

MAPPING STEM RUST RESISTANCE GENES IN 'KINGBIRD'

by

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Abstract

Stem rust, caused by the fungus *Puccinia graminis* f. sp. *tritici*, has historically been one of the most important diseases of wheat. Although losses have been much reduced in the last fifty years, new highly virulent races of the pathogen have recently emerged in East Africa. These new races are virulent on nearly all of the currently deployed resistance genes and therefore pose a serious threat to global wheat production. The spring wheat variety 'Kingbird' is thought to contain multiple quantitative trait loci (QTLs) that provide durable, adult-plant resistance against wheat stem rust. Stem rust-susceptible Kansas winter wheat line 'KS05HW14' was backcrossed to Kingbird and 379 recombinant lines were advanced to BC₁F₅ and then increased for testing. The lines were screened for stem rust resistance in the greenhouse and field in Kansas and in the field in Kenya over multiple years. We identified 16,237 single nucleotide polymorphisms (SNPs) with the Wheat 90K iSelect SNP Chip assay. After filtering for marker quality, linkage maps were constructed for each wheat chromosome. Composite interval mapping and multiple-QTL mapping identified seven QTLs on chromosome arms 2BL, 2DS, 3BS, 3BSc, 5DL, 7BL, and 7DS. Six QTLs were inherited from Kingbird and one QTL on 7BL was inherited from KS05HW14. The location of the QTL on 2BL is approximately at locus *Sr9*, 3BS is at *Sr2*, 3BSc is at *Sr12*, and 7DS is at *Lr34/Yr18/Sr57*. Although no QTL was found on 1BL, the presence of resistance gene *Lr46/Yr29/Sr58* on 1BL in both parents was indicated by the gene-specific marker *csLV46*. QTLs on 2DS and 5DL may be related to photoperiod or vernalization genes. Pairwise interactions were only observed with race QFCSC, most notably occurring with QTLs 2BL and 3BSc. These results confirm that there are multiple QTLs present in Kingbird. Ultimately, the identification of the QTLs that make Kingbird resistant will aid in the understanding of durable, non-race-specific resistance to stem rust of wheat.

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Dedication

I would like to dedicate this thesis to my loving fiancé and wonderful family, for their endless support and encouragement throughout this entire journey.

Chapter 1 - Literature Review

Introduction

Stem rust (*Puccinia graminis* Pers.:Pers. f. sp. *tritici* Erikss. & E. Henning) has devastated farmers' wheat fields for centuries. Although the effects of the disease are not often as detrimental as they once were in the United States, stem rust is still prevalent in many fields across the world today. With the rise of extremely virulent races of stem rust in Africa, awareness and concern for the pathogen has increased over the years and is a current topic of interest for researchers and growers. In the United States, stem rust causes less than 1% yield loss annually, while in some African countries there is a potential yield loss of up to 80%. These virulent races, specifically TTKSK, also named Ug99, are posing a great threat to world-wide wheat production. This is primarily due to the fact that TTKSK is virulent to almost 80% of the world's wheat varieties. The threat of these virulent strains coming to the United States, or other major wheat producing countries, is the reason that better resistance strategies for this pathogen are a necessity.

Wheat History and Cultivation

Wheat has been cultivated for millennia and is a significant food source for many individuals. The cultivation and evolution of wheat has gone hand-in-hand with the advancement of civilization. Wheat today is the product of years of selection and that continues to evolve along with society.

The place of origin for wheat is thought to have been near the Euphrates and Tigris rivers in the Middle East, but wheat is currently cultivated world-wide (Roelfs, Singh, Saari, & Broers, 1992). The domestication of wheat occurred thousands of years ago during the Neolithic Revolution, which was the transition from a hunter-gatherer society to a more sedentary one. The

revolution was primarily due to the cultivation of plants and the domestication of animals. Initial cultivation began about 10,000 years ago and was developed with wheat cultivars that were diploid and tetraploid. This then led to the development of the hexaploid wheat known today, which occurred about 9,000 years ago (Shewry, 2009). Domestication of wheat began from farmers taking wild types of wheat and selecting for desirable attributes. The most significant attributes were traits such as non-shattering, higher yield and better grain quality (Nesbitt, 2001). Selecting with the naked eye is still an important aspect of wheat improvement programs today, and is often used in conjunction with molecular techniques.

Wheat is primarily self-pollinated and is a member of the grass family *Poaceae*. Wheat is most easily grown in temperate climates. Countries that have regions for significant wheat production are: The United States, countries of the former Soviet Union, African countries, Canada, those in the Mediterranean Basin, China, India, Argentina, Brazil, Great Britain, Germany and Australia (Gustafson, Raskina, Ma, & Nevo, 2009). There are different characteristics of wheat grown throughout the world. The major characteristics are: red or white, hard or soft, and winter or spring. The most common class of wheat that is grown in the United States is hard red winter wheat. This is also the primary wheat grown in the state of Kansas.

Genetic Structure of Wheat

Wheat that is grown today is primarily hexaploid (*Triticum aestivum* L.), but some varieties are tetraploid (*Triticum turgidum* L. subsp. *durum* (Desf.)Husn.). Tetraploid wheat, or durum wheat, is often used for pasta and makes up about 5% of the world's wheat production. It is grown in North Dakota, Arizona, California and Montana in the United States. In contrast, hexaploid wheat has a higher gluten content and is used in the production of bread. It accounts

for about 95% of the world's wheat production and is the primary wheat grown in the United States (Shewry, 2009).

Tetraploid wheat contains 14 pairs of chromosomes ($2n = 4x = 28$) consisting of two copies of two genomes: AABB. Each genome consists of 7 chromosomes (Evans & Peacock, 1981). This genome arrangement first began with a hybridization of a *T. urartu* (AA) and *Aegilops speltoides* (SS genome, which is most closely related to the BB genome) which resulted in *T. turgidum* (AABB). *T. turgidum* was eventually hybridized with the wild diploid wheat *Aegilops tauschii* (DD) and ultimately formed the hexaploid wheat known today (Nesbitt, 2001). Hexaploid wheat contains 21 pairs of chromosomes ($2n=6x=42$, AABBDD). The combining of these three genomes is thought to have occurred around 8,000 years ago (Huang et al., 2002).

Economic Importance of Wheat

Wheat serves as an important food crop for many reasons. It is the third highest cereal produced world-wide behind rice and maize. Wheat is processed fairly easily, and lasts in storage for years; so long as the water content is below 15% (Shewry, 2009). The nutritional content of wheat primarily consists of starch. It contains about 60-70% starch in whole grain and 65-75% in white flour. The protein content is fairly low, ranging from about 8-15%, but is still a significant source of nutrients for many people. Aside from the primary composition of wheat being starch and protein, it also contains other important nutrients. Bread offers a person up to 15% of their daily iron and 11% of their daily zinc (Shewry, 2009). Wheat makes up an important part of many people's diets. It is a heavily relied upon source of calories, especially in countries where their main protein consumption comes from plants.

Besides its importance nutritionally, wheat production is an enormous industry. According to the USDA-ARS, the United States alone produced 2.05 billion bushels in 2015.

Each bushel ranged in price from \$4.90-\$5.10. The world's wheat production exceeded 7.3 billion bushels in the year of 2015 (including the United States). Billions of bushels are produced annually in this multi-billion dollar industry. This is why protecting the crop's yield from biotic and abiotic stressors will ultimately protect farmers and industry investment, along with helping feed the world.

Wheat is a high value crop that has many biotic and abiotic stressors that reduce yield, ultimately diminishing investment return. Economic losses to stem rust are primarily due to fewer and smaller kernel size, overall reduction of vigor, and reduced tiller number. The reduction in quality limits the possible uses for the wheat, ultimately lowering food value and milling capacity (Agrios, 2005). According to the USDA-ARS, losses to stem rust in the U.S. for 2013 were less than 1%. This is a small number but still significant. In 2015, the U.S. produced 2.05 billion bushels and with a 1% decrease would equate to a loss of about 20 million bushels. Although this seems to be an incredible loss, the races in the U.S. are not nearly as virulent as those in Africa. Ug99 races have caused great devastation to wheat production in countries where wheat serves as a primary source of nutrition. As of 2011, losses of up to 80% have been reported in countries such as Kenya and Uganda (Roy, Majumder, & Datta, 2011).

Stem Rust History

Stem rust was incredibly feared by farmers, due to the fact that a severe stem rust outbreak had the potential to ruin entire crops in as little as three weeks (Ravi P. Singh et al., 2008). The devastation of stem rust has been referred to in The Bible and also plagued the ancient Romans and Greeks. It has even been mentioned by prominent writers, such as Aristotle (Roelfs et al., 1992). The Romans made sacrifices to a god named Robigo, which was known as

the god of rust. Although they did not know much about the pathogen, they were aware that the orange, rust-like pustules on the plants were the reasons for their poor harvests (Roelfs et al., 1992). Unfortunately for them, sacrifices to Robigo did not help and many crops were lost to the devastating pathogen.

Today, stem rust is still an issue in the United States and the world. In the early 20th century, the disease took up to one-fifth of the harvest in the United states, over several epidemics (Sharma, 2012). Since the 1950's though, it has come under much better control due to the eradication efforts of its alternate hosts, common barberry (*Berberis vulgaris*), other *Berberis*, *Mahoberberis* and *Mahonia spp.* in the U.S. The eradication of barberry has removed the sexual stage of the fungus in northern states, limiting infection sources to only urediniospore dissemination via wind from southern states such as Texas or from Mexico (Kolmer, Jin, & Long, 2007). Recently, however, there has been the discovery of new races that possess a great threat if they spread to the United States.

Stem Rust Classification

In 1767, Fontana made the first drawings of *P. graminis* on the grass host (Roelfs et al., 1992). *P. graminis* is in the phylum *Basidiomycota* and has a very broad host range. The pathogen is able to infect at least 365 species of grass and cereals. It is known to infect primarily grains such as wheat, oats, barley and rye. Stem rust is an obligate parasite and cannot live as a saprophyte. This makes culturing impossible without live tissue. As a pathogen, it has a very complex life cycle. It is heteroecious which means it has a primary host (grass) and an alternate host (barberry). Aside from the complexity of it having two hosts, it is also macrocyclic (Ravi P. Singh et al., 2008). Stem rust goes through five spore stages to complete its lifecycle: teliospore, basidiospore, spermatia, aeciospore, and urediniospore. Since the eradication of barberry in the

United States, stem rust only goes through the urediniospore, basidiospore and teliospore stage. However, the eradication efforts for barberry are no longer in effect, which rekindles the possibility of the sexual stage occurring if barberry plants become prevalent again.

Symptoms and signs

Stem rust symptoms can greatly reduce vigor in the grass host. Generally, it reduces yield and quality of plants that are infected. Initial indicator symptoms are often chlorotic spots on the leaf sheath and stems. The level of resistance the plant has to stem rust directly correlates with infection severity. On a plant that is susceptible, urediniospores will develop. These pustules are orange, often in a diamond-shaped orientation, ranging from 1-10 mm in length (Leonard & Szabo, 2005). This visible manifestation of the fungus at the uredinial spore stage gives the pathogen its name. Another diagnostic sign for stem rust is the shiny black teliospores that are produced at the end of the growing season. These black spores are the reason for stem rust's alternate name, black rust.

Symptoms and signs of stem rust are not just limited to the grass host, they also develop on the alternate host, barberry. Some symptoms are similar to those that develop on the grass host, such as chlorotic leaf tissue, but it mainly differs in the signs that are present (Webster & Weber, 2007). The barberry host is where the spermogonium and aecium develop. These are fungal structures that are strictly produced on the barberry host. Spermogonia are flask-shaped and located on the tops of barberry leaves while aecia are on the bottom of leaves and appear cup-shaped (Ravi P. Singh et al., 2008).

Stem Rust Life Cycle

The life cycle of stem rust is very complex due to its heteroecious and macrocyclic nature. Once it was discovered that barberry was the alternate host, removal efforts led to the

eradication of the plant. This eliminated the sexual stage of stem rust which ultimately reduced diversity of the fungus in the United States (Roelfs & Groth, 1980). Although the pathogen is normally heteroecious and macrocyclic, the life cycle has currently been limited to one host in the United States and only three spore stages. The complete life cycle involves the fungus going through all five spore stages divided between the grass and alternate host. The macrocyclic life cycle starts with teliospores, which are the overwintering structure for stem rust. This spore is produced by *P. graminis* at the end of the wheat growing season in temperate climates. They are thick-walled structures and survive on wheat debris from the previous season. The spores are embedded in the host tissue, either in the mesophyll or epidermal region. Initially, teliospores are binucleate but as they mature, karyogamy takes place and joins the two nuclei (Leonard & Szabo, 2005). Meiosis begins soon after karyogamy, but is not completed until spring. Teliospores break dormancy in the spring and produce a basidium, completing meiosis. At the end of meiosis, four haploid nuclei are separated from each other by septa, which creates four basidiospores per basidium. A sterigma forms on the end of the basidium (Peterson, 1974). The basidiospores are located at the end of the basidium which expands as haploid nuclei enter. This portion of the life cycle ends with mitosis and leaves each basidiospore with two haploid nuclei. Basidiospores are the infecting spores of the alternate host barberry. After maturation, the spores are ejected and carried along wind currents. This occurs about the same time that fresh barberry leaves begin to unfold in the spring. Basidiospores can only infect young barberry leaves due to the increased thickness of the leaf cuticle that comes with age (Leonard & Szabo, 2005). Once the basidiospore has landed on a suitable host, infection begins. Basidiospore infection occurs through a haploid monokaryotic mycelium, which colonizes host tissue. This colonization leads to the production of a spermogonium. Spermogonia are flask-shaped structures where the narrow

end of the flask protrudes through the epidermal layer of the top of barberry leaves (Webster & Weber, 2007). Spermatia are produced in the spermogonium and protrude out the top on flexuous hyphae in a sticky sweet substance known as spermatial exudate (Webster & Weber, 2007). This nectar attracts insects and aids in dissemination of the haploid spermatia, which is also facilitated through rain splash. The spermogonia are either negative or positive in mating type and by moving the spermatia to opposite mating sites, nuclear fusion is able to take place. The introduced nucleus is able to migrate down the monokaryotic hyphae to the protoaecium which is located at the base of the spermogonium. The dikaryotic state is then established by repeated divisions which produced the cup-shaped aecium (Webster & Weber, 2007). The aecium develops directly beneath the spermogonium and protrudes through epidermal cells at the bottom of the leaf. Dikaryotic infectious aeciospores are then produced in chains (Peterson, 1974). They are not able to re-infect barberry but are able to infect the grass host via wind and water. This generally occurs in late spring (Agrios 2005).

