera, M., I. A. Khan, J. Kolmer, D. Lijavetzky, L. Zhong-qi <i>et al.</i> , 2003 PCR assays for the cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. <i>Crop Sci.</i> 43: 1839–1847.
<i>Yr56</i>	2A	2	6.3	Catalogue of Gene Symbols for Wheat
<i>Yrxy2</i>	2A	33.3	41.4	Zhou, X. L., W. L. Wang, L. L. Wang, D. Y. Hou, J. X. Jing <i>et al.</i> , 2011 Genetics and molecular mapping of genes for high-temperature resistance to stripe rust in wheat cultivar Xiaoyan 54. <i>Theor. Appl. Genet.</i> 123: 431–438.
<i>Yr32</i>	2A	48.3	59.5	Eriksen, L., F. Afshari, M. J. Christiansen, R. A. McIntosh, A. Jahoor <i>et al.</i> , 2004 Yr32 for resistance to stripe (yellow) rust present in the wheat cultivar Carstens V. <i>Theor. Appl. Genet.</i> 108: 567–575.
<i>Yr1</i>	2A	75.9	83.2	Catalogue of Gene Symbols for Wheat
<i>YrP81b</i>	2B	27.4	29.2	Catalogue of Gene Symbols for Wheat
<i>YrC51</i>	2B	27.5	29.1	Zheng, J., Z. Yan, L. Zhao, S. Li, Z. Zhang <i>et al.</i> , 2014 Molecular mapping of a stripe rust resistance gene in wheat line C51. <i>J. Genet.</i> 93: 443–450.
<i>Yr41</i>	2B	29.6	47.9	Catalogue of Gene Symbols for Wheat
<i>YrKK</i>	2B	30.4	44.3	Catalogue of Gene Symbols for Wheat
<i>YrH9014</i>	2B	32.3	33.5	Ma, D., D. Y. Hou, M. Tang, H. Wang, Q. Li <i>et al.</i> , 2013 Genetic analysis and molecular mapping of a stripe rust resistance gene YrH9014 in wheat line H9014-14-4-6-1. <i>J. Integr. Agric.</i> 12: 638–645.
<i>Yr27</i>	2B	34.4	36.4	Catalogue of Gene Symbols for Wheat

Table 1 *Yr* Resistance genes table found in paper by Maccaferri *et al.*, 2015 *contd.*

<i>YrH9014</i>	2B	32.3	33.5	Ma, D., D. Y. Hou, M. Tang, H. Wang, Q. Li <i>et al.</i> , 2013 Genetic analysis and molecular mapping of a stripe rust resistance gene YrH9014 in wheat line H9014-14-4-6-1. J. Integr. Agric. 12: 638–645.
<i>Yr5</i>	2B	62.1	64.5	McGrann, G. R., P. H. Smith, C. Burt, G. R. Mateos, T. N. Chama <i>et al.</i> , 2014 Genomic and genetic analysis of the wheat race-specific yellow rust resistance gene Yr5. J. Plant Sci. Mol. Breed. 3: http://dx.doi.org/10.7243/2050-2389-3-2 .
<i>Yr44</i>	2B	63.9	66.1	Xu, L. S., M. N. Wang, P. Cheng, Z. S. Kang, S. H. Hulbert <i>et al.</i> , 2013 Molecular mapping of Yr53, a new gene for stripe rust resistance in durum wheat accession PI 480148 and its transfer to common wheat. Theor. Appl. Genet. 126: 523–533.
<i>Yr53</i>	2B	66.1	78.1	Xu, L. S., M. N. Wang, P. Cheng, Z. S. Kang, S. H. Hulbert <i>et al.</i> , 2013 Molecular mapping of Yr53, a new gene for stripe rust resistance in durum wheat accession PI 480148 and its transfer to common wheat. Theor. Appl. Genet. 126: 523–533.
<i>Yr43</i>	2B	78.1	82.1	Xu, L. S., M. N. Wang, P. Cheng, Z. S. Kang, S. H. Hulbert <i>et al.</i> , 2013 Molecular mapping of Yr53, a new gene for stripe rust resistance in durum wheat accession PI 480148 and its transfer to common wheat. Theor. Appl. Genet. 126: 523–533.
<i>Yr3</i>	2B	86.8	90.7	Catalogue of Gene Symbols for Wheat
<i>Yr55</i>	2D	67.1	74.5	Catalogue of Gene Symbols for Wheat
<i>Yr54</i>	2D	70.7	80.3	Catalogue of Gene Symbols for Wheat
<i>Yr4</i>	3B	0	1.8	Catalogue of Gene Symbols for Wheat
<i>Yr57</i>	3B	0	10	Catalogue of Gene Symbols for Wheat
<i>Yr30</i>	3B	2.3	6.9	Suenaga, K., R. P. Singh, J. Huerta-Espino, and H. M. William, 2003 Microsatellite markers for genes Lr34/Yr18 and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93: 881–890.
<i>Yrns-B1</i>	3B	14.9	16.5	Catalogue of Gene Symbols for Wheat
<i>Yr66</i>	3D	0.4	2.4	Catalogue of Gene Symbols for Wheat
<i>Yr49</i>	3D	7.1	8.6	Catalogue of Gene Symbols for Wheat
<i>Yr45</i>	3D	60.2	72	Catalogue of Gene Symbols for Wheat

Table 1 *Yr* Resistance genes table found in paper by Maccaferri *et al.*, 2015 *contd.*

<i>Yr51</i>	4A	70.5	80.8	Randhawa, M., U. Bansal, M. Valárik, B. Klocová, J. Doležel <i>et al.</i> , 2014 Molecular mapping of stripe rust resistance gene <i>Yr51</i> in chromosome 4AL of wheat. Theor. Appl. Genet. 127: 317–324.
<i>Yr60</i>	4A	83.3	85.7	Catalogue of Gene Symbols for Wheat
<i>Yr50</i>	4B	39	68.6	Liu, J., Z. Chang, X. Zhang, Z. Yang, X. Li <i>et al.</i> , 2013 Putative <i>Thinopyrum</i> intermedium-derived stripe rust resistance gene <i>Yr50</i> maps on wheat chromosome arm 4BL. Theor. Appl. Genet. 126: 265–274.
<i>Yr62</i>	4B	52.1	58.1	Lu, Y., M. Wang, X. Chen, D. See, S. Chao <i>et al.</i> , 2014 Mapping of <i>Yr62</i> and a small-effect QTL for high-temperature adult-plant resistance to stripe rust in spring wheat PI 192252. Theor. Appl. Genet. 127: 1449–1459.
<i>Yr28</i>	4D	9.4	11.4	Catalogue of Gene Symbols for Wheat
<i>YrAS2388</i>	4D	14	15.9	Catalogue of Gene Symbols for Wheat
<i>Yr46/Lr67</i>	4D	49.5	50.8	Herrera-Foessel, S. A., E. S. Lagudah, J. Huerta-Espino, M. J. Hayden, H. S. Bariana <i>et al.</i> , 2011 New slow-rusting leaf rust and stripe rust resistance genes <i>Lr67</i> and <i>Yr46</i> in wheat are pleiotropic or closely linked. Theor. Appl. Genet. 122: 239–249.
<i>Yr48</i>	5A	86.8	100	Catalogue of Gene Symbols for Wheat
<i>Yr34</i>	5A	89.9	100	Catalogue of Gene Symbols for Wheat
<i>Yr47</i>	5B	3.3	10	Catalogue of Gene Symbols for Wheat
<i>YrExp2</i>	5B	53.8	55.2	Catalogue of Gene Symbols for Wheat
<i>Yr40</i>	5D	0	4	Catalogue of Gene Symbols for Wheat
<i>YrLM168</i>	6A	56.9	67.3	Feng, J., G. Chen, Y. Wei, Y. Liu, Q. Jiang <i>et al.</i> , 2014 Identification and genetic mapping of a recessive gene for resistance to stripe rust in wheat line LM168-1. Mol. Breed. 33: 601–609.
<i>Yr35</i>	6B	0	14.9	Catalogue of Gene Symbols for Wheat
<i>Yr36</i>	6B	23.1	25.1	Uauy, C., J. C. Brevis, X. Chen, I. Khan, L. Jackson <i>et al.</i> , 2005 High-temperature adult-plant (HTAP) stripe rust resistance gene <i>Yr36</i> from <i>Triticum turgidum</i> ssp. <i>dicoccoides</i> is closely linked to the grain protein content locus <i>Gpc-B1</i> . Theor. Appl. Genet. 112: 97–105.
<i>Yr61</i>	7A	19.5	32.4	Catalogue of Gene Symbols for Wheat

Table 1 *Yr* Resistance genes table found in paper by Maccaferri *et al.*, 2015 *contd.*

<i>Yrxy1</i>	7A	42	49.3	Zhou, X. L., W. L. Wang, L. L. Wang, D. Y. Hou, J. X. Jing <i>et al.</i> , 2011 Genetics and molecular mapping of genes for high-temperature resistance to stripe rust in wheat cultivar Xiaoyan 54. Theor. Appl. Genet. 123: 431–438.
<i>Yr63</i>	7B	0	1	Catalogue of Gene Symbols for Wheat
<i>Yr39</i>	7B	27.5	48.1	Lin, F., and X. M. Chen, 2007 Genetics and molecular mapping of genes for race-specific all-stage resistance and non-race-specific high-temperature adult-plant resistance to stripe rust in spring wheat cultivar Alpowa. Theor. Appl. Genet. 114: 1277–1287.
<i>Yr67</i>	7B	75.5	77.5	Catalogue of Gene Symbols for Wheat
<i>YrZH84</i>	7B	80.4	82.7	Catalogue of Gene Symbols for Wheat
<i>Yr59</i>	7B	80.9	84.7	Catalogue of Gene Symbols for Wheat
<i>Yr52</i>	7B	81.6	83.2	Catalogue of Gene Symbols for Wheat
<i>YrC591</i>	7B	82.7	87.8	Catalogue of Gene Symbols for Wheat
<i>Yr18/Lr34</i> (csLV23)	7D	26.1	33.6	Yang, E.-N., G. M. Rosewarne, S. A. Herrera-Foessel, J. Huerta-Espino, Z.-X. Tang <i>et al.</i> , 2013 QTL analysis of the spring wheat “Chapio” identifies stable stripe rust resistance despite inter-continental genotype × environment interactions. Theor. Appl. Genet. 126: 1721–1732.
<i>Yr33</i>	7D	49.8	60.2	Catalogue of Gene Symbols for Wheat

Genes are arranged by chromosomes. (Maccaferri *et al.*, 2015)

1.4 QTL Mapping

QTL maps are a common method used to discover the location of genes for complex traits. “A QTL is a region of any genome that is responsible for variation in the quantitative trait of interest” (Doerge 2002). The quantitative trait of interest in our case is resistance to stripe rust in LA841. Durable disease resistance is a complex trait, controlled by several genes, and it can be quantified. Qualitative resistance, or single gene, is normally not durable or effective for controlling diseases over time (Rossi, *et al.*, 2006). So the durable resistance found in LA841 may be due to the presence of multiple resistance genes, making it a quantitative trait.