After successful infection of the grass host, urediniospores are produced on sporophores. Urediniospores are single-celled dikaryotic spores that arise from a mat of hyphae beneath the epidermis. Once these spores rupture, they form a structure known as the uredinium (Webster & Weber, 2007). Urediniospores are able to re-infect grass hosts and typically occur on stems and leaf sheaths. They are disseminated by wind and rain. Late in the season, the uredinia mature and begin to produce two-celled teliospores, then the life cycle continues to repeat itself. The germination of teliospores can increase by thawing, freezing or wetting and drying (Roelfs & Bushnell, 1985).

The urediniospores are the spores that are able to keep re-infecting the grass host. These spores also travel along the Puccinia Pathway, which is a wind path that leads from The Gulf of

Mexico to the Great Plains (Kolmer, 2001). This is how northern states can become exposed and infected with stem rust. In northern states it is possible for the teliospores to break dormancy and produce basidiospores, but the eradication of barberry should limit the pathogen from developing further.

Races of Stem Rust

Stem rust has been an important disease for thousands of years, which shows the pathogen's strength and ability to be able to evolve and adapt in order to remain successful. There are multiple races of stem rust, some more virulent than others, that have and are infecting crops all over the world. The pathogen is constantly evolving, which increases difficulty in maintaining strong durable resistance.

In the U.S., major wheat producing states include: Kansas, Minnesota, Oklahoma, Texas, Nebraska, Colorado, Montana, and North and South Dakota. These states have all been plagued at some point by stem rust. Since the 1950's, the most common race of stem rust in the United States had been race TPMK. This race led to the discovery and use of *Sr6* and has rarely been detected since the 1980's due to the implementation of this resistance gene (Kolmer et al., 2007). A relatively recent race that has been discovered in the U.S. is race TTTT. This was first discovered in Texas in 2000. This race is virulent to the 16 *Sr* (Stem rust) differentials that were used at the time (Jin, 2005). Currently, there are 20 differentials used to determine stem rust races. Since 2003, the most common race of stem rust is QFCSC (Jin, 2005). This race is virulent on several resistance genes, including: *Sr5*, *Sr8a*, *Sr9a*, *Sr9d*, *Sr9g*, *Sr10*, *Sr17* and *Sr21* (Kolmer et al., 2007).

In the United States stem rust is managed fairly well, but the recent discovery of race Ug99 poses a much more serious threat than the races found in the U.S. Ug99 was discovered in

Uganda in 1998 and was found to have virulence to *Sr31* (Pretorius, Singh, Wagoire, & Payne, 2000). Ug99 is designated as race TTKSK, but now has eleven race variations that can be traced back to the Ug99 lineage. These races, most notably, have virulence for *Sr* genes *Sr24*, *Sr31*, *Sr36*, and *Sr38*. The virulence on these genes was very important because these genes are widely used in wheat lines as an essential source of stem rust resistance (R. P. Singh et al., 2015). Ug99 was initially found in Uganda, Kenya, Ethiopia, Sudan, and Yemen. It has now spread to Tanzania, Mozambique, Zimbabwe, South Africa, Rwanda, and Eritrea. In 2008, it was detected in Iran and even more recently, found in Egypt in 2014 (Patpour et al., 2015). TTKSK is the only race of the Ug99 lineage that has left Africa, except for race PTKSK which was found in Yemen in 2009. The Ug99 race group has been found to be virulent on a total of 34 *Sr* genes, however, there are still 39 resistance genes that confer moderate or full resistance (R. P. Singh et al., 2015). The most useful seedling resistance genes that are still agronomically viable are genes *Sr22*, *Sr25*, *Sr26*, *Sr33*, *Sr35*, *Sr45*, and *Sr50* (R. P. Singh et al., 2015). Further spread is expected by other races as well, either through air currents or human transmission. Fortunately, the speed of spread of this potentially devastating pathogen is not as high as was initially speculated.

Stem Rust Cultural and Chemical Control

In order to control diseases and pests, farmers generally have an integrated pest management system. An integrated pest management plan is multi-faceted and includes cultural, chemical and genetic resistance components (Roelfs, 1985). All three aspects play a major role in successfully growing wheat without much loss due to stem rust or other pests. Usually, the use of just one method is insufficient, which is the reason growers use multiple control and prevention

practices. The major focus of this section will be prevention and control methods, specifically for stem rust.

Cultural methods of prevention are primarily practices that are implemented by the grower. Cultural methods for controlling and preventing stem rust infection include: planting date, irrigation, fertilization application, and early maturing cultivars (Fetch, McCallum, Menzies, Rashid, & Tenuta, 2011). Planting date is important for both winter and spring wheat. Winter wheat varieties that are currently used in the central Great Plains mature about two weeks earlier than older varieties. This difference in maturation reduces the time frame for establishment from urediniospores coming from the south and ultimately limits the growth period of the fungus (Roelfs et al., 1992). For the same reason, farmers may use an earlier planting date for spring wheat varieties as an avoidance strategy (Roelfs, 1985).

Other cultural control methods are also helpful in the fight against stem rust. Growers need to take notice of fertilization and water application. Extra moisture on leaves and an excess of nitrogen can lead to a more severe stem rust infection because the fungus thrives in wet environments with lush foliage (Shumann & Leonard, 2000). In order to limit a potential infection from those factors, farmers need to ensure that their fields have proper row spacing and a properly timed irrigation and fertilization schedule, that does not correspond to the fungus' prime infection period; which is the end of April through June. The removal of wheat debris used to be an essential practice for stem rust control due to the fact that this is where the fungus produces its overwintering structures, known as teliospores. This practice is no longer essential in northern states for stem rust prevention because of the eradication of barberry (Roelfs, 1985).

Chemical control methods for stem rust are used, but often only in situations where the infection is severe. The most common fungicide for stem rust is sterol biosynthesis inhibitors

(SBIs) (Shumann & Leonard, 2000). SBIs work by inhibiting the biosynthesis of ergosterol which is essential for fungal growth. SBIs are very effective in dealing with stem rust, and can be a viable option for some growers. If a farmer decides to use fungicides as a part of their control regimen for stem rust, application timing is essential. It is best to apply foliar fungicides sometime between flag-leaf emergence and flowering. Foliar fungicides are being treated as an option for control in Kenya due to the lack of genetic resistance available. There has been several types of fungicides that were proven to reduce infection severity in rust-prone areas (Wanyera, Macharia, Kilonzo, & Kamundia, 2009). Although there are fungicides available, this has significantly increased the cost of production for farmers and is not an economically viable long term solution to Ug99 infection (Wanyera, Macharia J, & Kilonzo, 2010).

Stem Rust Control by Genetic Resistance

The major form of stem rust control in an integrated pest management program is genetic resistance. There are two primary classes of resistance that are used: seedling resistance and adult plant resistance (APR). Seedling resistance and APR are also known as vertical and horizontal resistance, which were first discerned from each other by James E. Vanderplank. Both have their pros and cons but are still most effective when they are combined with the other aspects of an integrated pest management plan.

Seedling resistance is a type of resistance that is controlled by one gene that confers resistance against a particular race of a pathogen. It is also known as race-specific resistance, vertical resistance, and major gene resistance. Seedling resistance can be responsible for a large amount of the resistance to a particular race of a pathogen through all stages of growth of the plant. This is because of the gene-for-gene interaction that occurs when the pathogen is detected in the plant. The gene-for-gene theory states that for every resistance gene in the plant host there

is a corresponding avirulence gene in the pathogen (Flor, 1971). When there is a reaction between the resistance gene of the plant and the avirulence gene of the pathogen, resistance is triggered, but if just one of those genes is present, in either the plant or the pathogen, resistance will not be triggered (Flor, 1971). Seedling resistance can be very powerful and can sometimes offer the plant near immunity against a specific race of the pathogen. This is the reason this type of resistance has been used for years and is frequently very successful, but in almost all cases is overcome eventually. This is because once a seedling gene is discovered it is often deployed over a broad area, which exposes the gene to incredible amounts of inoculum. There is no obvious way to predict the durability of these genes; for now, only time will tell if they are long-lasting (Hulbert & Pumphrey, 2014). Since this type of resistance is race-specific, and in most cases only one seedling gene is used, the pathogen will eventually overcome it. This is because of the ability of the pathogen to change by sexual recombination and mutation (Ayliffe, Singh, & Lagudah, 2008). Sexual recombination can occur in any areas where the alternate host is present. Once a pathogen overcomes a seedling gene, use of the gene often becomes futile (Keane, 2012). Defeated seedling genes can still prove useful if combined with other resistance genes, but at a reduced effect. This is the primary issue with seedling resistance that breeders are struggling with today. In order to reduce the probability of a pathogen overcoming this type of resistance, combining multiple effective seedling genes has the potential to achieve durable resistance, but this is can prove difficult due to the lack of effective of R genes available (Pederson, 1988). Many other R genes have virulence detected in other races, linkage with undesirable traits, low levels of resistance in high disease pressure or a lack of testing against Ug99 in field trials (R. P. Singh et al., 2011). Currently, many researchers are exploring other options in the pursuit for more durable resistance.

APR, also known as race-nonspecific resistance, horizontal resistance, and minor gene resistance, has great potential as a durable resistance strategy. For these types of genes, the effects are often small individually, but when combined with other APR genes, an additive effect could be observed. This is where the concept of pyramiding genes has proven beneficial. One or two APR genes may increase resistance slightly, but combining three, four or five could have the potential for even more significant resistance (R. P. Singh et al., 2010). Generally, for APR resistance, there is no gene-for-gene interaction, which means there is no host and pathogen effector recognition. Without this interaction, the pathogen cannot easily overcome the host's defenses; making this type of resistance more durable (Vanderplank, 1984). The mechanisms by which APR resistance works are mainly unknown. In a paper published in 2009, there were 6 different hypotheses listed as possibilities for the mechanism of APR resistance (Poland, Balint-Kurti, Wisser, Pratt, & Nelson, 2009). APR resistance is also theoretically effective against all races of a pathogen, but does not start to become effective until the plant reaches maturity in its growth. Although this type of resistance offers many benefits to growers and breeders, there are some drawbacks. APR genes frequently have small effects on their own, which makes them difficult for researchers to properly identify and to locate (Keane, 2012). Another issue with APR resistance is that even after locating and combining the genes, they may not achieve the complete immunity that seedling resistance offers. Often times there is an intermediate resistance response, which is still very beneficial, but is not usually as notable as seedling resistance (Vanderplank, 1984). Combining multiple APR genes though, (four or more), has the potential to give the resistance response that is desired and should dramatically improve the health of a plant that would have normally been infected.

Adult Plant Resistance Genes

Currently, most genes that control stem rust are seedling genes, but some are APR genes. APR genes are thought to be the type of resistance that has been most prevalently used for centuries due to farmer's selecting practices. This type of resistance does not prevent infection, but minimizes the intensity of the disease. Two particularly important APR genes are *Lr34/Yr18/Pm38/Sr57* and *Sr2/Yr30*. There have not been many specific APR genes catalogued, but these genes are particularly important because *Lr34* has been cloned and *Sr2* is a well-known and effective APR gene that confers resistance against stem rust. Another added benefit to APR genes is that some have the ability to confer resistance against all races of the pathogen and the ability to control all three types of rust: stem rust, stripe rust (*Puccinia striiformis* Westend.) and leaf rust (*Puccinia triticina* Erikss.), and some additional pathogens.

Lr34 is a well-known APR gene that has remained effective for over 50 years. It is inherited dominantly and located on the short arm of chromosome 7D. The gene was first described in 1966 in the cultivar 'Frontana' and was then later identified in 1977 by P.L. Dyck. It gives plants resistance to stripe rust, stem rust, leaf rust and powdery mildew and more recently, tolerance to barley yellow dwarf (Lagudah et al., 2006). On its own, this gene has a slight but significant reduction on spore production, which reduces the inoculum load for re-infection. It also decreases infectious period and increases latent period of the fungus (Drijepondt & Pretorius, 1989). Although this gene has many benefits, it does have some drawbacks. This gene is associated with leaf tip necrosis (LTN); although this could be used as a helpful diagnostic feature for the gene. The *Lr34* protein was found to function via an adenosine triphosphate-binding cassette transporter (ABC-transporter) after it was cloned (Krattinger et al., 2009). There has also been the development of successful markers for *Lr34* that have been validated across

many varieties, notably *csLV34* which was found by Lagudah et al in 2006. There was also the development of marker *SWM10* in 2006 that has shown to be a successful marker as well (Bossolini, Krattinger, & Keller, 2006).

The gene *Sr2* is located on the short arm of 3B and has been used as resistance for over 80 years; originating from the cultivar 'Hope'. *Sr2* has many of the same resistance mechanisms as *Lr34*, such as reduced pustule size and increased latent period of the fungus. A major difference from *Lr34* is that it is recessive in nature, versus dominant. This makes the gene much more difficult to work with in breeding programs. Another difference, from other APR genes discussed previously, is that *Sr2* is only effective for stem rust and stripe rust, unlike the others that are effective against all types of rust and some other types of pathogens. *Sr2* is thought to be present with other unknown APR genes which is termed the '*Sr2* complex' (R. P. Singh et al., 2011). Since *Sr2* can be difficult to work with and detect, there are some characteristics that can be diagnostic of its presence in a line. One of these characteristics is pseudo-black chaff (PBC). It is darkening of the chaff and stem due to a deposit of melanin pigments. This darkening occurs post-flowering, subsequently, confirmation of the presence of this trait is not known until fairly late in the plant's life. PBC can also be affected by the environment, which makes it difficult to detect and unreliable. This trait only affects the plant aesthetically, and has not been shown to interfere with yield. There have been attempts to dissociate PBC from *Sr2* but they were unsuccessful, most likely due to pleiotropy or its tightly linked nature (Kota, Spielmeier, McIntosh, & Lagudah, 2006). Another characteristic of *Sr2* is that it is tightly linked with a seedling chlorosis gene. This gene can be induced early on by rust infection and allows for earlier detection of *Sr2* than PBC, but this is also not completely reliable due to its dependence on temperature (Brown, 1997).