According to Cruzan (1998) one of the essential steps necessary to determine the location of a disease resistance gene is to find DNA markers that are genetically linked with the resistance gene in order to identify its chromosomal location, a procedure known as genetic mapping. A genetic or linkage map is like a road map of an organism's chromosome using molecular markers. These maps indicate the position and genetic distance between the markers in the different chromosomes (Patterson and Tanksley, 1991).

DNA markers are used to locate QTL on maps based on the principle of genetic linkage. "The importance of genetic linkage is that DNA markers that are sufficiently close to the disease resistance gene will tend to be inherited together with the disease gene in pedigrees—and the closer the markers, the stronger this association" (Patterson and Tanksley, 1991). So basically genes that are sufficiently close or tightly linked to markers will be inherited together from parent to progeny with a higher frequency than markers that are further apart. Since we know the location of the markers we can determine the location of the gene of interest by using the tightly linked marker.

Markers that are different in the parents (polymorphic) are used to construct the linkage map. The genotypic information obtained from scoring these markers is compared to the phenotypic information obtained in field trials. Once these calculations are completed a test of likelihood is used to determine the linkage between a location on the linkage map and the QTL. More specifically the linkage is determined by calculating an odds ratio (odds of linkage versus no linkage). This ratio is expressed as a logarithm of ratio and is known as the logarithm of odds (LOD) value. The threshold LOD value to consider a QTL as linked to a location on a map is typically around 3. A LOD value of 3 states that linkage is 1,000 times more likely to occur than

no linkage (Collard, *et al.*, 2005). So the position of a QTL can be determined with respect to the highest LOD value and the location of the flanking markers.

1.5 Molecular Markers

To develop a map, molecular markers known as Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) are often used. SSRs are among the most widely used for breeding and map development purposes (Cavalcanti and Wilkinson 2007). SSRs, or microsatellites, are PCR-based molecular markers used to indicate polymorphism in the DNA sequence between individuals and for genetic mapping (Liu, 1996). According to Liu (1996) SSRs are ideal markers for genetic mapping population studies for several reasons. Some of the most important reasons are their abundance, high level of polymorphism, wide dispersion in diverse genomes, ease of assay by the polymerase chain reaction (PCR) and ease of dissemination between laboratories. These markers work by detecting the number of copies of short DNA sequences that are repeated in order or in tandem. So SSRs may be different amongst individuals depending on the sequence, length of the unit and repeating tandem copies that occur in each individual's genome (Kumar, 1999).

AFLP markers are also PCR based however the methodology required for these markers is more complicated when compared to SSR markers (Collard, *et al.*, 2005). Steps prior to the use of primers and PCR are required for these markers to function properly. Initially the genomic DNA is digested using restriction enzymes, typically Eco RI. This produces a large number of DNA fragments flanked on both sides by the nucleotides or sites that remain from the enzyme used to digest. Afterwards specific adapters that match the ends of the fragments are ligated using the enzyme DNA ligase. The adapted product is then pre-amplified. The remaining double stranded fragments are ready to be used in PCR with selective primers (Mitton 1994).

The major advantage of AFLP markers is the large number of polymorphic bands that can be obtained in an analysis. However this system only detects dominant markers, meaning it can't distinguish heterozygous ones from the dominant. The complexity of the profiles produced by AFLPs makes it complicated to compare information from different laboratories and mapping populations. The use of SSR markers whose location are known and are locus specific helps overcome this problem by providing useful anchor points. These anchor points are used as a reference to associate different linkage groups produced in different mapping populations by different laboratories (Cavalcanti and Wilkinson 2007).

1.6 Previous Work

Over 140 QTL for stripe rust resistance in wheat have been described in over 30 publications. It is probable that many of these QTL contain identical genes which have been dispersed into different backgrounds and lines through breeding and germplasm exchange. These genes could have been selected by phenotypic evaluations in stripe rust infected locations. QTL have been found on every chromosome except for 5D (Rosewarne, *et al.*, 2013). However little is known about the extent and variation of resistance in soft red winter lines in the southeast United States, mostly due to the relatively new presence of the disease in the local varieties (Christopher, *et al.*, 2013). Two soft red winter wheat lines from the region which have been mapped for stripe rust resistance are Pioneer 26R61 and USG 3555. Both of these varieties have shown resistance to new stripe rust races prevalent in the southeast U.S. The Pioneer variety was found to have a major QTL in chromosome 2A which explained 56% of the phenotypic variation and a minor QTL in chromosome 6A responsible for 6-7% of the phenotypic variation (Hao, *et al.*, 2011). USG 3555 was found to have 3 QTL in chromosomes 1A, 4B and 7D (Christopher, *et al.*, 2013).

1.7 Justification

Rusts are caused by fungi that mutate and evolve very quickly so this causes problems with developing varieties with durable resistance (Yu, *et al.*, 2011). This is the reason that many resistance genes have been overcome by new races. There is a pressing need for discovering new sources and genes for resistance to this disease so they can be incorporated into already existing varieties or for the development of new resistant ones. With the availability of several molecular markers for resistance genes we can implement a gene pyramiding strategy where we incorporate several sources of resistance into one variety. This is why a goal of this project is to discover the new unidentified genes and their location.

1.8 Objectives

- 1) Map QTL linked to stripe rust resistance genes in the wheat variety Terral LA841.
- 2) Initialize work to identify the markers linked to gene or genes that confer resistance to stripe rust in the wheat variety Terral LA84.

Chapter II

Materials and Methods

2.1 Mapping Population

For this study, an F_2 population derived from a cross between wheat breeding line NC06BGTAG12 and Terral LA841 was used with 190 individuals planted in the field at Ben Hur Farm in Baton Rouge, LA. The wheat breeding line NC06BGTAG12, the susceptible parent, was developed at North Carolina State University by Dr. Paul Murphy for resistance to powdery mildew. It had 60% stripe rust infection in previous trials. Terral LA841, which was developed by the LSU AgCenter, has remained resistant to different races of stripe rust over many environments and years. Resistance in this variety was believed to be due to of the gene *Yr₁₇*, however, according to Milus (2009) this gene has become ineffective against new stripe rust races. Other varieties carrying this gene have become susceptible to new races of stripe rust, so this variety appears to contain additional/novel resistance genes. The objective is to identify genes that provide resistance to multiple races of stripe rust in this variety and develop molecular markers linked to the other genes for marker-assisted breeding.

2.2 DNA Extraction

The F_2 population was planted in the field at Ben Hur in November of 2011 and thinned in early seedling stage so individual plants could be selected. Leaf tissue was collected from 190 individual F_2 plants and the two parents for genomic DNA extraction. The total genomic DNA was extracted using the C-TAB mini prep protocol modified after Doyle and Doyle (1987). The quantity and quality of the DNA was determined by using ND-100 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). The total genomic DNA was diluted to a working concentration of 25ng/ μ l and stored at 4° C.

2.3 Seed Collection

The 190 F₂ plants were individually hand harvested and threshed. The F_{2:3} seed was stored in a freezer at 4° C to prevent insect or fungal damage.

2.4 Genotyping

2.4.1 Molecular markers

Polymorphism assay was performed on the parents of the mapping population with 501 SSR and 128 AFLP primer combinations. SSR markers were selected from the Grain-Genes data base for wheat (<http://wheat.pw.usda.gov/GG3/>). A total of 64 SSR markers were polymorphic, while only 13 AFLP primer combinations produced high number of polymorphic bands (152 total). All 216 polymorphic markers were selected to genotype the 190 F₂ lines.

Table 2. List of AFLP primer combinations used and number of polymorphic bands for each

AFLP Combination	Polymorphic Band
<i>Eco</i> RI AAG700 X <i>MSE</i> I ACC	7
<i>Eco</i> RI AGA800 X <i>MSE</i> I ACC	4
<i>Eco</i> RI AAG700 X <i>MSE</i> I ACT	4
<i>Eco</i> RI AGA800 X <i>MSE</i> I ACT	12
<i>Eco</i> RI AAG700 X <i>MSE</i> I ATA	9
<i>Eco</i> RI AGA800 X <i>MSE</i> I ATA	5
<i>Eco</i> RI AAG700 X <i>MSE</i> I TCC	9
<i>Eco</i> RI AGA800 X <i>MSE</i> I TCC	5
<i>Eco</i> RI AAG700 X <i>MSE</i> I TCG	6
<i>Eco</i> RI AGA800 X <i>MSE</i> I TCG	5
<i>Eco</i> RI AAG700 X <i>MSE</i> I TGT	4
<i>Eco</i> RI AGA800 X <i>MSE</i> I TGT	4
<i>Eco</i> RI AAG700 X <i>MSE</i> I GCG	12
<i>Eco</i> RI AGA800 X <i>MSE</i> I GCG	7
<i>Eco</i> RI AAG700 X <i>MSE</i> I GGT	11
<i>Eco</i> RI AGA800 X <i>MSE</i> I GGT	7

Table 2. List of AFLP primer combinations used and number of polymorphic bands for each *contd.*

<i>Eco</i> RI AAG700 X <i>MSE</i> I TCA	5
<i>Eco</i> RI AGA800 X <i>MSE</i> I TCA	5
<i>Eco</i> RI AGG700 X <i>MSE</i> I AGC	4
<i>Eco</i> RI ACC800 X <i>MSE</i> I AGC	4
<i>Eco</i> RI AGG700 X <i>MSE</i> I AAT	4
<i>Eco</i> RI ACC800 X <i>MSE</i> I AAT	5
<i>Eco</i> RI AGG700 X <i>MSE</i> I GTG	4
<i>Eco</i> RI ACC800 X <i>MSE</i> I GTG	4
<i>Eco</i> RI AGG700 X <i>MSE</i> I GTC	4
<i>Eco</i> RI ACC800 X <i>MSE</i> I GTC	3

Table 3. List of polymorphic SSR markers used in study.

SSR Marker	Primer (Forward)	Primer (Reverse)	Chr
BARC1020	5' GGG GCA ATC GTC GAC GCG TCC TC 3'	5' CCC GGC ACA AAA CCA CCATCG ACT 3'	\N
BARC120	5' CCC CCT CTC TTC CTC AT 3'	5' ATA TAG CTC CCC CAT TTC CT 3'	1A
BARC17	5' GCGCAACATATTCAGCTCAACA 3'	5' TCCACATCTCGTCCCTCATAGTTTG 3'	1A
BARC263	5' GGAAGCGCGTCAGCACTAGGCAAC 3'	5' GGCTTCTAGGTGCTGCGGCTTTTGTC 3'	1A
BARC61	5' TGCATACATTGATTTCATAACTCTCT 3'	5' TCTTCGAGCGTTATGATTGAT 3'	1B
BARC86	5' GCG CTT GCT TTA TTA GTA GGT AT 3'	5' TCC CAC GAT AGT ATT TGA TGT T 3'	1D
BARC99	5' CGCATCTTTTCGCATTCTCTGTCATA 3'	5' CGCATACTGTGTGTGTTCCTGGTTAGA 3'	1D
wmc24	5' GTGAGCAATTTTGATTATACTG 3'	5' TACCCTGATGCTGTAATATGTG 3'	1A
wmc336	5' GTCTTACCCCGCGATCTGC 3'	5'GTCTTACCCCGCGATCTGC 3'	1D
wmc432	5' ATGACACCAGATCTAGCAC 3'	5' AATATTGGCATGATTACACA 3'	1D
BARC35	5' GCGGTGTGCATGCTTGTGTGTAGGAGT 3'	5' GCGTAGTGTAGTATGTGGCCGATTATT 3'	2B
BARC159	5' CGCAATTTATTATCGGTTTTAGGAA 3'	5' CGCCCGATAGTTTTTCTAATTTCTGA 3'	2D
BARC219	5' GCG ATC CCA CAA TGATG ACA ACT TC 3'	5' GGA CGT CCG ATC GAA TTG GTT T 3'	2D
BARC297	5' GCG TAG GAG AGA C CCC AAA GGT T 3'	5' GCG TGC GGA CTC TG AAT CAT TAC A 3'	2D
Xgwm636	5' CGGTAGTTTTTAGCAAAGAG 3'	5' CCTTACAGTTCTTGCCAGAA 3'	2A
wmc25	5' TCTGGCCAGGATCAATATTACT 3'	5' TAAGATACATAGATCCAACACC 3'	2B
BARC1044	5' GCG TAT GTA TGTTA TTT TCC TAT CT 3'	5' CCC AAT TTT GCT AAG TTC ACT 3'	3A
BARC310	5' GGG CGG CGC ATG TGC ACC TA 3'	5' GCG TGG AAG CGA CTA AAT CAA CT 3'	3A
BARC45	5' CCCAGATGCAATGAAACCACAAT 3'	5' GCGTAGAACTGAAGCGTAAAATTA 3'	3A
BARC57	5' GCGACCACCTCAGCCAACTTATTATGT 3'	5' GCGGGGAGGCACATTCATAGGAGT 3'	3A
BARC1111	5' CGC TTC TCA TCA GTA TGT ATC CAA T 3'	5' CGC AAT CCC AATT CCA TT TCT ACT T 3'	3B
BARC164	5' TGCAAACTAATCACCAGCGTAA 3'	5' CGCTTTCTAAACTGTTTCGGATTTCTAA 3'	3B
BARC187	5' GTGGTATTTCAGGTGGAGTTGTTTTA 3'	5' CGGAGGAGCAGTAAGGAAGG 3'	3B
BARC71	5' GCGCTGTTCCTCACCTGCTCATA 3'	5' GCGTATATCTCTCGTCTCTTGTTGGTT 3'	3D
wmc623	5' ACGATTGGCCACAGAGGAG 3'	5' CAGTGACCAATAGTGGAGGTCA 3'	3B

Table 3. List of polymorphic SSR markers used in study. *contd.*

BARC106	5' GCCCTCAAATAATTACGCCAATCCTATG 3'	5' GCGTCAAGATCAGAAGGCTCATTATTG 3'	4A
BARC153	5' CGCGCCTTGCTTTATTAGTATTAGTATT 3'	5' GCGGCATGCACATATAATTCTCTTGACT 3'	4A
BARC170	5' CGCTTGACTTTGAATGGCTGAACA 3'	5' CGCCCACTTTTTACCTAATCCTTTTTGAA 3'	4A
BARC184	5' TTCGGTGATATCTTTTCCCCTTGA 3'	5' CCGAGTTGACTGTGTGGGCTTGCTG 3'	4A
BARC190	5' CCG TAT GCA AAT CTG ACA AAG TTA 3'	5' GCG ATC GTT CTC TTC TCTC CTA CTC 3'	4A
BARC236	5' GCG AAA ATT GTC ACC CTT C CAG TA 3'	5' CGT TCG TAC ACA CCA TG TCA CTT C 3'	4A
BARC343	5' GGC CTA ATT ACA AGT CCA AAA G 3'	5' GCT CAA AGT AAA GTT CAC GAA TAT 3'	4A
BARC78	5' CTCCCCGGTCAAGTTTAATCTCT 3'	5' GCGACATGGGAATTTTCAAGTGCCTAA 3'	4A
BARC163	5' GCGTGTTTAAAGGTATTTTCCATTTTCT 3'	5' GCGCATCCTGTTTCTCCATTCATA 3'	4B
BARC1118	5' CGC AGT TGC CTC CCT TGTAAG ATG TT 3'	5' CGC TTA TTC CTT TCCAT TGG GTT TG 3'	4D
wmc47	5' GAAACAGGGTTAACCATGCCAA 3'	5' ATGGTGCTGCCAACAACATACA 3'	4B
Xgwm251	5' CAACTGGTTGCTACACAAGCA 3'	5' GGGATGTCTGTTCCATCTTAG 3'	4B
BARC122	5' CCCGTGTATATCCAGGAGTG 3'	5' CAGCCCTTGTGATGTGATG 3'	5A
BARC141	5' GGCCCATGGATAATTTTGAATG 3'	5' CAATTCGGCCAAAGAAGAAGTCA 3'	5A
BARC151	5' TGAGGAAAATGTCTCTATAGCATCC 3'	5' CGCATAAACACCTTCGCTCTTCCACTC 3'	5A
BARC301	5' CGC AAC TAT CCA ACG CAG ACC A 3'	5' CGG TGA TCG GCA AAT AAA TA 3'	5A
BARC40	5' GCCGCCTACCACAGAGTTGCAGCT 3'	5' GCGGCATTGACAAGACCATAGC 3'	5A
BARC142	5' CCGGTGAGAGGACTAAAA 3'	5' GGCCTGTCAATTATGAGC 3'	5B
BARC4	5' GCGTGTTTGTGTCTGCGTTCTA 3'	5' CACCACACATGCCACCTTCTTT 3'	5B
BARC59	5' GCGTTGGCTAATCATCGTTCCTTC 3'	5' AGCACCTACCCAGCGTCAGTCAAT 3'	5B
BARC143	5' TTGTGCCAAATCAAGAACAT 3'	5' GGTTGGGCTAGGATGAAAAT 3'	5D
BARC161	5' GCG AAA GGG GCT AAG TAA CAC TAA 3'	5' GCG CAG AAC ACA GGG TAT CGT C 3'	5D
BARC177	5' GCG ATC CTG TTG TTG AGC TGC ATA A 3'	5' TCC CGT TTT CCC GTG TGT TAG TCT A 3'	5D
BARC44	5' CCCTACAAAATACGAACATGAAGTCAG 3'	5' GGGTCCTACTCAGAAGTGACAGTCAAC 3'	5D
wmc713	5' ACATAGCATCCCATCTGAGAGAGG	5' ATGCGGGGAATAGAGACACAC 3'	5A
BARC118	5' AGT TGC CGC TTC TTT TCA TTT TT 3'	5' AGA GGT CCA TTT TTC GTC CTT TGA C 3'	6A
BARC134	5' CCGTGCTGCAAATGAACAC 3'	5' AGTTGCCGGTTCCCATTGTCA 3'	6B
BARC1121	5' GCG AGC AAA CTG ATC CCA AAA AG 3'	5' TAT CGG TGA GTA CGC CAA AAA CA 3'	6D
BARC204	5' CGCAGAAGAAAAACCTCGCAGAAAACC 3'	5' CGCAGTGTATCCAAATGGGCAAGC 3'	6D
BARC54	5' GCGAACAGGAGGACAGAGCACGAGAG 3'	5' GCGCTTTCCACGTTCCATGTTTCT 3'	6D
BARC96	5' AAGCCTTGTGTTCCTGATTATT 3'	5' GCGGTTTATATTTTGGTTGAGCATTTT 3'	6D
wmc419	5' GTTTCGGATAAAACCGGAGTGC 3'	5' ACTACTTGTGGGTATCACCAGCC 3'	6B
cf49	5' TGAGTTCTTCTGGTGAGGCA 3'	5' GAATCGGTTTACAAGGGAAA 3'	6D
BARC108	5' GCGGGTCGTTTCCCTGGAATTCATCTAA 3'	5' GCGAAATGATTGGCGTTACACCTGTTG 3'	7A
BARC22	5' GCG ACA TCC GAT TTC TAC AAC A 3'	5' CAC GCA TAACG AAC ACG CAT CTG 3'	7A
BARC275	5' GCG TTT GGT CAG AAT AG GAA GAT 3'	5' GCG TAT GTT CGTT AGT GTGT TAT GC 3'	7A
BARC255	5' GTGGCGGCTTGCGGGTGGCTGAGTA 3'	5' GGGTCGGCTAGCCTTCTTTTATGTT 3'	7B
BARC172	5' GCGAAATGTGATGGGGTTTATCTA 3'	5' GCGATTTGATTAACTTTAGCAGTGAG 3'	7D
Xgwm350	5' ACCTCATCCACATGTTCTACG 3'	5' GCATGGATAGGACGCCC 3'	7A
wmc479	5' GACCTAAGCCCAGTGCATCAG 3'	5' AGACTCTTGGCTTTGGATACGG 3'	7A