There are other important APR genes, one of which is *Lr68*. *Lr68* is one of the most recent APR genes discovered and was found in 2012 in the cultivar ‘Parula’. This gene is located on 7BL and currently, is only known to give resistance to leaf rust (Herrera-Foessel et al., 2012). *Lr68* shares many of the same phenotypic characteristics as *Lr34*. It reduces pustule size, spore production, and increases the latent period. *Lr68* is also associated with slight LTN ((Juliana et al., 2015). *Lr46/Yr29/Pm39/Sr58* is another important APR gene and was catalogued in 1998 by R.P. Singh at CIMMYT. It was found to be located on chromosome 1B in the cultivar ‘Pavon 76’ (R. P. Singh, Mujeeb-Kazi, & Huerta-Espino, 1998). *Lr46* also has similar effects as *Lr34* but has a weaker response to the pathogen. The gene is associated with a higher percentage of aborted urediniospore and overall reduction in infection frequency (Martinez, Niks, Singh, & Rubiales, 2001). This gene was found to have pleiotropic effects with stripe rust and so was given the additional designation as resistance gene *Yr29* (William, Singh, Huerta-Espino, Ortiz Islas, & Hoisington, 2002). *Lr46* has also been found to provide plants with resistance to powdery mildew (Lillemo et al., 2008). *Lr67/Yr46/Pm46/Sr55* was found in 2010 and has many of the same attributes as *Lr34* (Hiebert et al., 2010). Researchers are currently still looking to identify additional APR genes in hopes of increasing the probability of creating a durable form of stem rust resistance.

Genotyping Methods

There are many types of DNA sequencing technologies that are available today. It is an ever-changing field that has made tremendous progress since its start. In this study, genotyping was performed with the iSelect 90k SNP assay (Illumina Inc., www.illumina.com). Single gene markers were also used for the identification of some APR genes that already have markers

developed. The iSelect 90k SNP assay gives SNP data from the entire genome while the gene-specific markers will only give data on a few specific known genes.

SNP microarrays are a popular method for genotyping by detecting known polymorphisms that were incorporated in the chip design. The detection of these polymorphisms among individuals supply a great amount of information for researchers. The type of SNP microarray used in this study is the iSelect 90k SNP assay, which is a new technology that was characterized in 2014 (Wang et al., 2014). It was specifically developed for allohexaploid and allotetraploid wheat populations and contains 81,587 gene-associated SNPs. 46,977 of these markers were genetically mapped and can be used as a resource for genotyping studies (Wang et al., 2014). The microarray contains many of the common SNPs that are found across different populations of wheat from all around the world. By using this technology, thousands of SNPs will be identified that can be used for the construction of molecular maps. A particularly important use of discovering SNPs is that it can help better understand disease resistance. This is due to the fact that the presence or absence of particular SNPs are associated with different genes, which can be related to disease resistance or susceptibility. Once the presence or absence and location are determined and markers are developed, breeders can then use the markers for marker-assisted selection (MAS). The use of MAS will ultimately accelerate the breeding process by enabling breeders to no longer solely rely on phenotypic-based selection; which can take years.

Gene-specific markers were used for APR genes *Lr34*, *Lr68*, *Lr46*, and *Sr2*. This is a PCR-based method which will indicate the presence or absence of the particular markers, ultimately demonstrating the presence or absence of the APR genes. The marker for *Lr34* is *Lr34Exon-11* and is a Kompetitive Allele Specific PCR (KASP) marker which was developed in

2009 (Lagudah et al., 2009). *Lr68-csGS* is a codominant marker that was used in this study. *Lr68-csGS* is a cleaved amplified polymorphic sequence (CAPS) marker; after amplification of DNA the PCR product is digested with enzymes which prepares it for gel separation. *Lr68-csGS* was developed in 2012 by Herrera-Foessel et al (Herrera-Foessel et al., 2012). The *Lr46* and *Sr2* makers are also CAPS markers. The *Lr46* marker used is *csLV46G22* developed by Lagudah in 2010 and the *Sr2* marker is *csSr2-CAP* which was developed in 2011 (Mago et al., 2011).

QTL Analysis

Quantitative trait loci (QTL) are regions of the genome that are associated with quantitative traits such as yield and disease resistance. A quantitative trait causes a continuous range of phenotypic variation among a population. The genes responsible for quantitative traits are difficult to locate because their effects can be small and they can also be dictated by environmental factors. QTL analysis is an association of genotype to phenotype which will identify the QTLs. Performing QTL analysis can allow researchers to locate these complex and minor traits if the population size is large enough (Young, 1996). QTL analysis is primarily used for the development of molecular markers for MAS, but is also used for identifying genes in a population (Collard, Jahufer, Brouwer, & Pang, 2005). In order to perform QTL analysis, a genetic map must be constructed first. The mapping program that was used in this study is Kyazama JoinMap 4.1. This program allowed for the identification of linkage groups which were used for the QTL analysis.

There are multiple types of mapping that are used to identify QTLs. There is simple interval mapping (SIM), composite interval mapping (CIM) and multiple QTL mapping (MQM). SIM is often the first type of mapping that is performed and it analyzes the intervals between linked markers (Collard et al., 2005). CIM is a more preferred method of mapping compared to

SIM because it allows for the addition of covariates. Its power come from the combination of linear regression and interval mapping (Collard et al., 2005). Both SIM and CIM are only able to detect one QTL on a linkage group. MQM is higher-powered method compared to the previous two methods. It allows for the discovery of multiple QTLs on a linkage group and for interactions between QTLs.

Current Stem Rust APR Breeding Efforts

Due to extremely virulent races and the lack of effective R genes available for pyramiding, many researchers are looking for more durable types of resistance and placing multiple APR genes into cultivars. For example, organizations, like CIMMYT, are turning their focus to developing resistant lines to stem rust through APR genes.

Genes such as *Lr34* and *Sr2* have been used separately for years in breeding programs, but combining these genes in addition to other APR genes, has the potential to give complete resistance to multiple stem rust races. CIMMYT has recently been developing such lines and has had success. CIMMYT developed a Mexico-Kenya breeding shuttle in 2006 in order to achieve strong resistance quickly by exposing the lines to high disease pressure in Kenya and two crops per year in Mexico to accelerate breeding (R. Singh, 2006). This shuttle breeding strategy was initiated by Dr. Norman Borlaug in the 1940's, between Ciudad Obregon and Toluca, Mexico (R. P. Singh et al., 2015). CIMMYT combined multiple APR genes and were able to achieve high yield and near immunity in 298 lines to Ug99 (R. P. Singh et al., 2011). The Borlaug Global Rust Initiative (BGRI) and Durable Rust Resistance in Wheat (DRRW) project have increased breeding efforts drastically in the past decade as well. Genetic resources and breeding materials have been tested from over 32 countries in Ug99 hotspots like Kenya and Ethiopia (R. P. Singh et al., 2015).

CIMMYT has multiple lines that confer APR to the Ug99 group of stem rust. Some of these lines are ‘Kingbird’, Kiritati’, ‘Huirivis#1’, ‘Juchi’, ‘Muu’ and ‘Pavon 76’. Kingbird is a spring wheat that was developed by CIMMYT in 2007 and is one of the parents of the population in this study. According to CIMMYT, this line does not have resistance at the seedling stage and is known to contain the APR genes *Sr2* and *Lr34*. It is also suspected to contain an additional three APR loci. Kingbird has been successful in CIMMYT field trials against the Ug99 group of stem rust races and has been grown in Kenya since 2012. Kingbird’s pedigree is TAM-200/TUI/6/PAVON-F-76//CARIANCA-422/ANAHUAC-F-75/5/BOBWHITE/ CROW// BUCKBUCK/ PAVON-F-76/3/YECORA-F-70/4/TRAP-1. Kingbird was released in Ethiopia in the fall of 2015 after the devastating stem rust epidemics of 2013 and 2014 that had crop losses of up to 100% (Olivera et al., 2015). The BGRI reported that the Ethiopian farmers’ considered Kingbird a success due to the variety’s disease resistance and good bread making qualities. ‘Digalu’ was the most common variety grown in Ethiopia and despite its resistance to Ug99 was extremely susceptible to race TKTTF (R. P. Singh et al., 2015). TKTTF was the primary cause of the epidemics but belongs to a different genetic lineage than the Ug99 race group (Olivera et al., 2015).

Ug99 Group QTL Analysis

QTL analysis has proven to be an effective method for detecting and locating APR genes for stem rust resistance. There have been numerous studies focusing on locating stem rust resistance genes in order to develop markers or to identify novel stem rust resistance genes. Current QTL analysis research for stem rust is primarily focused on the Ug99 race group. In a paper by Singh et. al, a cross between Muu and ‘PBW343’ yielded four significant QTLs that were found to be effective against the Ug99 group. The QTLs were 2BS, 3BS, 5BL, and 7AS.

3BS was identified as *Sr2*. 5BL came from PBW343, and all other QTLs were from Muu (S. Singh, Singh, Bhavani, Huerta-Espino, & Eugenio, 2013). In a paper published in 2014 by Yu et al., Kingbird was crossed with PBW343. QTLs were found on chromosomes 1A, 3BS, 5BL, 7A, and 7DS (L. X. Yu et al., 2014). This study created a consensus map for the location of stem rust resistance loci. A total of 141 stem rust resistance loci were found that are considered resistant to Ug99 (L. X. Yu et al., 2014). In 2012, the variety Pavon 76 was crossed with `Avocet` and was also found to contain APR genes that confer resistance to Ug99. Pavon 76 is a part of the pedigree for Kingbird. Pavon 76 was found to carry three QTLs, one of which was *Sr2*. The other two QTLs were found on 1B and 3D (Njau, Bhavani, Huerta-Espino, Keller, & Singh, 2012). Three APR genes for Ug99 were found in the cultivar `Thatcher` in 2014. Thatcher is an important variety that has provided stem rust resistance for over 80 years. Virulence to the seedling genes occurred around the 1950's, but the APR genes still gave the variety moderate resistance (Rouse, Talbert, Singh, & Sherman, 2014). The locations of the QTLs were found on chromosomes 1AL, 2BS and 3B. The QTL on 3B was located in the same location as the APR gene *Sr12*, which only conferred resistance to TTKSK when combined with other resistance genes (Rouse, Talbert, et al., 2014).

Rationale for this Study

Stem rust is an ever-changing threat to the world's wheat producing countries. The Ug99 group, in particular, causes immense concern. Although, there are other varieties that have performed well against the Ug99 threat, Kingbird has performed exceptionally well. Kingbird has proven to be a successful variety, not only against the Ug99 group, but to other virulent races of stem rust as well. Unfortunately, only part of the resistance that is found in Kingbird is understood, that is why it is imperative to locate all of the resistance genes that have enabled

Kingbird to remain a success. Ultimately, uncovering the resistance that lies behind Kingbird will allow us to better understand what types of genes and it what combination will lead to a durable resistant variety.

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Chapter 2 - Mapping Stem Rust Resistance Genes in 'Kingbird'

Abstract

Stem rust, caused by the fungus *Puccinia graminis* f. sp. *tritici*, has historically been one of the most important diseases of wheat. Although losses have been much reduced in the last fifty years, new highly virulent races of the pathogen have recently emerged in East Africa. These new races are virulent on nearly all of the currently deployed resistance genes and therefore pose a serious threat to global wheat production. The spring wheat variety 'Kingbird' is thought to contain multiple quantitative trait loci (QTLs) that provide durable, adult-plant resistance against wheat stem rust. Stem rust-susceptible Kansas winter wheat line 'KS05HW14' was backcrossed to Kingbird and 379 recombinant lines were advanced to BC₁F₅ and then increased for testing. The lines were screened for stem rust resistance in the greenhouse and field in Kansas and in the field in Kenya over multiple years. We identified 16,237 single nucleotide polymorphisms (SNPs) with the Wheat 90K iSelect SNP Chip assay. After filtering for marker quality, linkage maps were constructed for each wheat chromosome. Composite interval mapping and multiple-QTL mapping identified seven QTLs on chromosome arms 2BL, 2DS, 3BS, 3BSc, 5DL, 7BL, and 7DS. Six QTLs were inherited from Kingbird and one QTL on 7BL was inherited from KS05HW14. The location of the QTL on 2BL is approximately at locus *Sr9*, 3BS is at *Sr2*, 3BSc is at *Sr12*, and 7DS is at *Lr34/Yr18/Sr57*. Although no QTL was found on 1BL, the presence of resistance gene *Lr46/Yr29/Sr58* on 1BL in both parents was indicated by the gene-specific marker *csLV46*. QTLs on 2DS and 5DL may be related to photoperiod or vernalization genes. Pairwise interactions were only observed with race QFCSC, most notably occurring with QTLs 2BL and 3BS. These results confirm that there are multiple QTLs present

in Kingbird. Ultimately, the identification of the QTLs that make Kingbird resistant will aid in the understanding of durable, non-race-specific resistance to stem rust of wheat.

Introduction

Stem rust (caused by *Puccinia graminis* f. sp. *tritici*) has been a devastating pathogen of wheat for centuries. With the rise of extremely virulent races of stem rust in Africa, awareness and concern for the pathogen has increased over the years and is a current topic of interest for researchers and growers. In the United States, stem rust only causes about a 1% yield loss annually, while in some African countries there is a potential yield loss of up to 90%. These virulent races, specifically TTKSK, also named Ug99, are posing a great threat to world-wide wheat production (R. P. Singh et al., 2011). Ug99 was discovered in Uganda in 1998 with virulence to stem rust resistance gene *Sr31* (Pretorius et al., 2000). Since then, it has spread to a total of thirteen countries and is continuing to move and evolve. Currently, there are eleven races that are derivatives from the original race, TTKSK, which are referred to as the Ug99 race group. The Ug99 race group has been found to be virulent on a total of 34 *Sr* genes, however, there are still 39 resistance genes that confer moderate or full resistance (R. P. Singh et al., 2015). Most of these are alien-derived and only a few of these have been tested adequately for linkage drag and effectiveness in the field. Ug99 poses an immense threat to world-wide wheat production due to its virulence to the majority of common wheat varieties grown.

The threat of Ug99 has directed research focus towards more durable forms of stem rust resistance. Adult plant resistance (APR) has become an ideal alternative to seedling resistance because of its more durable nature and lack of race specificity (R. P. Singh et al., 2010). Seedling resistance has been the major form of stem rust resistance for years; due to its strong resistance

effect that can be achieved by the presence of only one gene. APR genes, often have miniscule effects on their own, but when combined can offer durable, nearly complete resistance. APR genes also frequently offer resistance to multiple types of pathogens. Two APR genes that are common in many wheat varieties are *Sr2/Yr30* and *Lr34/Yr18/Pm38/Sr57*. According to MASWheat (www.maswheat.ucdavis.edu), the gene *Sr2* is located on the short arm of chromosome 3B and has been used as resistance for over 80 years; originating from the cultivar ‘Hope’. *Lr34* is a well-known APR gene that has remained effective for over 50 years and is located on the short arm of chromosome 7D. The gene was first described in 1966 in the cultivar ‘Frontana’ and was then later identified in 1977 by P.L. Dyck.