2.4.2 Polymerase chain reaction (PCR)

For the SSR markers, 100 ng of genomic DNA were used in a final volume of 25 µl for every reaction which contained 5 µl of 5X PCR buffer, 2.5 µl of 25 mM MgCl₂, 2.5 µl of 2 mM dNTP mix, 1 µl of forward and reverse primer, 0.2 µl of 5U/µl *Taq* DNA polymerase (Promega, San Luis Obispo, CA) and 8.8 µl of ddH₂O. Amplifications of DNA was performed in a Thermocycler (Biorad, Hercules, Ca) using a profile: (1) initial denaturation 95° C, 4 min; (2) 35 cycles of 95° C, 45 sec; 52-55° C, 45 sec; 72° C, 1 min; and (3) final extension 72° C, 7 min. The PCR product was resolved on a 4.5 % SFR agarose (Amresco, Solomon, OH) gel, already containing ethidium bromide, in 0.5 X TBE buffer for approximately 4 hours at 100 volts. The gel was photographed using a KODAK Gel Logic 200 Imaging system (Kodak, New Haven, CT).

For the AFLP marker analysis, 375 ng of genomic DNA were digested overnight at 37° C using the restriction enzymes *Mse* I (5,000 units) and *Eco* RI (5,000 units). The digested fragments were ligated at room temperature for 5 hours with *Eco* RI and *Mse* I adapters using 20 units of the enzyme ligase. The adapted fragments were pre-amplified using primers *Eco* RI + A oligo and *Mse* I + C oligo. The pre-amplification product was used for selective amplification. The final volume for each reaction of the selective amplification was 9.4 µl, which contained 1 µl of pre-amplification product, 2 µl of 5X PCR buffer, 1.2 µl of 25 mM MgCl₂, 1 µl of 5 mM dNTP mix, 0.165 µl of each primer with IR 700 dye, 0.335 of each primer with IR 800 dye, 0.2 µl of *Taq* DNA polymerase and 3 µl of ddH₂O. PCR was performed in a Thermocycler (Biorad) using a profile: (1) initial denaturation 94° C, 2 min; (2) 12 cycles of 94° C, 30 sec; 65° C (decreasing by 0.7° C every cycle), 30 sec; 72° C, 1 min; (3) 23 cycles of 94° C, 30 sec; 56° C, 30 sec; 72° C, 1 min and (4) final extension 72° C, 5 min. The selective amplification product was

loaded onto a 6% acrylamide gel placed and run in a Licor 4300 genetic analyzer for electrophoresis and image documentation of the gels.

2.5 Disease Screening

For the disease screening the $F_{2:3}$ seed was grown in two replications of a randomized complete block design at three locations where stripe rust is commonly found. We planted a test on the Macon Ridge Research Station near Winnsboro, Louisiana in November of 2012. The remaining tests were conducted at the Coastal Plains Research Station in Plains, Georgia by Dr. Jerry Johnson; and in the Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas by Dr. Eugene Milus. The lines along with the susceptible variety NC06BG TAG12, used as disease spreaders, were planted in one-meter rows using a headrow magazine planters. Artificial inoculation of stripe rust was used at Winnsboro and Plains to help spread the disease uniformly. Spores collected from nearby fields with disease infested plants were suspended in Soltrol oil and sprayed throughout the field using a pressurized CO₂ tank. Inoculation was conducted once in February, on an afternoon when night conditions were expected to be favorable for infection.

Lines were rated once, at adult stage when disease symptoms were clearly visible on the plants. Ratings were conducted in late March in Louisiana, early May in Arkansas and early April in Winnsboro. The rating scale used was on a range from 0 to 9 where the most resistant lines were given a score of 0 and the most susceptible ones were given a 9. Lines with rating between 0 and 4 were considered resistant while lines with rating between 5 and 9 were susceptible. Lines segregating for resistance were given two different scores.

2.6 Data Analysis

The amplified fragments from the AFLP and SSR primers on gels were scored and the data was merged in an Excel (Microsoft Corp., Redmond WA) worksheet for further analysis.

JoinMap 3.0 (Kyazama Inc., Netherlands) software was used for development of linkage map. The complete set of polymorphic markers and scoring information from the Excel worksheet was converted into a text file in Word Pad. The Word Pad file containing the marker data was saved as a .loc file in JoinMap. The .loc file was uploaded as a new project into JoinMap to calculate the genotype and locus frequencies. After calculating the recombination frequencies, the Kosambi mapping function was used to calculate the genetic distance between markers from the frequencies. Groups with LOD above 4 were chosen. The selected linkage groups were highlighted and the corresponding group maps were viewed using the “groups” tab.

QTL Cartographer version 2.5 was used to develop the QTL map. The linkage groups along with the average disease rating of each line were used to locate the QTL regions. A new text file was created containing scoring data only from the markers found within the linkage groups along with the average disease rating for each line. The text file was uploaded into the software using the “source file” option. The CIM (composite interval mapping) option, was chosen for the analysis. A walking speed of 0.5 cM was selected, as opposed to the default 1 cM, to ensure a finer coverage. A significant difference was established at $P = 0.05$ with 1000 iterations. The default LOD value of 2.5 for QTL was maintained.

2.7 Expected Output

QTL with a significant effect on stripe rust resistance would be found on specific linkage group(s) corresponding to a chromosome with LOD value over the threshold of 2.5.

Chapter III

Results

3.1 Disease Ratings

The number of $F_{2:3}$ lines in each location with different infection types or segregating for resistance is presented in Table 4. Based on phenotypic data obtained from rating the $F_{2:3}$ lines, there were more resistant lines than susceptible ones, which indicates resistance genes were probably dominant. None of the non-segregating lines showed major differences in severity over different locations. A total of 78 lines had ratings between 0-4 (resistant) averaged across locations while only 24 received ratings from 5-9 (susceptible) and 88 were segregating (heterozygous) for resistance within the line. This suggests the presence of more than one resistance gene in the resistant parent, LA841. We believe that LA841 contains resistance gene Yr_{17} and some other gene or genes that provide resistance to the new stripe rust races based on the reaction of LA841 to stripe rust in environments where other varieties with Yr_{17} showed a susceptible reaction type. This assumption agrees with the disease screening data obtained in the field trials. Data obtained from screening complex traits controlled by multiple genes would not necessarily follow a normal distribution as is the case with the rust resistance phenotypic data (Young 1996). The data is skewed towards having more resistant lines because of the multiple resistance genes in the resistant parent LA841.

Table 4 Stripe rust ratings over three locations.

ENT	Line	Arkansas		Georgia		Louisiana		Mean	Class
		REP1	REP2	REP1	REP2	REP1	REP2		
58	LA11242-60	0	1	0	1	1	1	0.67	Res
114	LA11242-119	1	1	0	0	1	2	0.83	Res
130	LA11242-136	1	1	1	0	1	1	0.83	Res
11	LA11242-11	1	1	1	1	1	1	1.00	Res

Table 4 Stripe rust ratings over three locations. *contd.*

46	LA11242-48	1	1	0	1	2	1	1.00	Res
57	LA11242-59	1	1	1	1	1	1	1.00	Res
66	LA11242-69	1	1	0	1	2	1	1.00	Res
74	LA11242-77	1	1	1	1	1	1	1.00	Res
125	LA11242-130	1	1	1	1	1	1	1.00	Res
183	LA11242-194	1	1	1	1	1	1	1.00	Res
8	LA11242-8	1	1	1	1	1	2	1.17	Res
34	LA11242-35	1	1	1	1	1	2	1.17	Res
89	LA11242-92	1	1	1	1	1	2	1.17	Res
93	LA11242-96	1	1	1	1	2	1	1.17	Res
95	LA11242-98	1	1	2	1	1	1	1.17	Res
150	LA11242-159	1	1	1	1	1	2	1.17	Res
153	LA11242-163	1	1	1	1	2	1	1.17	Res
156	LA11242-167	1	1	1	1	1	2	1.17	Res
158	LA11242-169	1	1	2	1	1	1	1.17	Res
68	LA11242-71	1	1,4	1	1	1	1	1.25	Res
37	LA11242-38	1	1	1	1	3	1	1.33	Res
59	LA11242-61	1	1	1	2	2	1	1.33	Res
63	LA11242-66	1	1	1	1	2	2	1.33	Res
111	LA11242-116	1	1	1	1	2	2	1.33	Res
136	LA11242-142	1	1	1	1	2	2	1.33	Res
142	LA11242-150	1	1	2	1	1	2	1.33	Res
146	LA11242-155	1	2	2	1	1	1	1.33	Res
161	LA11242-172	1	1	2	1	1	2	1.33	Res
179	LA11242-190	1	1	1	1	2	2	1.33	Res
184	LA11242-195	1	1	1	2	1	2	1.33	Res
189	LA11242-200	1	1	1	2	2	1	1.33	Res
2	LA11242-2	1	1	1	2	1	3	1.50	Res
26	LA11242-27	1	1	2	1	2	2	1.50	Res
44	LA11242-46	1	2	1	1	2	2	1.50	Res
51	LA11242-53	1	1	1	2	3	1	1.50	Res
69	LA11242-72	1	1	2	1	2	2	1.50	Res
84	LA11242-87	1	1	2	1	2	2	1.50	Res
145	LA11242-153	1	1	3	1	2	1	1.50	Res
151	LA11242-160	1	1	1	1	3	2	1.50	Res
190	LA11242-201	1	1	2	2	2	1	1.50	Res
10	LA11242-10	1	1	2	2	2	2	1.67	Res
19	LA11242-20	3	1	1	2	1	2	1.67	Res
50	LA11242-52	2	1	1	1	2	3	1.67	Res
94	LA11242-97	1	1	1	1	3	3	1.67	Res