The CIMMYT variety Kingbird is known to contain the APR genes *Lr34* and *Sr2* is also suspected to contain an additional three resistance loci and has no reported resistance at the seedling stage (R. P. Singh et al., 2011). Kingbird has been successful in CIMMYT field trials against the Ug99 group of stem rust races (R. P. Singh et al., 2010). According to the Borlaug Global Rust initiative (BGRI), Kingbird was recently released in Ethiopia in the fall of 2015 after the devastating stem rust epidemics of the past two years, which resulted in crop losses of up to 100% (Olivera et al., 2015). The variety was reported as a successful alternative to other common wheat varieties of the area and also offered resistance against stem rust races outside of the Ug99 lineage (Olivera et al., 2015). A study published in 2011, reported that a cross between Kingbird and ‘PBW343’ resulted in the identification of 5 QTLs all derived from Kingbird: 1A, 3BS, 5BL, 7A, and 7DS (Bhavani et al., 2011). The QTL located on 3BS was determined to be *Sr2* and 7DS was reported as *Lr34* (Bhavani et al., 2011). The success of the variety Kingbird against the Ug99 group, and other virulent stem rust races, has been an impressive feat. However, the exact composition of Kingbird’s resistance is still somewhat unknown and it is

important to understand the resistance behind this variety's success. The identification of the QTLs that make Kingbird resistant will aid in the understanding of durable, non-race-specific resistance to stem rust of wheat.

Materials and Methods

Plant Materials

The stem rust-susceptible Kansas hard white winter wheat line `KS05HW14` was backcrossed to the spring wheat Kingbird and 379 lines (recombinant inbred lines, RILs) were advanced to a BC₁F₅ and then increased for testing. KS05HW14 was developed in 2005 and has a pedigree of KS98HW452/CO960293//KS920709B-5-2. Kingbird was developed in 2007 by CIMMYT. The pedigree of `Kingbird` is TAM-200/TUI/6/PAVON-F-76//CARIANCA-422/ANAHUAC-F-75/5/BOBWHITE/ CROW// BUCKBUCK/ PAVON-F-76/3/YECORA-F-70/4/TRAP-1.

Seedling Evaluation

Kingbird and KS05HW14 were sent to the USDA-ARS Cereal Disease Laboratory in St. Paul, Minnesota to be screened against the Ug99 race group at the seedling stage. The parents were evaluated against multiple stem rust races. The evaluations for races TTKSK (isolate 04KEN156/04), TKTTF (13ETH08-3), and TRTTF (06YEM34-1) were performed in a BSL-3 greenhouse. The evaluations for races QFCSC (06ND76C), QTHJC (75ND717C), RKQQC (99KS76A-1), SCCSC (09ID73-2), TPMKC (74MN1409), and TTTTF (01MN84A-1-2) were carried out in a BSL-2 greenhouse. All urediniospores used for inoculation were stored at –80°C. The spores were then heat shocked at 45°C for 15 min and rehydrated and maintained at 80% relative humidity by a KOH solution, and mixed with mineral oil (Rouse, Wanyera, Njau,

& Jin, 2011). Seedlings were inoculated 7-9 days after planting and then placed in a dew chamber for 14 h at 18°C in darkness. The plants were then placed in a greenhouse maintained at 18 ± 2°C with 16 h light (Rouse et al., 2011). At 13 to 15 days after inoculation, the infection types were recorded according to Stakman et al. (Stakman, Stewart, & Loegering, 1962).

Field Evaluation

The F₅ generation-derived RILs were evaluated in two locations over multiple years. In 2013, a partial set of the population, along with the parents, were sent to Njoro, Kenya USDA-ARS Stem Rust Nursery to be evaluated against the Ug99 group of stem rust races. Due to shortages of seed, a slightly different subset of the population was sent in 2014 to the same location to also be evaluated for stem rust resistance. The seed that was sent to Kenya was vernalized for 8 weeks before being planted by hand. The seedlings were then planted in a block of 65' x 150'. Plant samples were placed in single rows, spaced 18 inches from each other and surrounded by a spreader row on either side. Each plant was located within 6 inches of an inoculum source. The field was inoculated with a composite of race TTKSK and TTKST in addition to the natural infection that occurs.

Full sets of the population were tested at Rocky Ford Research Farm, Manhattan, Kansas over three years: 2013, 2014, and 2015. Approximately, 1.0 g of seed for each wheat line was planted in single-row plots that were 1.2-m long. Four single-row plots were planted in ranges with 20 cm between plots laterally and 60 cm between ranges lengthwise. Each drill pass was 2.8-m wide and approximately 50-m long and consisted of four drill rows. The outer border and every third pass of the drill were planted with a susceptible spreader cultivar consisting of 'KS05HW14', 'McNair 701', 'Winterhawk', and/or 'Pioneer 25R40'. Parents were planted every twentieth range. The field was inoculated several times during the jointing to early boot

growth stages with urediniospores from a composite of three Kansas isolates of race QFCSC. Field inoculations consisted of urediniospores that were stored at -80°C that were then heat-shocked in a water bath at 42°C for 6 minutes and suspended in Soltrol-170 mineral oil (Chevron-Phillips chemical company) at a rate of 2 ml dried urediniospores per liter. Beginning with the first inoculation date, plots were irrigated nightly with overhead rotor sprinklers (10 minutes every two hours for 6 hours) to maintain canopy wetness.

The stems were scored for stem rust infection type (IT) when plants were between Zadoks (Zadoks et al, 1974) growth stage 70.5 (kernels in middle spike half formed) and 71 (watery ripe, clear liquid). IT was rated on a 0-9 scale defined as follows: 0) no symptoms; 1) small chlorotic or necrotic flecks, no sporulation; 2) small to medium chlorotic or necrotic flecks, no sporulation; 3) small pustules with chlorosis or necrosis, trace of sporulation; 4) small pustules with chlorosis or necrosis, light sporulation; 5) small to medium pustules with chlorosis or necrosis, intermediate sporulation; 6) small to medium pustules with chlorosis or necrosis, moderately high sporulation; 7) medium to large pustules with chlorosis and necrosis, abundant sporulation; 8) medium to large pustules with chlorosis, abundant sporulation; 9) medium to large pustules with little or no chlorosis, abundant sporulation. Percent severity was estimated using the modified Cobb scale (Peterson et al, 1948) at the early dough (Zadoks 83) to hard dough stages (Zadoks 87).

Greenhouse evaluations of stem rust were also performed with races RKQQC (isolate 99KS76A-1) and QFCSC (SR12-1) on adult plants. Two evaluations were done with RKQQC in 2014 and 2015, while only one year of data were obtained with race QFCSC in 2013. Greenhouse inoculations were conducted on adult plants at the flowering stage. Pots were sprayed with a 1% (v/v) suspension of 0.18 ml of fresh or frozen dried urediniospores in 18 ml of

Soltrol 170 oil using an atomizer (G-R Manufacturing, Manhattan, KS). Frozen spores were heat-shocked at 42°C for 6 minutes prior to use. Each pot received approximately 0.2 ml of inoculum suspension. The inoculated plants were placed in Percival dew chambers (Model I-36DL) overnight maintained at 20±1°C at 100% humidity. Plants were removed the next morning and placed in greenhouses with 16 h light and 8 h dark maintained at 18±2°C. Percent severity and infection type were taken 14 days after inoculation. The modified Cobb scale was used at all locations to assess the amount of stem rust severity as the percentage of leaf area affected (0-100%). Infection type (0-9) was also taken at all locations. Best linear unbiased predictions (BLUPs) for phenotypic data were calculated in SAS (SAS version 9.4, SAS Institute, Cary, NC) Proc Mixed by treating genotype as a random effect and environment as a fixed effect. BLUPs were made by first dividing the phenotypic data sets into either severity or infection type and then grouping by the race used (TTKSK, QFCSC, or RKQQC). Broad-sense heritability was calculated on an entry-mean basis where σ_g^2 is the covariance due to genotype, σ_e^2 is the residual covariance, and r is the number of environments for each BLUP:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}}$$

Molecular Marker Analysis

Leaf tissue was collected at 6-7 weeks post emergence from the BC₁F₅ generation RILs and lyophilized before processing. Total genomic DNA was extracted from the ground tissue using the BioSprint 96 Workstation (QIAGEN) with Biosprint 96 DNA Plant kits (QIAGEN). Purified stock DNA aliquots were diluted in 1× TE buffer and normalized to a final concentration of 50 ng/μL with a final volume of 20 μL with PicoGreen dsDNA assay kit. Extracted DNA samples from 379 lines and the parents were sent for further analysis by the Wheat 90K SNP iSelect Chip assay (Wang et al., 2014). 16,237 single nucleotide

polymorphisms (SNPs) were initially identified from the Wheat 90K iSelect Chip assay, these SNPs were then further analyzed with GenomeStudio Polyploid Clustering Module v1.0 (Illumina) for cluster assignments. Once clusters were designated, alleles were assigned based on which parent they were derived from. Heterozygous markers were not used for map construction.

Gene-specific Markers

Extracted DNA was also used for gene-specific markers for resistance genes *Sr2/Yr30*, *Lr34/Yr18/Pm38/Sr57*, *Lr68* and *Lr46/Yr29/Pm39/Sr58*. The *Sr2* marker, *csSr2*, was used and is a cleaved-amplified polymorphic sequence (CAPS) marker developed in 2011 (Mago et al., 2011). The marker for *Lr34* is *Lr34Exon11*, and is a Kompetitive Allele Specific PCR (KASP) marker which was developed in 2009 (Lagudah et al., 2009). *Lr68-csgs* is a CAPS marker that was used for the detection of resistance gene *Lr68*. To detect the presence of *Lr46*, the marker *csLV46G22* was used (Dr. E. Lagudah, personal communication).

Map Construction and QTL Analysis

A linkage map was assembled in JoinMap 4.1 (Kyazma B.V., Wageningen, Netherlands) by using the Kosambi mapping function and the maximum likelihood independence LOD algorithm (Van Ooijen, 2006, 2011). A total of 30 linkage groups were acquired from JoinMap 4.1, which were aligned to the 90K wheat consensus map for chromosome assignment. The linkage groups were then fit together into the 21 chromosomes of the wheat genome in JoinMap 4.1. This was accomplished by combining linkage groups that aligned to the same chromosome. Some linkage groups were inflated in cM length and were reduced in size by removing duplicate markers, or markers at a very similar location. Other linkage groups contained gaps that could not be closed, which were then anchored to the 90K consensus map to assess the gap length and adjust positions accordingly. This was necessary for the following chromosomes: 2A, 2D, 3D,

5D, 7B, and 7D. The gene-specific markers for *Sr2* and *Lr34* were also added to the linkage map. A total of 3,339 SNP markers were used in the final linkage map. Although there was very little missing genotypic data for markers (<10%), the missing data were imputed with Beagle 4.1 (<https://faculty.washington.edu/browning/beagle/beagle.html>) to aid in QTL analysis.

QTLs were mapped using the R-package R/qtl (K. Broman & Sen, 2009; K. W. Broman, Wu, Sen, & Churchill, 2003). Phenotypic data sets were run individually by year and location and then combined into BLUPs based on the stem rust race. Simple interval mapping (SIM) was performed for the initial detection of QTLs via Haley-Knot regression. The results from SIM allowed for the selection and designation of covariates to be used in composite interval mapping (CIM). CIM was also implemented through the use of Haley-Knott regression. The marker positions and identification of QTLs detected from SIM and CIM were used for multiple quantitative trait loci mapping (MQM). MQM was conducted in R/qtl to obtain QTL effects and possible QTL x QTL interactions. The presence of QTL x QTL interactions was determined by the difference in the lod.fv1 and lod.av1 statistics (K. Broman & Sen, 2009). MQM models were then fit to each phenotypic data set and QTLs were determined to act either additively or with interactions. Bayesian 95% confidence intervals for each QTL for each dataset were calculated with R/qtl. The genome-wide logarithm of odds (LOD) thresholds for declaring significant QTLs were calculated using 1000 permutations and a Type 1 error set to 0.05 for each phenotypic data set and all BLUPs.

In some cases, locations of markers associated with QTLs were compared to markers from the literature by blasting both sets against the POPSEQ database for ‘Chinese Spring’ and ‘W7984’ as discussed by Edae et al., and as implemented by the E. Akhunov lab (Edae, Bowden, & Poland, 2015), (<http://129.130.90.211/wpdb/gbsloc>).

Results

Stem Rust Phenotypic Response

At the seedling stage, KS05HW14 was susceptible to all races tested (Table 2.1). Kingbird gave a consistent infection response of 3- with low infection frequency (lif) against the races TTKSK and TKTTF. Kingbird had an IT of 2+3 lif or 3-1 lif to race TRTTF. The IT in one replicate was 3-; for race TTTTF. For all other races the IT was very low.

Disease response at the adult stage differed greatly between the parents and among members of the population of the KS05HW14*2/Kingbird cross. Kingbird, at the adult plant stage, was found to have very high resistance against all races at each field location and all greenhouse evaluations. Adult plants of KS05HW14 were observed to be susceptible to all races at all locations. The 379 accessions varied greatly in their stem rust resistance response, but were correlated by location and common race (Figure 2.1). The phenotypic response for the population varied by race. One example of each phenotypic distribution observed for each race is in Figure 2.2. Transformation of phenotypic data was attempted by the implementation of Box-Cox, logit, and arcsine square-root. The transformations showed no significant difference in the identification and detection of QTLs, subsequently, phenotypic data sets were left non-transformed. Broad-sense heritability was calculated for severity and infection type data for the BLUPs and are shown in Table 2.2.

Gene-specific Markers

Gene-specific markers for resistance genes *Sr2* and *Lr34* were found to be present in Kingbird and absent for KS05HW14. The marker for *Lr46* was found in both Kingbird and KS05HW14. The gene-specific marker for resistance gene *Lr68* was not found to be present in either parent.

Linkage Maps

The completed linkage map consisted of 3339 SNP markers with an average of 1.4 cM interval spacing and total map length of 4664 cM distributed over all 21 chromosomes (Figure 2.3). The linkage maps were validated in two ways. First, linkage heat maps showed that linkage groups were in high linkage internally, but independent of other linkage groups (Figure 2.4). Second, the linkage maps for this study were compared with the 90K consensus map and found to be linearly aligned (Figure 2.5). The linkage groups in the current study tended to be longer than the consensus map, so both map positions are presented for comparison in the QTL list (Tables 2.2 and 2.3).