Table 4 Stripe rust ratings over three locations. *contd.*

97	LA11242-100	2	1	1	3	1	2	1.67	Res
105	LA11242-109	3	1	2	2	1	1	1.67	Res
137	LA11242-143	1	2	1	2	3	1	1.67	Res
31	LA11242-32	2	1	2	1	2	3	1.83	Res
33	LA11242-34	1	2	2	1	3	2	1.83	Res
54	LA11242-56	2	2	2	1	2	2	1.83	Res
67	LA11242-70	2	1	1	1	4	2	1.83	Res
71	LA11242-74	1	1	1	2	2	4	1.83	Res
79	LA11242-82	1	1	4	2	1	2	1.83	Res
91	LA11242-94	2	1	2	2	2	2	1.83	Res
104	LA11242-108	1	1	3	2	2	2	1.83	Res
118	LA11242-123	2	1	2	3	2	1	1.83	Res
181	LA11242-192	2	1	2	2	2	2	1.83	Res
6	LA11242-6	3	1	2	1	4	1	2.00	Res
47	LA11242-49	2	1	2	3	3	2	2.17	Res
72	LA11242-75	2	1	2	1	3	4	2.17	Res
85	LA11242-88	2	2	2	3	2	2	2.17	Res
88	LA11242-91	2	1	3	1	4	2	2.17	Res
121	LA11242-126	1	1	3	3	2	3	2.17	Res
173	LA11242-184	3	1	1	2	3	3	2.17	Res
83	LA11242-86	3	2	2	0	4	3	2.33	Res
186	LA11242-197	4	2	2	1	3	2	2.33	Res
62	LA11242-64	1	3	3	3	2	3	2.50	Res
82	LA11242-85	1	2	1	2	5	4	2.50	Res
96	LA11242-99	3	2	2	2	2	4	2.50	Res
64	LA11242-67	3	1	4	3	3	2	2.67	Res
103	LA11242-107	1	3	1	3	3	5	2.67	Res
169	LA11242-180	4	3	3	2	2	2	2.67	Res
28	LA11242-29	1	2	3	2	4	5	2.83	Res
70	LA11242-73	2	2	3	4	4	2	2.83	Res
24	LA11242-25	3	2	2	3	5	3	3.00	Res
109	LA11242-113	3	2	4	2	4	3	3.00	Res
157	LA11242-168	4	2	3	3	4	4	3.33	Res
102	LA11242-106	4	3	4	3	5	5	4.00	Res
49	LA11242-51	5	4	6	6	6	4	5.17	Susc
176	LA11242-187	5	4	4	7	3,7	6	5.20	Susc
21	LA11242-22	4	5	7	5	7	5	5.50	Susc
38	LA11242-39	6	4	4	6	7	6	5.50	Susc
131	LA11242-137	6	5	6	4	7	5	5.50	Susc
56	LA11242-58	4	4	8	6	5	7	5.67	Susc

Table 4 Stripe rust ratings over three locations. *contd.*

36	LA11242-37	4	5	7	7	7	5	5.83	Susc
92	LA11242-95	7	6	7	4	6	6	6.00	Susc
124	LA11242-129	6	5	6	6	7	7	6.17	Susc
165	LA11242-176	7	4	7	7	6	6	6.17	Susc
175	LA11242-186	6	5	7	6	7	6	6.17	Susc
12	LA11242-12	5	6	7	6	8	6	6.33	Susc
162	LA11242-173	7	7	7	4	6	7	6.33	Susc
45	LA11242-47	6	6	5	6	8	8	6.50	Susc
52	LA11242-54	8	7	3	6	8	8	6.67	Susc
170	LA11242-181	7	8	4	7	8	6	6.67	Susc
187	LA11242-198	6	6	8	6	8	6	6.67	Susc
53	LA11242-55	8	6	5	6	8	8	6.83	Susc
43	LA11242-45	7	7	5	7	8	8	7.00	Susc
20	LA11242-21	7	7	8	7	8	7	7.33	Susc
110	LA11242-114	7	6	8	7	8	8	7.33	Susc
128	LA11242-133	8	8	6	6	8	8	7.33	Susc
55	LA11242-57	8	8	7	6	8	8	7.50	Susc
60	LA11242-62	8	8	7	5	9	8	7.50	Susc
1	LA11242-1	4	2,6	2,6	1,5	2,6	6	Het	Het
3	LA11242-3	3	2,6	1,5	1,4	6	6	Het	Het
4	LA11242-4	2,5	3	1,4	1,5	6	2,7	Het	Het
5	LA11242-5	3,7	2	6	1,5	2,6	5	Het	Het
7	LA11242-7	2,6	5	7	4	3,7	3,8	Het	Het
9	LA11242-9	1,5	1,5	4,8	3,7	2,6	2,7	Het	Het
13	LA11242-13	2,6	1,6	6	3	7	3,7	Het	Het
14	LA11242-14	1,5	2,5	2,7	5	2,7	1,5	Het	Het
15	LA11242-16	1,5	1,4	1,4	1,5	2,6	1,4	Het	Het
16	LA11242-17	2,5	2,5	6	2,6	6	2,7	Het	Het
17	LA11242-18	2	1,4	1,5	1,7	1,4	2,6	Het	Het
18	LA11242-19	5	2,6	6	6	2,7	1,5	Het	Het
22	LA11242-23	1,5	1,6	4	1,5	3,8	2,6	Het	Het
23	LA11242-24	3,7	2,8	5	6	2,7	1,7	Het	Het
25	LA11242-26	1,5	3	1,5	1,7	1,5	1,5	Het	Het
27	LA11242-28	1,5	6	6	6	2,7	3,7	Het	Het
29	LA11242-30	1,6	1	6	3	1,5	2,6	Het	Het
30	LA11242-31	1,4	1,5	3,8	4	2,6	3,7	Het	Het
32	LA11242-33	1,5	1,5	2	3	4	2,6	Het	Het
35	LA11242-36	3	2	7	6	3,7	4,8	Het	Het
39	LA11242-40	2,7	2,5	6	3	2,7	2,7	Het	Het
40	LA11242-41	3	1,4	1,5	2	2,7	1,6	Het	Het

Table 4 Stripe rust ratings over three locations. *contd.*

41	LA11242-42	3	1,4	5	2,5	2,5	2,6	Het	Het
42	LA11242-43	6	2	2	4	2,6	2,7	Het	Het
48	LA11242-50	4	2,7	1,5	1,5	2,5	6	Het	Het
61	LA11242-63	2,6	1,5	1,6	2,5	2,6	1,7	Het	Het
65	LA11242-68	1,4	2	1,5	4	2,6	5	Het	Het
73	LA11242-76	1,4	2,6	6	6	3	3,7	Het	Het
75	LA11242-78	3	2,5	2,6	2	7	1,5	Het	Het
76	LA11242-79	1,5	1,4	5	1,5	3	1,7	Het	Het
77	LA11242-80	4	2	1,6	4	7	7	Het	Het
78	LA11242-81	1,4	1,5	1,6	1,4	2,7	2,6	Het	Het
80	LA11242-83	2	2	1,4	2,5	2,6	2,6	Het	Het
81	LA11242-84	2	3	2	2,6	2,6	3,7	Het	Het
86	LA11242-89	2,6	1,5	2,7	2,6	1,7	5	Het	Het
87	LA11242-90	1	3	1,4	1,5	2,5	2,6	Het	Het
90	LA11242-93	3	2,6	2,7	1,5	1,4	2,6	Het	Het
98	LA11242-101	2	2	1,5	2,6	3,7	7	Het	Het
99	LA11242-103	1,5	1,6	4	4	1,6	6	Het	Het
100	LA11242-104	1,5	1,5	1,4	1,5	4	3,7	Het	Het
101	LA11242-105	4	2	2,7	1,4	7	2,6	Het	Het
106	LA11242-110	4	2,7	6	7	3,7	6	Het	Het
107	LA11242-111	1	1,6	1,4	1,5	2,7	2,6	Het	Het
108	LA11242-112	3	2,5	2,5	1,5	1,6	1,7	Het	Het
112	LA11242-117	2,5	2,5	6	3	6	3,7	Het	Het
113	LA11242-118	1,4	3	1,4	1,5	2,6	2,6	Het	Het
115	LA11242-120	3	2,5	6	2	6	2,7	Het	Het
116	LA11242-121	6	2,6	6	8	1,6	2,5	Het	Het
117	LA11242-122	1,4	1	2,5	2,5	2,5	1,4	Het	Het
119	LA11242-124	1,4	1,5	1,6	1,6	2,6	6	Het	Het
120	LA11242-125	1,5	3	2,5	1,4	2,7	2,6	Het	Het
122	LA11242-127	2,5	3,6	3	4	2,6	2,5	Het	Het
123	LA11242-128	1,5	1	1	2	2,6	1,5	Het	Het
126	LA11242-131	1,6	2,5	4	1,5	3,7	2,5	Het	Het
127	LA11242-132	4	2,6	1,5	5	2,7	2,6	Het	Het
129	LA11242-135	7	7	5	5	3,7	2,8	Het	Het
132	LA11242-138	2	1,6	1,5	2,6	2	3	Het	Het
133	LA11242-139	3	3,7	2	1	2,5	1,5	Het	Het
134	LA11242-140	1,5	2,7	3	2	1,5	2	Het	Het
135	LA11242-141	1,4	1,5	1,4	1,4	1,5	1,6	Het	Het
138	LA11242-144	1,4	4	6	6	3,7	3,6	Het	Het
139	LA11242-145	1,4	5	2,5	2,5	5	3,6	Het	Het