QTL Mapping

QTL mapping revealed the repeated identification of seven QTLs, with major and minor effects, located on chromosomes 2BL, 2DS, 3BS, 3BSc, 5DL, 7BL, and 7DS (Table 2.3). Bayesian 95% confidence intervals were calculated for QTLs that were significant and repeatedly detected (Figure 2.6). All seven QTLs were detected and repeated with race QFCSC, either at Rocky Ford or in the greenhouse evaluation from 2013. A QTL was found on 6B from Rocky Ford 2013 infection type data (Table 2.3), however, it was not detected in other phenotypic data sets. QTLs on 2BL and 3BSc sometimes had exceptionally high LOD scores and accounted for a large amount of the phenotypic variation against race QFCSC. Notably, 2BL had a severity additive effect of 9.66-19.33%. The QTL located on 3BSc was only detected with race QFCSC. For race RKQQC, QTLs on 2BL, 2DS, 3BS, and 5DL were detected and repeated except for 2BL, which was only identified once. Exposure to the Ug99 race group lead to the detection of five QTLs: 2BL, 2DS, 3BS, 7BL, and 7DS. QTLs on 2BL and 2DS were only significantly identified once. Although the QTL on 2BL was detected with all three races, the

effect of the QTL was substantially lower against other races compared to QFCSC. 3BS was the only other QTL that was significantly identified with all three races. The phenotypic variation explained by 3BS ranged from 2.15-18.92% with a severity additive effect of 4.3-11.14%. The QTL on 2DS was also identified with all three races, but only statistically significantly with races QFCSC and the Ug99 race group. The BLUPs for severity and infection type for race QFCSC located all seven QTLs (Table 2.4). RKQQC severity and infection type BLUPs identified the same four QTLs that were detected with the independent phenotypic data sets, with the addition of a QTL found on chromosome 4A. QTLs on chromosomes 2BL, 3BS, 7BL, and 7DS were located by the severity and infection type BLUPs for Kenya with the Ug99 race group. Additive effects for the BLUPs were a much more conservative estimate compared to the effects from the independent phenotypic data sets.

Epistatic analysis

MQM was used for the exploration of QTL x QTL interactions and estimated effects. Independent phenotypic interactions and complete phenotypic variation are reported in Table 2.5. Severity data for race QFCSC identified four pairwise interactions between QTLs. Interactions occurred between QTLs 2BL and 3BS, 2BL and 3BSc, and 3BS and 3BSc. The interaction between 2BL and 3BSc was observed over two years. Infection type data for race QFCSC detected five pairwise interactions. Interactions were observed between QTLs 2BL and 6B, 2BL and 7DS, 3BS and 3BSc, 2BL and 3BSc and 3BSc and 7DS. Full models, for race QFCSC, with seven QTLs explained from 67.05-78.95% of the complete phenotypic variation observed. The only QFCSC model that was additive was Rocky Ford 2013 severity. This model explained 50.73% of the phenotypic variation. Race RKQQC models were additive and explained 5-11.25% of the phenotypic variation observed. Additive models for the Ug99 race

group explained from 15.55-35.04% of the observed phenotypic variation. BLUPs interactions were also identified and are shown in Table 2.5. Interactions were observed for both severity and infection type for race QFCSC. Severity interactions observed were between QTLs 2BL and 3BSc, and 2BL and 3BS. Infection type interactions observed were between QTLs 2BL and 7DS, and 3BS and 3BSc. Full models for the BLUPs of race QFCSC both explained at least 77% of the phenotypic variation. The severity for race RKQQC was an additive model that explained 16.71% of the variation while the infection type was a full model that accounted for 27% of the phenotypic variation. The interaction that was detected for race RKQQC infection type was between QTLs 5DL and 4A. The Ug99 race group severity and infection type BLUPs were strictly additive and explained 24.8-31.59% of the phenotypic variation.

Discussion

The results of this study have helped identify the stem rust resistance loci that are in the highly resistant cultivar, Kingbird. This study identified seven QTLs consistently on chromosomes 2DS, 2BL, 3BS, 3BSc, 5DL, 7BL, and 7DS from a cross of Kingbird and KS05HW14. QTL detection varied by exposure to stem rust races QFCSC, RKQQC, and the Ug99 Group. QTLs were found in both parents, with six derived from Kingbird and one, 7BL, derived from KS05HW14. Both parents were found to contain the gene-specific marker *csLV46G22* for the resistance gene *Lr46*. The QTL identified on 3BS was consistently found with all races and located directly over the *cs-Sr2* marker in the linkage map. The QTL located on 7DS was repeatedly identified over the *Lr34* marker *Lr34Exon11*. These results confirmed the presence of resistance genes *Sr2* and *Lr34* in Kingbird. The additional QTLs that were identified here only have a few possible *Sr* genes as candidates based on previous mapping studies.

Stem rust evaluations varied from race to race and from location to location. The most consistent and highest heritability explained was achieved with QFCSC at Rocky Ford Kansas. For race RKQQC, disease was difficult to establish in the greenhouse setting. This resulted in poor phenotypic data and ultimately, reducing the potential for QTL detection. The broad-sense heritability reflected the poor phenotypic data. The low percent variability and low heritability for the Kenya phenotypic data sets could be explained by the small subsets of the population that were evaluated, the vernalization issues, or variations in photoperiod. QTLs were still identified with all three races, but repetition could have increased with improved phenotypic data from race RKQQC and the Ug99 group. BLUPs gave more conservative estimates of additive effects compared to the independent phenotypic data sets.

The QTL found on the long arm of 2B is located at the position of 109 cM according to the 90K Wheat Consensus Map. This QTL had a very large effect against race QFCSC and very small, non-duplicated, effect against the Ug99 race group. The most likely resistance gene that is found in this location is *Sr9*. According to the consensus map for Ug99 stem rust resistance loci, *Sr9* is located at 104 cM (L. X. Yu et al., 2014). The *Sr9* locus has seven characterized alleles: *Sr9a*, *Sr9b*, *Sr9d*, *Sr9e*, *Sr9f*, *Sr9g*, and *Sr9h* (Green, Knott, Watson, & Pugsley, 1960; Knott, 1966; Loegering, 1975; McIntosh & Luig, 1973; Rouse, Nirmala, et al., 2014). *Sr9h* is the only characterized allele that has proven to be completely effective against the Ug99 race group (Rouse, Nirmala, et al., 2014). The other alleles of *Sr9*, have a differential response to varying races of stem rust. In a previous study, *Sr9a*, *Sr9b*, *Sr9d*, and *Sr9g* were found to be ineffective against Ug99 but *Sr9e* appeared to show some moderate level of resistance at the adult stage (Jin et al., 2007). The QTL on 2BL showed substantial race specificity which could help with identifying the allele of *Sr9*. However, two QTLs, 2BL and 3BSc, were determined to have race specificity and

major effects making it difficult to discern between the alleles for *Sr9* solely based on the seedling data. Further testing with other races of stem rust in the segregating progeny will need to be evaluated in order to reveal the identity of the allele of *Sr9* and explain the seedling data results.

The gene identified on the short arm of chromosome 2DS was only significant at two locations with two races: Kenya 2014 with the Ug99 race group and greenhouse 2013 with race RKQQC. The QTL showed a weak additive effect and explained little phenotypic variation. The markers for this QTL were not anchored to the Wheat 90K Consensus Map. In order to obtain a general location for these markers, the markers directly under the QTL peak were blasted against the POPSEQ database for cultivars ‘Chinese Spring’ and ‘W7984’ and found to be located on the short arm of 2D at the positions of 17.34 cM and 13.64 cM respectively. Previously reported stem rust resistance genes at this general location have been *Sr32* and *Sr46*, which are both qualitative genes (Mago et al., 2013; G. Yu et al., 2015). Both *Sr32* and *Sr46* have been reported as having resistance against races of the Ug99 group. However, *Sr32* has not been widely deployed due to the deleterious effects of the genes associated with it (Friebe, Jiang, Raupp, McIntosh, & Gill, 1996). *Sr46* is a relatively recently identified *Sr* gene and since it is alien, it is unlikely that it would be found in Kingbird at this time. The location of the QTL on 2DS is also very close to the location of the photoperiod gene *Ppd-D1*. The molecular sequence tag for *Ppd-D1* is readily available on GenBank. We blasted part of the sequence to POPSEQ and found that it aligned with W7984 on chromosome 2DS at position 27.05cM. We also blasted the sequence of the flanking markers of the QTLs found on 2DS and found that the *Ppd-D1* position fell within the interval. This suggests that this gene could be *Ppd-D1*, however, there is a significant gap in the linkage map near this QTL. The gap in this section of the map and low frequency of the QTL make it

difficult to determine the identity of the QTL, consequently, there is still a possibility that this is a novel stem rust resistance gene.

The second QTL identified on 3BSc was only identified with race QFCSC and maps to the general position of 67-68cM on the 90K Consensus Map. According to the consensus map for Ug99, (L. X. Yu et al., 2014), the only *Sr* resistance gene in that general location is *Sr12*, which is located at 70.6 cM. *Sr12* is a seedling gene with race specificity and major effects. *Sr12* is effective to races QFCSC and SCCSC which correlates with the results from the seedling evaluation and the field evaluations. These results make *Sr12* a likely candidate for the second QTL on 3B.

A QTL was consistently identified on chromosomes 5DL with races QFCSC and RKQQC. The QTL on 5DL was detected five times with the most repeated marker under the QTL peak mapping to the 90K Consensus Map at the position of 131.54 cM. This QTL had a relatively small additive effect of about 5% and generally explained a small amount of the phenotypic variance (1-5%). There are no known *Sr* genes at this location, but it is in close proximity to the vernalization gene *VRN-D1*. The molecular sequence tag for *VRN-D1* was located on GenBank and then used to blast against POPSEQ for cultivars Chinese Spring and W7984. The *VRN-D1* gene was located on chromosome 5DL for both Chinese Spring and W7984 at positions 138.39cM and 102.46cM respectively. This is in close proximity to the QTL that we identified on 5DL, and since there are no other *Sr* genes at this location, it is a likely candidate. 5DL was derived from Kingbird, indicating that it could be a spring vernalization allele. The association with the vernalization gene and stem rust disease response could be related to the fact that spring wheat matures more quickly compared to winter wheat, subsequently, shortening the infection period for the pathogen, resulting in less disease.

A resistance gene was repeatedly mapped to the long arm of chromosome 7B and was identified with race QFCSC and the Ug99 group. 7BL was the only QTL mapped that was derived from the winter wheat parent KS05HW14. The QTL has minor, but significant effects. The markers directly beneath the QTL peak of 7BL, were found to be located between the distances of 148.65-166.99 cM on 90K Wheat Consensus Map. The only rust APR gene that is known to be on the long arm of 7B in this general location is resistance gene *Lr68*. The meta-analysis of Yu et al (2014) showed a stem rust resistance QTL in four different studies near this position on 7BL as well. The gene-specific marker for *Lr68* came back negative for both parents, however, the close proximity to this gene led us to investigate the possibility of *Lr68* further. We blasted markers that were found directly under the QTL peak for 7BL against POPSEQ and found that these markers were located in the same W7984 recombination bin (118.6 cM) on 7BL as the best marker for *Lr68* (Herrera-Foessel et al., 2012). This suggests that even though the gene-specific marker came back negative, it is likely that the gene we identified on 7BL is *Lr68*. The presence of *Lr68* in KS05HW14 can be confirmed in subsequent studies with this population using leaf rust adult plant phenotypic data.

According to the seedling evaluation, the avirulence to TTTTF for Kingbird cannot be explained by the alleles of seedling genes *Sr9* or *Sr12*. This likely suggests that there is at least one additional seedling resistance gene present in Kingbird that went undetected. A potential identity for this resistance gene could be *Sr7a* due to its resistance to race TTTTF (Dr. M. Rouse, personal communication, February 20, 2016). *Sr7a* on chromosome 4A is a seedling resistance gene that is commonly found in North American wheat lines, consequently, most North American stem rust races are virulent to the gene. However, some virulent African races as well as race TTTTF of

stem rust are avirulent on *Sr7a*. *Sr7a* has also been reported to have complementary epistasis with resistance gene *Sr12* (R. P. Singh & McIntosh, 1987).

There have been other QTLs identified in mapping studies with Kingbird. A cross with Kingbird and 'PBW343' resulted in the identification of five QTLs: 1A, 3BS, 5BL, 7A, and 7DS (Bhavani et al., 2011). We only identified two of the five QTLs previously detected, which were 3BS and 7DS (*Sr2* and *Lr34*). This could be for a number of reasons. Firstly, the five QTLs previously identified were found solely with races belonging to the Ug99 group. The resistance genes that we identified on 2BL and 3B were race-specific seedling genes and not identified with the Ug99 group. The QTLs we located on 2DS and 5DL are most likely related to photoperiod and vernalization. The previous QTL identified on 5BL had a large effect in the paper from 2014 and is thought to be derived from Kingbird. A paper published in 2013 with a cross of PBW343 and 'Muu' found a QTL on 5BL as well, but it was derived from PBW343 (S. Singh et al., 2013). The meta-analysis study published in 2014 by Yu et al, found the 5BL QTL coming from Muu and not PBW343 (L. X. Yu et al., 2014). This could suggest that the 5BL QTL identified in the Kingbird/PBW343 cross could have been derived from PBW343, which would explain why it was not detected in our study. No significant QTL peaks were identified on chromosomes 1A and 7A. This could be due to the small subsets of the population that were sent to Kenya for testing against the Ug99 group, along with the difficulty associated with growing a winter wheat in Kenya. Alternatively since our study was much larger, the QTLs reported in the literature on 1A and 7A could be spurious.

The identification of the QTLs that make Kingbird resistant was not the only objective of this study. Understanding how these resistance genes might possibly interact with each other, either positively or negatively, is crucial for breeding efforts for stem rust resistance. All

pairwise interactions were only observed with race QFCSC. The most significant interactions were those involving the QTL on 2BL. The 2BL QTL was found to have interactions with 3BS, 3BSc, 6B, and 7DS. The largest interaction was found with 3BSc, but the estimated effect was positive, indicating a less than additive effect. This could also suggest that since the resistance genes on 2BL and 3BSc have major effects, the presence of either gene would greatly reduce disease and ultimately provide very similar effects. The only beneficial interactions observed with 2BL were with infection type QFCSC data and with QTLs 6B and 7DS. Both interactions were small and unrepeated, indicating that they may not be real or of any great value for breeding. The combining of multiple resistance genes could result in a more than additive effect, but with most of the resistance genes that were identified here, that was not the case.

Kingbird has proven to have effective, durable resistance across multiple races of stem rust. We were able to explain up to 77% of the phenotypic variability observed against race QFCSC. However, the majority of the variability was explained by 2BL and 3BSc which did not explain the phenotypic variance in Kenya. For the Ug99 race group, most of the resistance was conferred by genes *Lr34* and *Sr2*. The combination of *Lr34* and *Sr2* alone does not explain enough of the variability that would account for the complete resistance that is observed with Kingbird. The nature of quantitative genes makes them very difficult to detect and with less than perfect phenotype data, the identification of all QTLs and their interactions can be nearly impossible. Part of the resistance that could not be captured for Kingbird could be attributed to *Lr46*. Since *Lr46* was detected in both parents and not able to be mapped, we were unable to determine the additive effect of this resistance gene or any possible epistatic interactions that could have occurred. Previous studies have shown that resistance gene *Sr12*, even when defeated, still has complementary epistatic effects when found in combination with other APR

genes, specifically *Lr34* (Rouse, Talbert, et al., 2014). We identified this interaction only once with Rocky Ford infection type data for race QFCSC. The QTL located on 3BSc also had complementary epistasis with 3BS. However, the effects were small and unrepeated. The 2BL QTL was detected in Kenya, but only for one year. This could suggest that conquered seedling genes still might aid in resistance when combined with other resistance genes, but at a reduced effect. Previous studies have identified complementary epistasis with resistance genes *Sr9* and *Lr34* (Kolmer, Garvin, & Jin, 2011). We observed this interaction only once, with QFCSC infection type data. These findings suggest that defeated seedling genes interacting with APR genes could be responsible, in part, for Kingbird's resistance.

Table 2.1 Seedling phenotypic response to multiple races of stem rust.

Race	Replicate	Chinese Spring	Kingbird	KS05HW14
TTKSK	1	3+ ^a	3- lif ^b	3+
TTKSK	2	3+	3- lif	33+
TKTTF	1	3+	3	3+
TKTTF	2	3+	3- lif	3+
TRTTF	1	3+	2+3 lif	3+
TRTTF	2	3+	3-1 lif	3+
SCCSC	1	3+	;	3
SCCSC	2	3+	;	33+
QTHJC	1	3+	;1	3+
QTHJC	2	3+	0;	3+
TTTTF	1	3+	3-;	3+
TTTTF	2	3+	;1+	3+
QFCSC	1	3+	0;	3+
QFCSC	2	3+	;	3+
TPMKC	1	3+	;2-	3+
TPMKC	2	3+	12-	3+
RKQQC	1	3+	;1-	3+
RKQQC	2	3+	;2-	3+

^a Ratings of infection type on the Stakman scale. 0 = no symptoms, ; = hypersensitive flecks, 1 = small uredinia with necrosis, 2 = small to medium-sized uredinia with green islands and surrounded by necrosis or chlorosis, 3 = medium-sized uredinia with or without chlorosis, and 4 = large uredinia without chlorosis, + = uredinia larger than average for infection type, - = uredinia smaller than average for infection type, / = plants heterogeneous for infection type, with most common type listed first. A range of infection types on a single leaf is written by combining the high and low infection types, with the most common type listed first.

^b low infection frequency

Table 2.2 Analysis of variance and broad-sense heritability.

SV^a Data Set	σ^2_g	σ^2_e	Replicates	Broad Sense Heritability H^2
Kenya (Ug99 Group)	89.511	197.3	2	0.476 ^b
Rocky Ford & Greenhouse 2013 (QFCSC)	565.5	449.13	4	0.834
Greenhouse (RKQQC)	24.4449	357.79	2	0.120

IT Data Set	σ^2_g	σ^2_e	Replicates	Broad Sense Heritability H^2
Kenya (Ug99 Group)	-0.07562	4.7654	2	-0.033
Rocky Ford & Greenhouse 2013 (QFCSC)	2.2627	1.5179	4	0.856
Greenhouse (RKQQC)	0.4385	1.8755	2	0.319

^a SV=severity, IT=infection type.

^b The broad-sense heritability was calculated for each stem rust race.

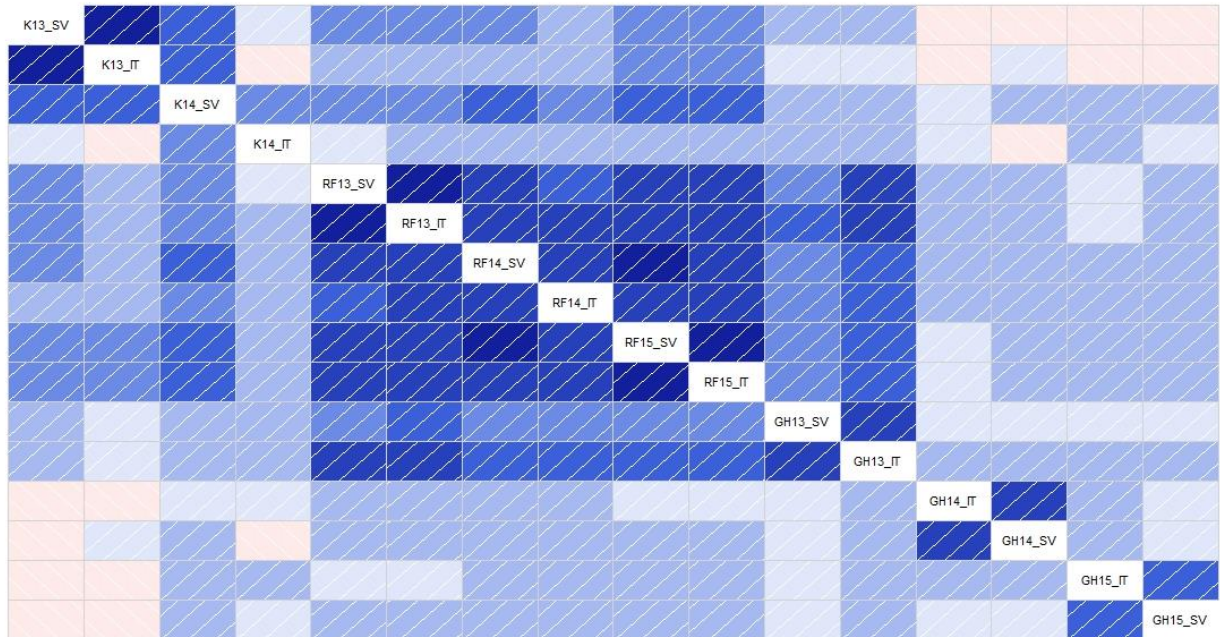


Figure 2.1 Correlation between independent phenotypic data sets

Correlation between independent phenotypic data sets for both severity (SV) and infection type (IT). The darker the blue the more closely related the data sets are to each other. The correlation coefficient is color-coded with 7 categories ranging from light pink to dark blue. The pink color is a negative correlation coefficient while all blue colors are positive. There are 6 shades of blue from lightest to darkest that represent the correlation coefficient value ranges which are: 0-.10, .11-.30, .31-.50, .51-.60, .61-.75, and .76-1 respectively. K=Kenya, R=Rocky Ford, and G=Greenhouse. The number indicates the year. Top left are the Kenya data sets (Ug99 group). Middle section is race QFCSC and the bottom right corner is race RKQQC. This shows correlation between common races over different locations.

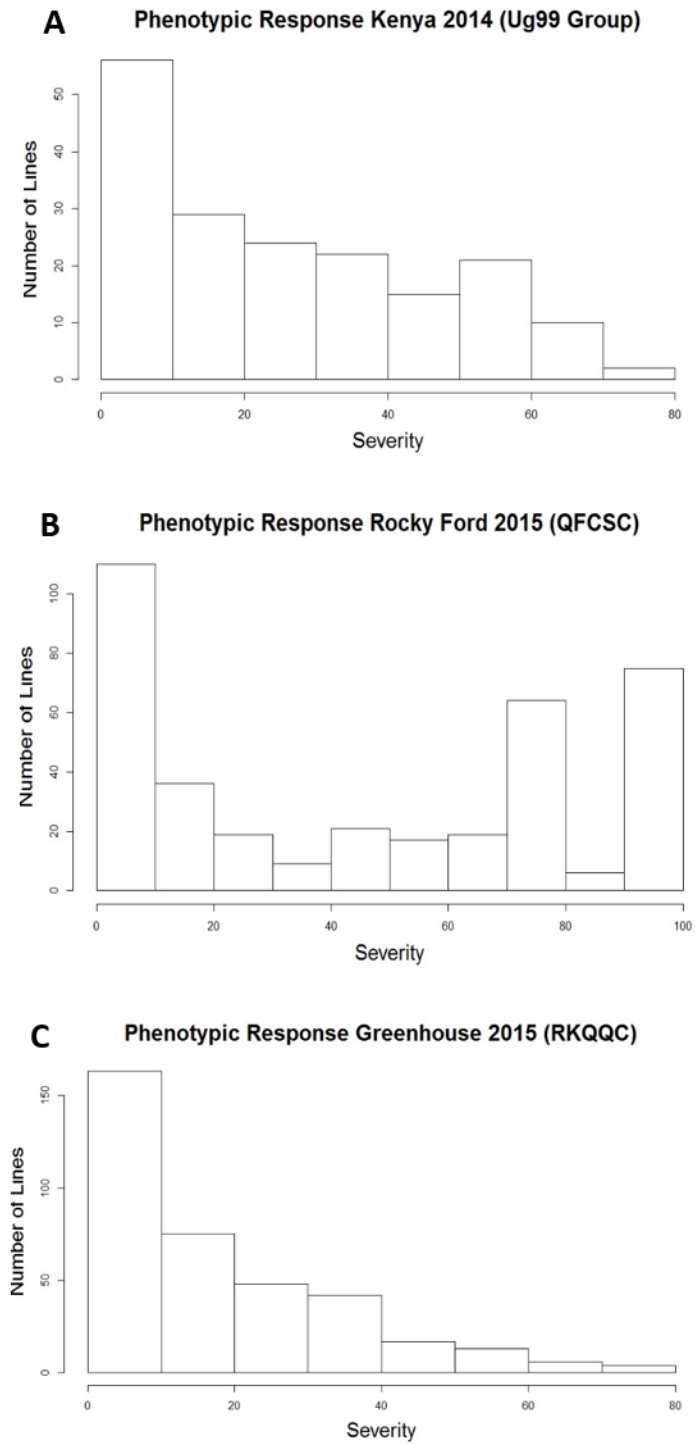


Figure 2.2 Phenotypic severity response of adult plants at all three locations.

(A) Kenya 2014 severity response to the Ug99 race group. (B) Rocky Ford Kansas 2015 severity response with race QFCSC. (C) Greenhouse 2015 phenotypic response to race RKQQC.

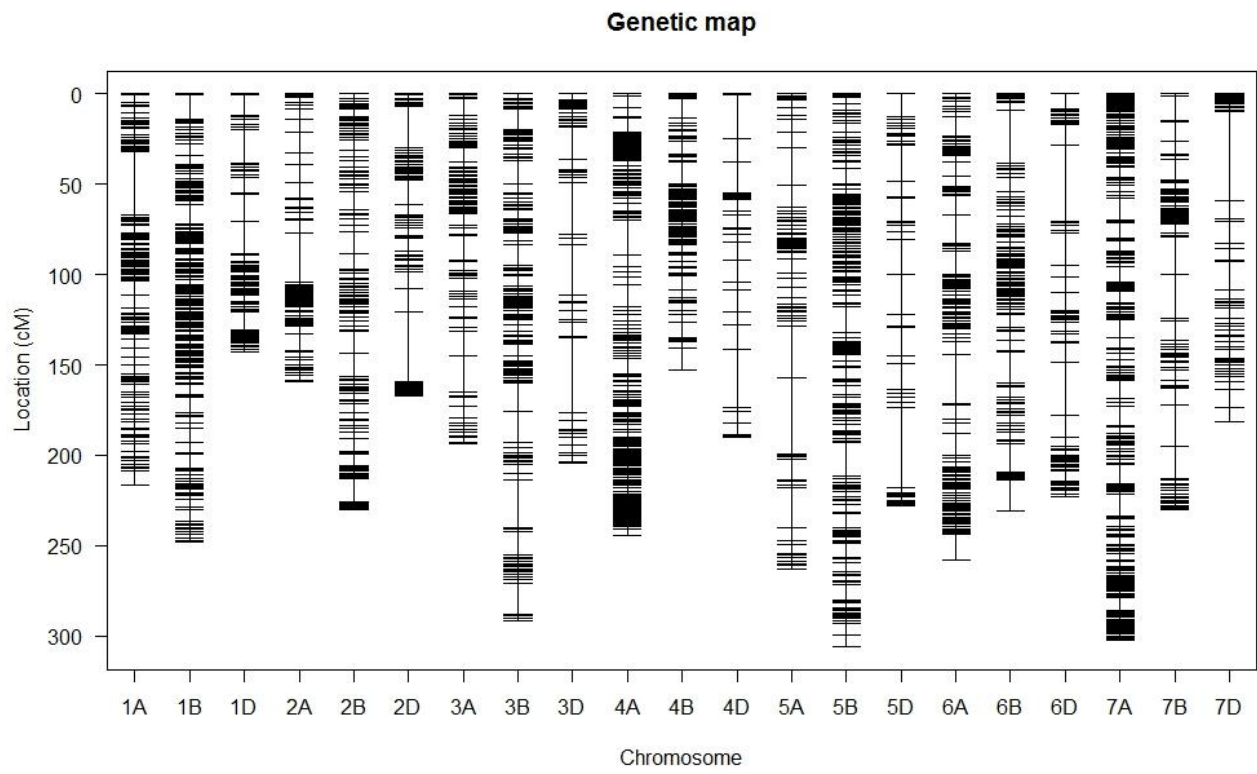


Figure 2.3 Distribution of markers over all 21 wheat chromosomes.