Table 4 Stripe rust ratings over three locations. *contd.*

140	LA11242-147	2,5	1,6	1,4	1,5	3	4,8	Het	Het
141	LA11242-148	2,6	5	2,6	1,4	3,7	3,7	Het	Het
143	LA11242-151	2,7	3,7	1	6	7	2,7	Het	Het
144	LA11242-152	3	2,6	6	2,6	4	2,7	Het	Het
147	LA11242-156	1	1	1,5	1,4	1,5	1,7	Het	Het
148	LA11242-157	2	1,5	2,7	3	1	2,7	Het	Het
149	LA11242-158	1,6	1,7	1,4	1,5	2,6	1,4	Het	Het
152	LA11242-162	2	1,5	1,6	1,4	2,7	2,6	Het	Het
154	LA11242-164	7	1,5	3,7	5	2,7	3,7	Het	Het
155	LA11242-166	3,8	2,5	1,5	6	3,7	3,7	Het	Het
159	LA11242-170	3,7	1,5	7	4	3,7	2,7	Het	Het
160	LA11242-171	1,5	2	1	1,4	2,7	3	Het	Het
163	LA11242-174	2,6	2	3	4	7	4,7	Het	Het
164	LA11242-175	1,5	2	1,4	1,4	1,5	1,6	Het	Het
166	LA11242-177	4	1	5	2,5	4	3,7	Het	Het
167	LA11242-178	3	1	1,4	3	1,5	2,5	Het	Het
168	LA11242-179	1,5	2,5	7	3,7	2,6	4	Het	Het
171	LA11242-182	2,6	2	5	3	2,6	5	Het	Het
172	LA11242-183	1,7	3	1,4	1,4	6	2,7	Het	Het
174	LA11242-185	1,5	2,6	2,6	2	3,7	2,6	Het	Het
177	LA11242-188	1,5	3	6	4	1,5	2,5	Het	Het
178	LA11242-189	2,5	2,6	2	1,5	2,6	3	Het	Het
180	LA11242-191	1,6	1	1,5	1	2	2,6	Het	Het
182	LA11242-193	3,6	3	7	2	3,7	2,6	Het	Het
185	LA11242-196	1	1	1,6	1,5	1,6	2	Het	Het
188	LA11242-199	2,6	2,7	8	3	3,8	2,5	Het	Het

Ratings from a 0-9 scale: 0= Resistant, no symptoms; 9= highly susceptible.

Ratings taken at adult stage when symptoms were clearly visible (between March and April 2012).

Lines with two ratings per rep are segregating for stripe rust resistance.

Means stating 'Het' indicate entries segregating for stripe rust resistance.

Analysis of variance (ANOVA) test were performed, using the general linear model, for the disease rating obtained from each location. For the individual location analysis reps were treated as blocks and only lines which were not segregating (heterozygous) for resistance were used. A total of 101 entries were used in the analysis. The tables for the ANOVA are presented below.

Table 5

ANALYSIS OF VARIANCE					
Winnsboro					
LA					
Variable: Stripe Rust rating					
Source	df	SS	MS	F-value	Pr> F
Total	203	1090.706			
BLOC	1	2.373	2.373	3.95	0.0495
ENTRY	101	1027.706	10.175	16.95	0.0000
Residual	101	60.627	0.600		
Grand mean = 3.235 C.V. = 23.95% LSD for ENTRY = 1.2862					

Table 6

ANALYSIS OF VARIANCE					
Plains					
GA					
Variable: Stripe Rust rating					
Source	df	SS	MS	F-value	Pr> F
Total	203	927.980			
BLOC	1	0.961	0.961	1.35	0.2485
ENTRY	101	854.980	8.465	11.87	0.0000
Residual	101	72.039	0.713		
Grand mean = 2.657 C.V. = 31.79% LSD for ENTRY = 1.4020					

Table 7

ANALYSIS OF VARIANCE					
Fayetteville					
AR					
Variable: Stripe Rust rating					
Source	df	SS	MS	F-value	Pr> F
Total	203	980.602			
BLOC	1	3.982	3.982	9.77	0.0023
ENTRY	101	935.477	9.262	22.74	0.0000
Residual	101	41.143	0.407		
Grand mean = 2.527 C.V. = 25.26% LSD for ENTRY = 1.0595					

Based on results from the ANOVA tables, there was a highly significant difference in entries for all locations in the study. This indicates the population was segregating for stripe rust resistance. Also observed was a significant difference in block effect at Winnsboro and Arkansas, but not at Georgia. The highest mean rating for stripe rust incidence was found at Winnsboro and while the lowest was at Fayetteville. Coefficients of variation varied from 24 to 32% which can be considered normal for visual ratings of a disease.

3.2 DNA Polymorphism

A total of 216 polymorphic markers were obtained between the two parents with both SSR and AFLP primers. Polymorphism was low with the SSRs; only 63 of the 501 (12.5%) SSRs tested were polymorphic. Out of the 128 AFLP primer combinations tested, 13 combinations showing high polymorphism between parents were selected for genotyping of the mapping population. A total of 153 polymorphic AFLP markers were scored. The number of polymorphic markers for each AFLP primer combination ranged from 3 to 12.

3.3 Linkage Groups

A total of 24 linkage groups (LG) were obtained with the 216 polymorphic markers (SSR and AFLP) with a LOD threshold of 4 (Figure 1). The chromosomes represented in the LG were 1A, 2A, 4A, 2B, 3B, 5B, 7B, and 3D. The remaining 16 LG were made up of only AFLP markers with unknown locations. The number of markers per LG ranged from two to eight. The largest LG had a size of 74 cM while the smallest was only 5 cM.

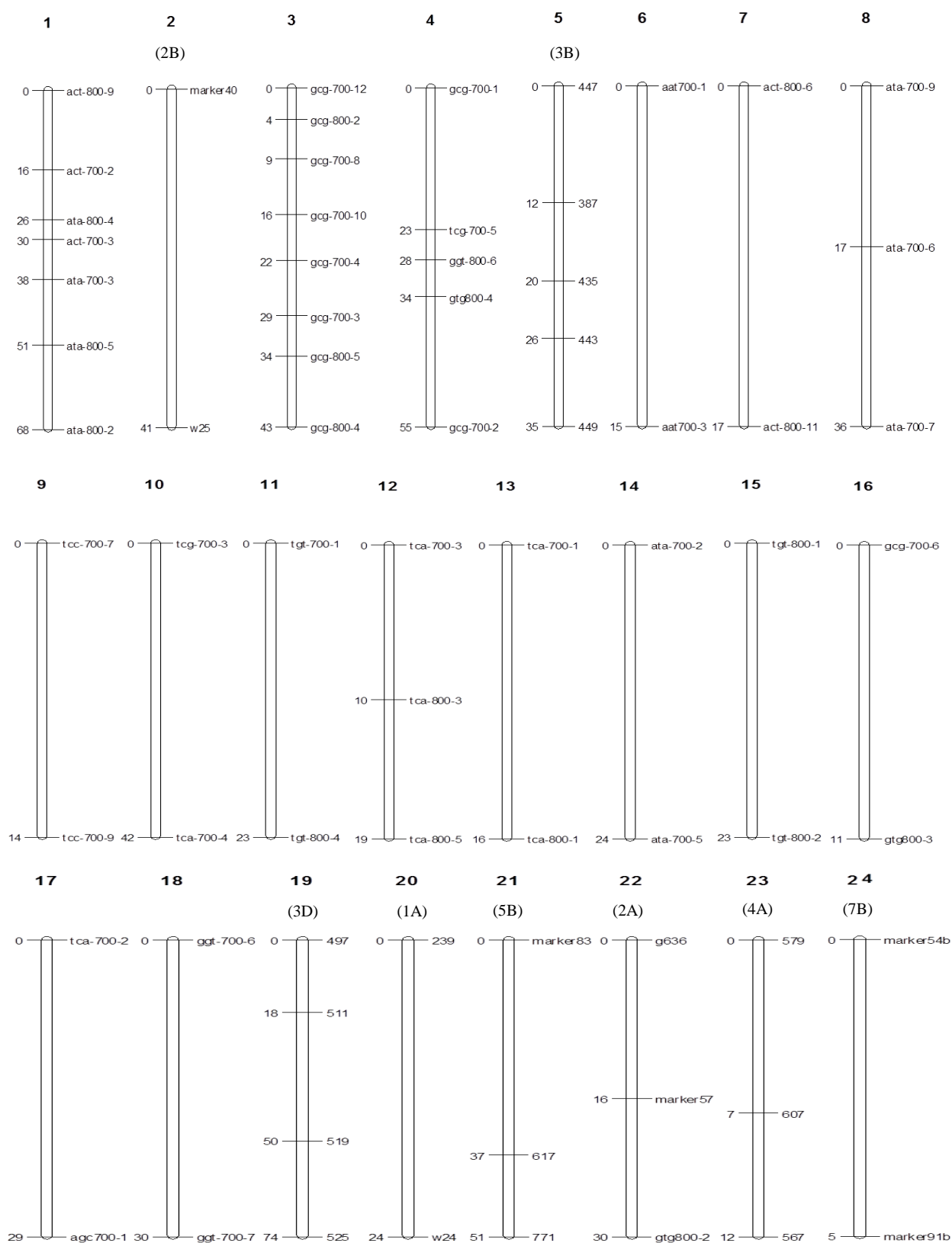


Figure 1. Graphical representation of the 24 LG and the chromosome it represents in parenthesis. Distance in cM.