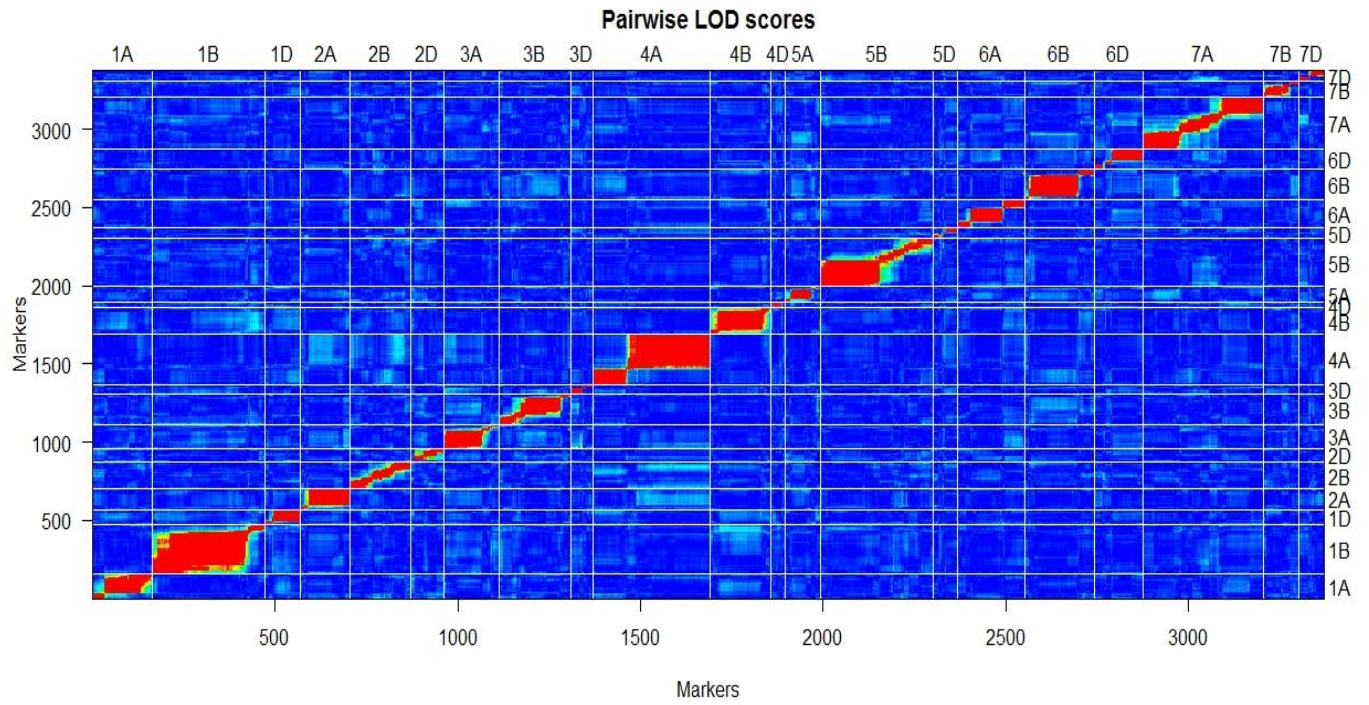


Figure 2.4 Linkage heat map

Represents all 21 chromosomes of wheat and was made in the R/qtl package of the R Software.

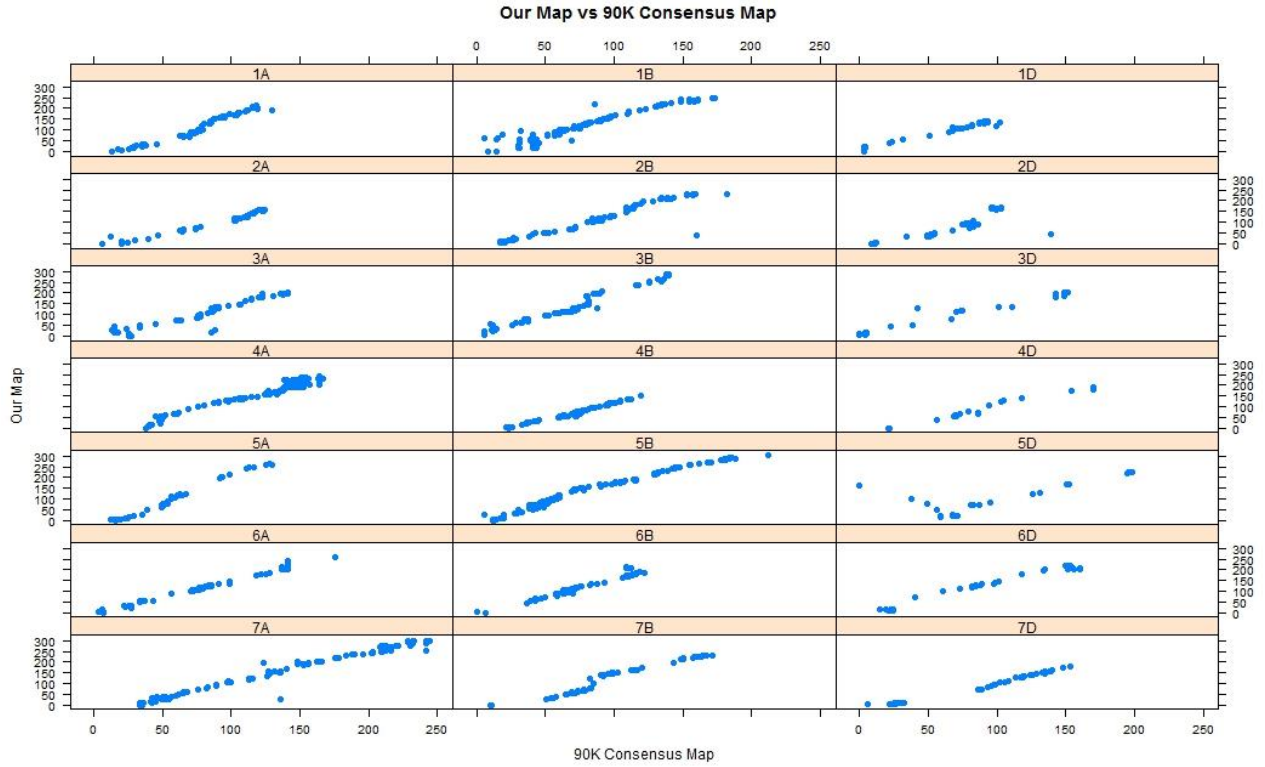


Figure 2.5 Correlation between our map and 90K Consensus Map

Only corresponding points are shown between our map and the 90K Consensus map. Our map has additional markers that did not have a corresponding position with the 90K consensus map and our not listed here.

Table 2.3 Significant QTLs from independent phenotypic data sets.

Chr	Race	Environments	Trait ^a	Position cM ^b	90K Consensus ^c	LOD	Phenotypic Variance (R ²)	Additive effect ^d
2BL	QFCSC	Rocky Ford 2015	SV	167.00	109.25	86.33	39.55	-18.12
	QFCSC	Rocky Ford 2015	IT	167.00	109.25	81.80	43.96	-1.53
	QFCSC	Rocky Ford 2014	SV	167.00	109.25	56.73	32.45	-11.85
	QFCSC	Rocky Ford 2014	IT	168.00	109.25	47.04	28.21	-1.51
	QFCSC	Rocky Ford 2013	SV	168.00	109.25	37.57	29.08	-19.33
	QFCSC	Rocky Ford 2013	IT	168.00	109.25	48.15	34.79	-1.22
	Ug99 Group	Kenya 2014	SV	157.60	109.48	4.36	7.71	-7.28
	RKQQC	Greenhouse 2015	SV	164.00	NA	2.42*	2.73	-3.40
	QFCSC	Greenhouse 2013	SV	160.00	109.24	6.95	7.41	-9.66
	QFCSC	Greenhouse 2013	IT	168.00	109.25	22.90	22.70	-1.20
2DS	QFCSC	Rocky Ford 2014	SV	14.00	NA	2.90*	1.16	-5.74
	Ug99 Group	Kenya 2014	IT	10.00	NA	4.63	10.69	-1.20
	RKQQC	Greenhouse 2015	SV	10.00	NA	2.87*	3.25	-4.82
	RKQQC	Greenhouse 2015	IT	5.40	NA	2.47*	2.78	-0.31
	RKQQC	Greenhouse 2014	IT	10.00	NA	3.10*	3.60	-0.44
	QFCSC	Greenhouse 2013	SV	20.00	NA	4.13	4.32	-11.63

Table 2.3 Continued

Chr	Race	Environments	Trait	Position cM	90K Consensus	LOD	Phenotypic Variance (R²)	Additive effect	
3BS	QFCSC	Rocky Ford 2015	SV	27.00	11.56	28.24	8.70	-8.64	
	QFCSC	Rocky Ford 2015	IT	30.00	4.00	6.61	2.15	-0.45	
	QFCSC	Rocky Ford 2014	SV	27.00	11.56	34.29	16.82	-9.58	
	QFCSC	Rocky Ford 2014	IT	30.00	4.00	34.38	18.92	-1.37	
	QFCSC	Rocky Ford 2013	SV	27.00	11.56	14.83	9.90	-11.14	
	QFCSC	Rocky Ford 2013	IT	20.00	11.26	16.68	9.80	-0.56	
	Ug99 Group	Kenya 2014	SV	35.00	14.10	5.42	9.73	-8.02	
	Ug99 Group	Kenya 2013	SV	30.00	4.00	6.25	12.00	-4.30	
	Ug99 Group	Kenya 2013	IT	30.00	4.00	6.03	12.59	-0.99	
	RKQQC	Greenhouse 2015	SV	25.00	5.86	4.85	5.55	-4.74	
	RKQQC	Greenhouse 2015	IT	15.00	11.56	3.71	4.22	-0.39	
	QFCSC	Greenhouse 2013	IT	30.00	4.00	6.01	5.77	-0.57	
	3BSc	QFCSC	Rocky Ford 2015	SV	117.50	68.87	62.99	24.48	-13.28
		QFCSC	Rocky Ford 2015	IT	117.50	68.87	49.90	19.25	-1.15
QFCSC		Rocky Ford 2014	SV	117.50	68.87	20.26	9.22	-8.41	
QFCSC		Rocky Ford 2014	IT	115.00	67.20	30.45	16.33	-1.10	
QFCSC		Rocky Ford 2013	SV	117.50	68.87	10.31	6.69	-10.20	
QFCSC		Rocky Ford 2013	IT	118.00	69.53	10.49	5.93	-0.55	
QFCSC		Greenhouse 2013	SV	116.00	68.87	7.36	7.87	-9.53	
QFCSC		Greenhouse 2013	IT	115.00	67.20	6.11	5.40	-0.56	

Table 2.3 Continued

Chr	Race	Environments	Trait	Position cM	90K Consensus	LOD	Phenotypic Variance (R²)	Additive effect
5DL	QFCSC	Rocky Ford 2015	SV	142.00	NA	3.98	1.05	-5.44
	QFCSC	Rocky Ford 2014	IT	135.00	131.54	4.98	2.27	-0.55
	RKQQC	Greenhouse 2015	IT	128.00	NA	2.84*	3.22	-0.42
	RKQQC	Greenhouse 2014	SV	130.00	131.54	4.11	5.01	-5.60
	RKQQC	Greenhouse 2014	IT	137.00	131.54	3.96	4.60	-0.48
7BL	QFCSC	Rocky Ford 2015	SV	225.00	163.16	10.91	3.00	7.73
	QFCSC	Rocky Ford 2015	IT	227.00	162.53	10.40	3.47	0.49
	QFCSC	Rocky Ford 2014	SV	222.00	158.51	5.47	2.23	5.23
	Ug99 Group	Kenya 2014	SV	230.00	166.99	4.54	8.05	7.16
	Ug99 Group	Kenya 2013	SV	230.00	166.99	4.04	7.56	3.26
Ug99 Group	Kenya 2013	IT	215.00	148.65	4.08	8.31	0.81	
7DS	QFCSC	Rocky Ford 2015	SV	62.00	NA	8.01	2.17	-7.37
	QFCSC	Rocky Ford 2015	IT	62.00	NA	8.70	2.87	-0.50
	QFCSC	Rocky Ford 2014	SV	60.00	NA	4.24	1.71	-4.91
	QFCSC	Rocky Ford 2014	IT	62.00	NA	6.08	2.79	-0.55
	QFCSC	Rocky Ford 2013	SV	62.00	NA	3.17	1.97	-5.66
	QFCSC	Rocky Ford 2013	IT	65.00	86.19	2.63*	1.42	-0.27
	Ug99 Group	Kenya 2014	SV	62.00	NA	6.00	10.84	-9.24
	Ug99 Group	Kenya 2014	IT	60.00	NA	3.44	7.81	-0.96
	Ug99 Group	Kenya 2013	SV	62.00	NA	1.85*	3.35	-2.44
	6B	QFCSC	Rocky Ford 2013	IT	45.00	36.69	4.60	2.58

All seven repeated QTLs that were found among the independent phenotypic data sets at each location. Not all QTLs that are listed are significant; some are only shown to indicate repetition. 5% genome-wide LOD thresholds ranged from 3.3-3.4 and can be viewed in Table 2.5.

^a SV=Severity, IT=Infection Type

^b cM position based off of map that was calculated in JoinMap 4.1

^c Position of map that was made relative to positions from the 90K Consensus Map

^d Additive effect of QTL; if negative, QTL is derived from Kingbird, if positive, QTL is derived from KS05HW14

*QTLs that are not significant based off of 5% genome-wide LOD thresholds.