3.4 QTL Mapping

Three QTL were found to control stripe rust resistance with a LOD value of 2.5 or greater (Figure 2). The three QTL were distributed over two LGs (chromosomes). Two QTL were found in Chr 2A (LG6) while Chr3 B (LG2) contained the third QTL. The rest of the LGs did not contain any QTL with a LOD values greater than or equal to 2.5. The three QTL together accounted for 43.2% of the total phenotypic variation in stripe rust resistance.

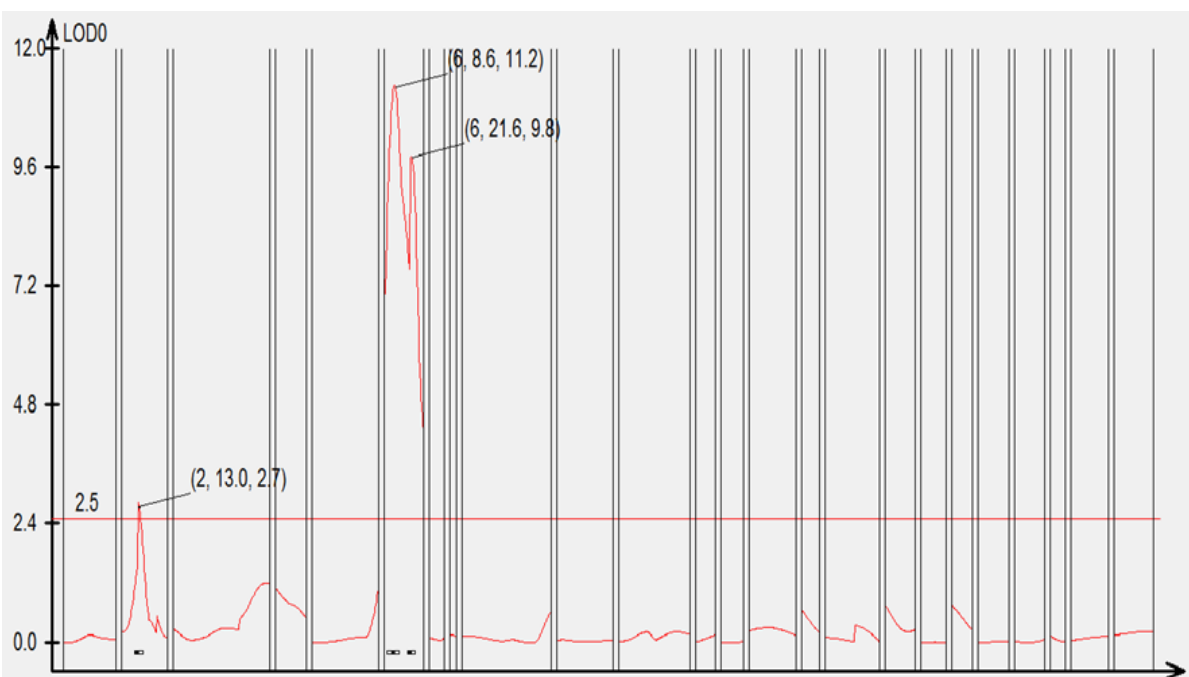


Figure 2. Chart showing the three QTL peaks above the 2.5 threshold LOD. The numbers in parentheses correspond to linkage group, percentage of phenotypic variation explained, and LOD value.

One of the QTL (*Yr841_3B.1*) was found in LG 2, which corresponded to Chr3 B (Figure 3), which was 35 cM long and contained 5 SSR markers (BARC164, BARC1044, BARC1111, BARC1103, BARC187). *Yr841_3B.1* had a LOD value of 2.7 and explained 13.0% of the total

phenotypic variation for stripe rust resistance. The length of the QTL is 4 cM long, while the peak is 12 cM from the edge of the LG.

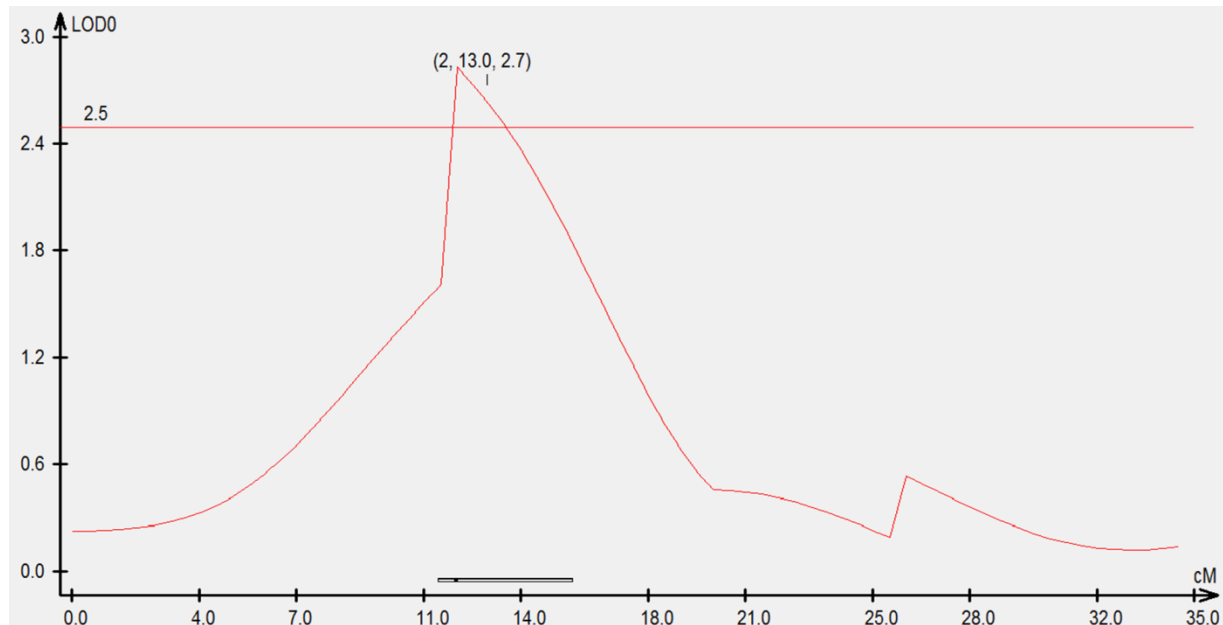


Figure 3. Cartogram showing the *Yr841* 3B.1 peak in chromosome 3B. The numbers in parenthesis correspond to linkage group, percentage of phenotypic variation explained, and LOD value.

The other two QTL were found in LG 6 which corresponded to Chr2A. This linkage group was 30 cM long and contained two SSR markers and one AFLP marker (Xgwm636, Xgwm359, gtg800-2). The QTL peaks were 13 cM apart. The first of the two QTL (*Yr841_2A.1*) accounted for 8.6% of the total phenotypic variation for stripe rust resistance. The QTL had a LOD value of 11.2, which was the highest out of all three QTL identified. The QTL is 7 cM long. The second QTL (*Yr841_2A.2*) found in Chr 2A with a LOD of 9.8 accounted for 21.6% of the trait phenotypic variation, which was the highest of the three QTL in the present study. This QTL is 4 cM long.

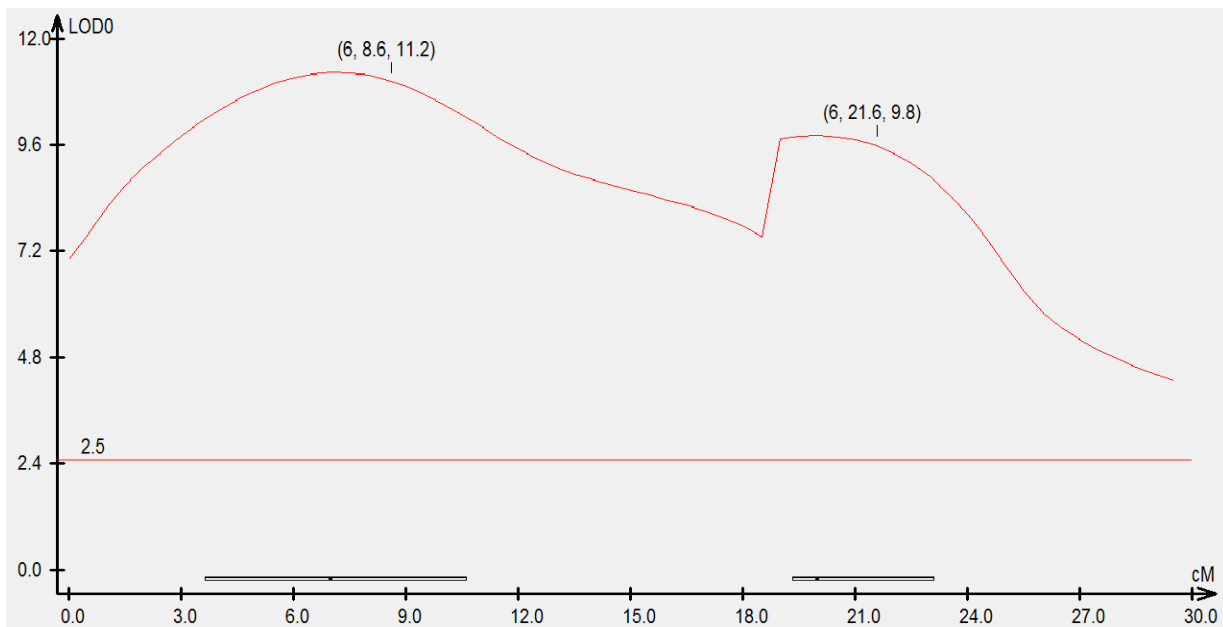


Figure 4. Cartogram showing two QTL peaks (*Yr841_2A.1* and *Yr841_2A.2*), found in Chr-2A (LG-6). The numbers in parenthesis correspond to linkage group, percentage of phenotypic variation explained, and LOD value.

3.5 Discussion

QTL mapping is considered a highly effective approach for studying genetically complex traits such as plant disease resistance (Young, 1996). The role and influence of specific resistant loci can be determined with QTL mapping. QTL mapping is considered an initial step to developing specific markers, which can be used in breeding programs for selection and advancement of lines. QTL mapping also provides an opportunity for QTL-assisted breeding for complex disease resistance traits and positional cloning of partial resistance genes. Therefore, the success of QTL (marker)-assisted selection in breeding depends on adequate prediction models and sufficient number of markers for trait phenotypes (Cooper *et al.*, 2009).

Disease Rating

Traits expressed on a continuous scale are considered quantitative. This continuous variation of a trait is usually caused by the segregation of various genes which have an effect on

the gene. Quantitative resistance slows down the development of an epidemic, in several ways, which reduces the severity of the disease. Resistance from the host on the disease can be attributed to effects on latent period, infection efficiency, spore production, and other epidemiological traits (Geiger and Heum, 1989). Our trait of interest, stripe rust resistance, is considered a quantitative trait.

After reviewing our field ratings for the trait we noticed they did not behave in a typical Mendelian fashion. The lines did not segregate in the traditional 1:2:1 ratio expected in $F_{2:3}$ lines. This is probably due to the presence of several genes for resistance. Young (1996) stated that “most complex resistance traits (i.e. resistance traits that cannot be fitted to simple Mendelian ratios) are controlled by multiple loci. These resistance phenotypes tend to be measured quantitatively”. We also found there were more resistant lines than susceptible ones. This could be explained by the multiple QTL that were found which increases the chances of having some level of resistance.

There are significant differences in stripe rust reaction among lines (Tables 5,6,7). Average stripe rust ratings over three locations (Table 4) of the lines ranged from 0.67 to 7.50. Stripe rust was present at high levels as shown by the number of high ratings (≥ 7) in each of the three environments. There are seventy-eight lines with mean ratings of ≤ 4.0 and 24 lines with ≥ 5.0 . Approximately $\frac{1}{2}$ (88/190) of the lines were classified as heterozygous as $F_{2:3}$ lines in the field.

The ratings of lines across environments were pretty consistent with a few exceptions. For example, line 82 (LA11242-85) has a mean of 1.5 in Arkansas and Georgia, but a mean of 4.5 in Louisiana. Line 169 had a mean rating of 3.5 in Arkansas versus 2.0 in Louisiana. The

average rating in Louisiana for all non-heterozygous lines was 3.23 compared to 2.53 and 2.66 for Arkansas and Georgia, which indicates that disease pressure was similar, so differences in ratings is probably a function of virulence differences across environments.

The classification of lines as resistant or susceptible is somewhat arbitrary. The lines with consistent ratings of '1' or '2' probably have at least one major gene that is effective in that environment. Those lines with '7', '8', and '9' ratings probably do not have any effective major genes. Those lines with intermediate reactions probably have at least one gene present that provides partial resistance to the races present.

DNA Polymorphism Discussion

Marker polymorphism in wheat is reportedly lower than in other major crops such as corn and soybean. Studies have shown that SSR marker polymorphism in corn (51.8%) and soybean (50.7%) is over 50% (Sa *et al.*, 2012; Singh, *et al.*, 2010), whereas it is around or even below 40% in wheat (37.5%) (Cregan, 2012). Common hexaploid wheat also has extremely low levels of polymorphism at DNA marker loci compared to its parent species, especially *Aegilops squarrosa* (Nishikawa *et al.*, 1980). An average of 3.4 alleles was found in 20 RFLP loci in *A. squarrosa* while only 1.1 alleles on average were found for the same RFLP loci in a sample of common hexaploid wheat. The low polymorphism in wheat can be attributed to the findings that the worldwide gene pool of cultivated wheat comes from a small number of interspecific hybrids (Lubbers *et al.*, 1991).

The number of polymorphic markers found in the cross between NC06BGTAG12 and LA 841 (12.5%) was lower than reported for wheat. This could be due to the fact that both varieties share common ancestors, such as Coker-68-15, Norin-10 and Brevor in their pedigree.

Linkage and QTL Mapping Discussion

The number of LGs (24) obtained in the present study is similar to the number produced in other wheat mapping experiments. Messmer *et al.*, (1999) found 23 LGs using F5 recombinant inbred lines derived from a cross between Swiss winter wheat and Swiss winter spelt.

The QTL found in Chr 3B (*Yr841_3B.1*) was flanked by markers BARC164 and BARC1044. However the peak was almost directly aligned with the marker BARC1044 whose physical location according to GrainGene is 115.5 cM. When looking at the consensus map of stripe rust QTL (Rosewarne, *et al.*, 2013) there is no QTL in this location so *Yr841_3B.1* is potentially a new source of resistance. However there are other resistances QTL in this chromosome (Rosewarne, *et al.*, 2013). Further fine mapping of the previously uncharacterized *Yr841_3B.1* QTL must be conducted to confirm if it is not linked to other previously known markers. Other previously discovered BARC markers in this chromosome include Xbarc87, 133, and 147 (Rosewarne, *et al.*, 2013). However none of these markers are close to *Yr841_3B.1* found in LA841.

In Chr 2A, two QTL were discovered. The QTL with a LOD value of 11.2 (*Yr841_2A.1*) was closely located to SSR marker Xgwm 636 whereas the QTL with an LOD value of 9.8 (*Yr841_2A.2*) was closely flanked by SSR marker Xgwm 359. Both SSR markers were located in the short arm of Chr-2A. Both of these markers have been discovered to be linked to stripe rust resistance QTL in other varieties.

Yr841_2A.2 proximal to marker Xgwm 359 could possibly represent *Yr₆₁*. Marker Xgwm 359 was discovered to be flanking a previously discovered QTL known as *Yr₆₁* in Pioneer variety 26R61. This locus, designated *QYr.uga-2AS*, was detected in the *QYr.ufs-2A* interval in variety

26R41 (Hao, *et al.*, 2011). It explained 56 % of the phenotypic variation in 26R61 making it a major QTL. LOD values of up to 24.5 were detected for this QTL when 26R61 was tested in Plains, GA in 2009 (Hao, *et al.*, 2011). *Yr841_2A.2* with a LOD of 9.8 explained 21.6% of the phenotypic variation. *Yr₆₁* and *Yr841_2A.2* in LA841 are both located in the same region of the short arm in Chr2A and flanked by the same marker (Xgwm359). *Yr₆₁* was assigned to the distal 22% of the short arm of wheat Chr2A (Hao, *et al.*, 2011). Both varieties (LA841 and 26R61) have common ancestors in their pedigree from soft red winter lines found in the southeast such as Coker-68-15, suggesting the similarities in their genetic background.

The SSR marker Xgwm359 has been validated for the region QYr.uga-2AS with preliminary test of two cultivars and two advanced lines. The test results indicated that the QTL was present in the material, and was consistent with stripe rust resistance responses found in the field. The present study and previous reports indicate that Xgwm359 could be used in marker assisted selection for stripe rust resistance (Hao, *et al.*, 2011).

The other QTL (*Yr841_2A.1*) in Chr-2A short arm, closest to SSR marker Xgwm636 with a LOD of 9.8, could represent the previously characterized stripe rust resistance gene *Yr₁₇*. SSR marker Xgwm636 and *Yr₁₇* are found in the same region of the short arm in Chr-2A and have been declared linked (Jahier, *et al.*, 2011). *Yr₁₇* is the only formally known *Yr* gene found in this specific chromosome region. Through previous screening, it is known that LA841 carries *Yr₁₇* gene, which provides resistance to some stripe rust races. So it is likely that *Yr841_2A.1* in Chr2AS is *Yr₁₇*. *Yr₁₇* is distinct from *Yr₆₁* found in 26R61, which is also located in the short arm of chromosome 2A. This is known because the variety 26R61 has tested negative for *Yr₁₇* when screened with linked markers Xgwm636 and VENTRIUP/LN2 (Hao, *et al.*, 2011; Agenbag, *et*

al., 2012). Further finer mapping is necessary to determine the exact position of these QTL and discover markers inside/flanking the region that could be used for marker assisted selection.

3.6 Conclusions

- Resistance to stripe rust in wheat is a complex quantitative trait, primarily controlled by many genes with major effects.
- *Yr₁₇* (*Yr841_2A.1*) still provides resistance to some of the stripe rust races in the region. This QTL had the highest LOD value of all in the present mapping population.
- The gene *Yr₆₁* previously discovered in 26R61 appears to be found in Terral LA841. *Yr841_2A.1* was considered a major QTL in this case, explaining over 20% of the phenotypic variation, the highest of the three QTL found in the present study.
- The QTL found in chromosome 3B (*Yr841_3B.1*) was not previously described in other varieties. It potentially presents a new source of resistance which can be pyramided with other previously identified genes in new varieties.
- The three QTL identified accounted for 43.2% of the total phenotypic variation for stripe rust resistance. The results imply that there are additional QTL in LA841, which can further be fine mapped with a large number of markers to identify the markers tightly linked to the resistance gene.



Figure 5. Picture of wheat variety Terral LA841

<http://www.terralseed.com/Portals/0/downloads/2012-Terral-proof-9-26.pdf>

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Vita

Alejandro Castro Aviles was born in Tegucigalpa, Honduras in March of 1988. He graduated from the Mazapan School, a bilingual school in La Ceiba, Honduras in June of 2006. He entered Zamorano Pan-American Agricultural School in January of 2007. During the spring semester of 2010 he traveled to participate in an internship in the Department of Plant Pathology and Crop physiology at LSU, where he worked in the plant disease diagnostic laboratory. In December 2010, he completed his undergraduate degree in agronomical sciences and production from Zamorano. During his time as an undergraduate student he developed an interest for plant breeding and genetics so he decided to return to Baton Rouge to further his study of plant breeding and pursue a graduate degree. In 2011 he joined the LSU AgCenter wheat and oat breeding program working for Dr. Stephen Harrison. He is expected to graduate with a Master of Science degree in Agronomy from the School of Plant, Environmental, and Soil Sciences in December, 2015. After he completes he's master's degree he will continue his research as a Ph.D. student.