Table 2.4 Significant QTLs identified with BLUPs

Chr	Race	Environments	Trait^a	Position cM^b	90K Consensus^c	LOD	Phenotypic Variance (R²)	Additive effect^d
2BL	QFCSC	Rocky Ford & Greenhouse 13	SV	167.00	109.25	84.09	39.63	-10.40
	QFCSC	Rocky Ford & Greenhouse 13	IT	167.00	109.25	89.25	44.52	-1.18
	Ug99 Group	Kenya	SV	160.00	109.24	3.79	5.23	-1.72
	RKQQC	Greenhouse	SV	167.00	109.25	3.83	4.02	-0.40
2DS	QFCSC	Rocky Ford & Greenhouse 13	SV	17.00	NA	2.72*	0.75	-3.58
	RKQQC	Greenhouse	SV	10.00	NA	3.26*	3.41	-0.48
	RKQQC	Greenhouse	IT	15.00	NA	4.34	4.06	-0.14
3BS	QFCSC	Rocky Ford & Greenhouse 13	SV	30.00	4.00	35.34	11.96	-7.03
	QFCSC	Rocky Ford & Greenhouse 13	IT	30.00	4.00	31.51	10.83	-0.65
	Ug99 Group	Kenya	SV	35.00	14.10	7.76	11.09	-2.37
	Ug99 Group	Kenya	IT	29.00	29.00	5.99	9.34	0.02
	RKQQC	Greenhouse	SV	30.00	4.00	3.20*	3.34	-0.37
	RKQQC	Greenhouse	IT	30.00	4.00	3.70	3.45	-0.08
3BSc	QFCSC	Rocky Ford & Greenhouse 13	SV	117.50	68.87	45.63	16.47	-8.26
	QFCSC	Rocky Ford & Greenhouse 13	IT	117.50	68.87	38.71	13.94	-0.73

Table 2.4 Continued

Chr	Race	Environments	Trait^a	Position cM^b	90K Consensus^c	LOD	Phenotypic Variance (R²)	Additive effect^d
5DL	QFCSC	Rocky Ford & Greenhouse 13	SV	155.00	NA	5.07	1.41	-3.95
	QFCSC	Rocky Ford & Greenhouse 13	IT	135.00	131.54	5.27	1.53	-0.24
	RKQQC	Greenhouse	SV	135.00	131.54	4.04	4.25	-0.48
	RKQQC	Greenhouse	IT	130.00	131.54	13.06	12.70	-0.16
7BL	QFCSC	Rocky Ford & Greenhouse 13	SV	216.00	NA	3.12*	0.86	2.38
	Ug99 Group	Kenya	SV	216.00	NA	5.26	7.34	1.90
	Ug99 Group	Kenya	IT	215.00	148.65	3.47	5.27	-11.00
7DS	QFCSC	Rocky Ford & Greenhouse 13	SV	62.00	NA	5.65	1.58	-3.61
	QFCSC	Rocky Ford & Greenhouse 13	IT	62.00	NA	7.23	2.13	-0.30
	Ug99 Group	Kenya	SV	62.00	NA	6.05	8.49	-2.24
	Ug99 Group	Kenya	IT	64.00	NA	2.57*	3.87	0.01
	Ug99 Group	Kenya	IT	125.00	82.14	3.44	5.22	0.012
4A	RKQQC	Greenhouse	IT	202.80	NA	10.9	10.50	-0.13

All seven QTLs for all BLUPs. BLUPs were made based off of common races and either severity or infection type. Not all QTLs that are listed are significant; some are only shown to indicate repetition. 5% genome-wide LOD thresholds ranged from 3.3-3.4 and can be viewed in Table 2.6.

^a SV=Severity, IT=Infection Type

^b cM position based off of map that was calculated in JoinMap 4.1

^c Position of map that was made relative to positions from the 90K Consensus Map

^d Additive effect of QTL; if negative, QTL is derived from `Kingbird`, if positive, QTL is derived from `KS05HW14`

*QTLs that are not significant based off of 5% genome-wide LOD thresholds.

Table 2.5 Interactions for independent phenotypic data sets.

Environment	Trait^a	LOD 5%	Model^b	Interactions	LOD	Phenotypic Variance (R²)^c	EST Effect^d	Phenotypic Variance (R²)^e
Rocky Ford 2013(QFCSC)	SV	3.36	Additive					50.73
Rocky Ford 2013(QFCSC)	IT	3.43	Full	2BLx6B	3.61	2.05	-0.28	55.76
Rocky Ford 2014(QFCSC)	SV	3.46	Full	2BLx3BS	5.64	2.40	6.39	67.05
				2BLx3BSc	3.97	1.67	5.67	
Rocky Ford 2014(QFCSC)	IT	3.41	Full	2BLx7DS	3.55	1.60	-0.46	64.68
				3BSx3BSc	11.68	5.54	-0.68	
Rocky Ford 2015(QFCSC)	SV	3.41	Full	2BLx3BSc	18.37	5.31	12.93	78.95
				3BSx3BSc	4.57	1.21	5.86	
Rocky Ford 2015(QFCSC)	IT	3.38	Full	2BLx3BSc	3.91	1.25	0.39	74.49
				3BSx7DS	4.45	1.43	-0.38	
Kenya 2013 (Ug99 Group)	SV	3.52	Additive					29.06
Kenya 2013 (Ug99 Group)	IT	3.47	Additive					22.65
Kenya 2014 (Ug99 Group)	SV	3.50	Additive					35.04
Kenya 2014 (Ug99 Group)	IT	3.63	Additive					15.55
Greenhouse 2013(RKQQC)	SV	3.48	Additive					40.23
Greenhouse 2013(RKQQC)	IT	3.40	Additive					38.25
Greenhouse 2014(RKQQC)	SV	3.45	Additive (1QTL)					5.01
Greenhouse 2014(RKQQC)	IT	3.60	Additive					8.79
Greenhouse 2015(RKQQC)	SV	3.50	Additive					11.25
Greenhouse 2015(RKQQC)	IT	3.54	Additive					11.12

Only significant interactions are listed.

^a SV=Severity, IT=Infection Type

^b Model selection, either additive (no interaction) or full (interaction)

^c phenotypic variation explained for that particular interaction.

^d Estimated effect for that interaction; if negative; interaction is less than additive, if positive; interaction is greater than additive.

^e phenotypic variation explained by entire model (additive or full).

Table 2.6 Interactions identified with BLUPs.

Environment	Trait^a	LOD 5%	Model^b	Interaction	LOD	Phenotypic Variance(R²)^c	EST Effect^d	Phenotypic Variance(R²)^e
Rocky Ford & Greenhouse 2013 (QFCSC)	SV	3.43	Full	2BLx3BS	3.68	1.01	3.12	77.99
				2BLx3BSc	9.41	2.69	5.25	
Rocky Ford & Greenhouse 2013 (QFCSC)	IT	3.46	Full	2BLx7DS	3.17	0.91	-0.20	77.01
				3BSx3BSc	4.87	1.41	-0.24	
Kenya (Ug99 Group)	SV	3.44	Additive					31.59
Kenya (Ug99 Group)	IT	3.35	Additive					24.80
Greenhouse (RKQQC)	SV	3.28	Additive					16.71
Greenhouse (RKQQC)	IT	3.34	Full	5DLx4A	7.71	7.25	-0.13	27.00

All interactions that are listed are significant.

^a SV=Severity, IT=Infection Type

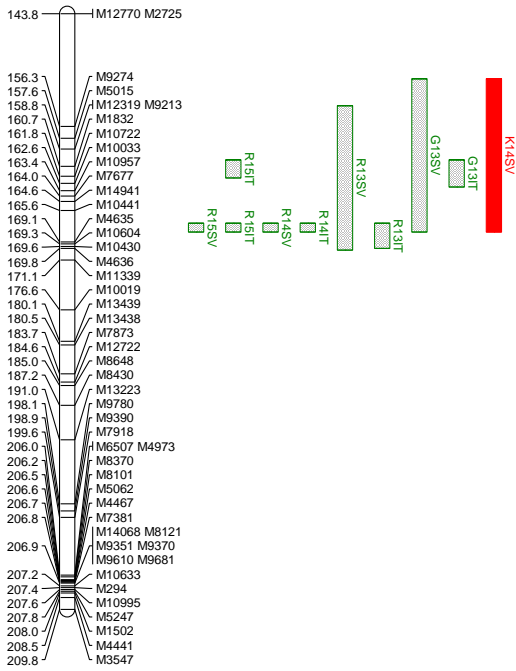
^b Model selection, either additive (no interaction) or full (interaction)

^c phenotypic variation explained for that particular interaction.

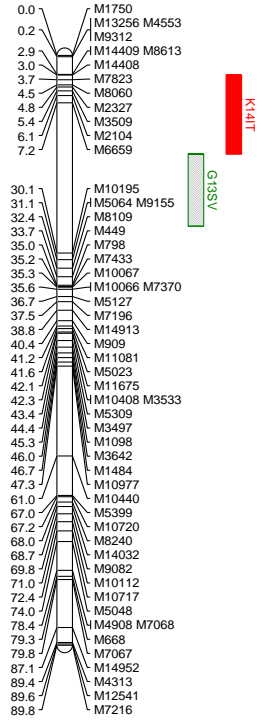
^d Estimated effect for that interaction; if negative; interaction is less than additive, if positive; interaction is great than additive.

^e phenotypic variation explained by entire model (additive or full).

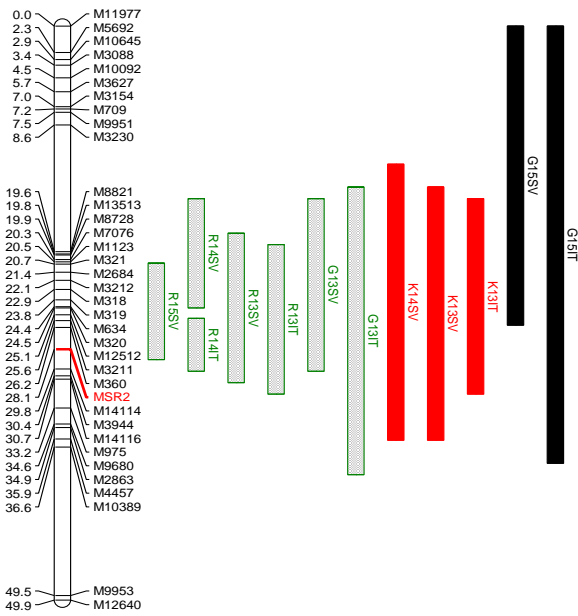
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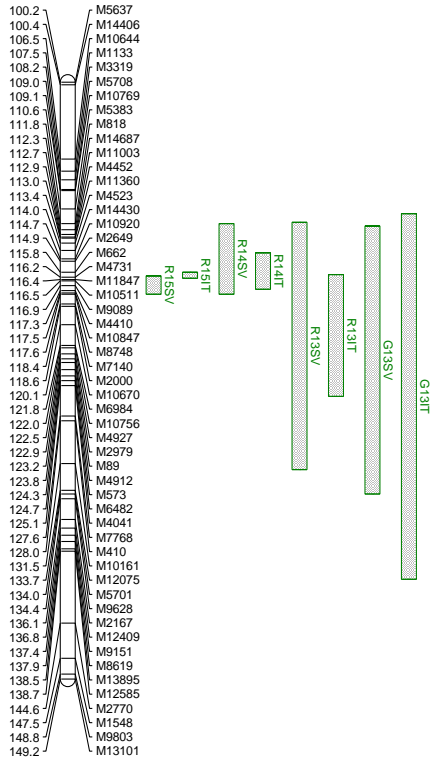
2DS



3BS



3BSc



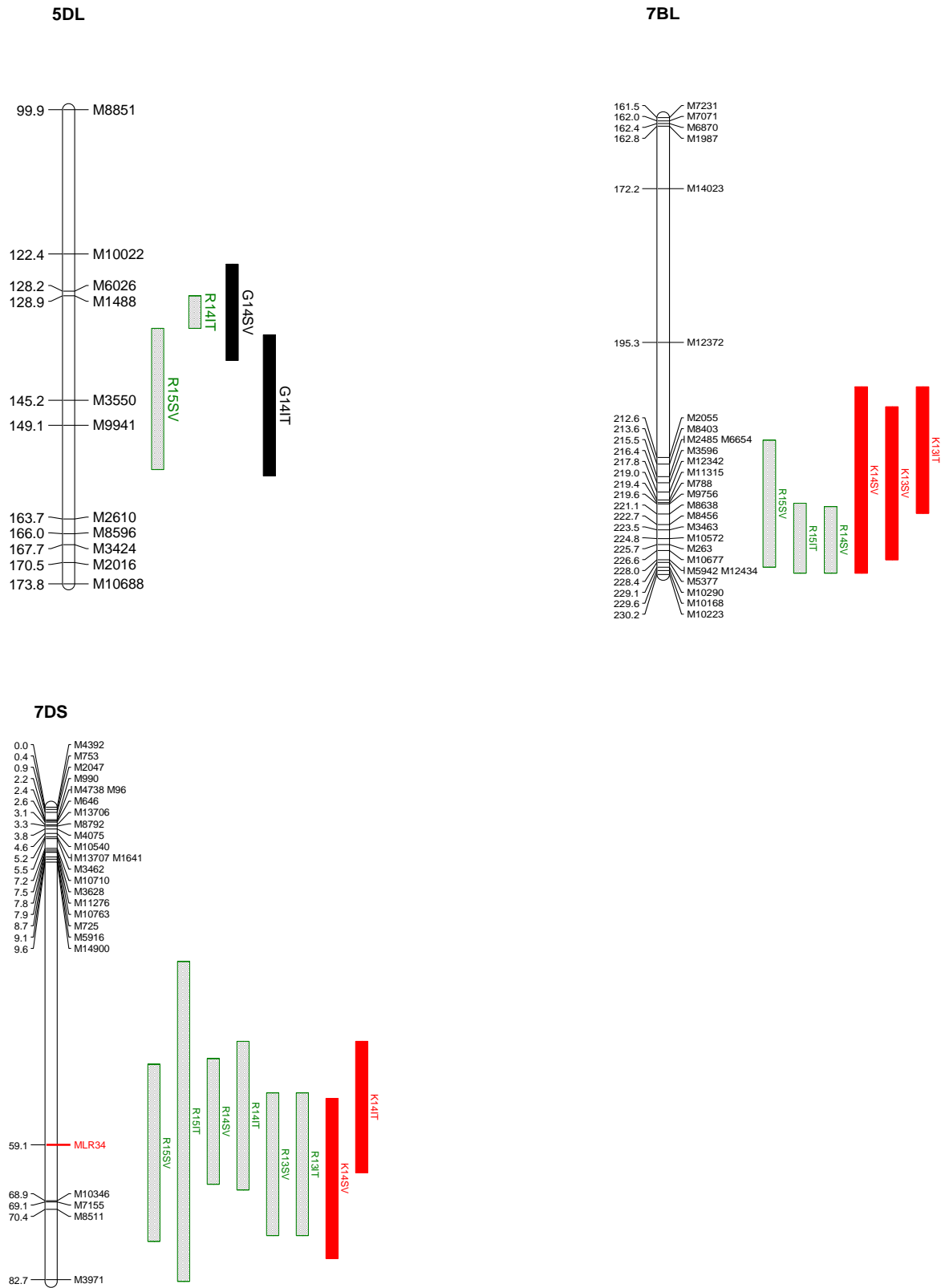


Figure 2.6 95% Bayesian confidence intervals

Linkage groups are partial sections of the chromosome where the QTL was located. 95% Bayesian confidence intervals were calculated with R/qtl package of the R Software. The linkage maps were constructed with MapChart 2.3. All bars are from independent phenotypic data sets by year and location. R=Rocky Ford, K=Kenya and G=Greenhouse. The number indicates the year that the phenotypic data was recorded. The green bars with stripes represent QTLs that were located with race QFCSC. The bars that are in red, represent QTLs located with the Ug99 race group. The black bars indicate QTLs located with race RKQQC. Confidence intervals that are shown are only for significant QTLs.

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