GENETICS OF RESISTANCE TO LEAF AND STRIPE RUST DISEASES IN THE SPRING WHEAT ‘AMADINA’

by

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B.Sc. (Hons.), University of Juba, Sudan, 1996
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Abstract

In this research, a recombinant inbred line (RIL) population derived from cross between a leaf rust- and stripe rust-susceptible spring wheat 'Avocet S' and a slow leaf- and stripe-rusting resistant spring wheat 'Amadina' was used to postulate and map leaf rust seedling resistance genes, identify quantitative trait loci (QTL) for slow-rusting resistance against leaf and stripe rust, and study slow leaf-rusting components, latent period and infection frequency. Two known *Lr* genes (*Lr23*, and *Lr26*) were identified to be present in 'Amadina' through gene postulation, pedigree, cytogenetic, and polymerase chain reaction analyses. One unknown gene associated with seedling resistance was also mapped on chromosome 1BL. In greenhouse experiment, it was estimated that at least five genes conditioning final disease severity (FS) and latent period (LP), and four genes conditioning infection frequency (IF), segregated in the population. Correlations between LP and FS, and LP and IF were moderately negative, and that between IF and FS was moderately positive, indicating inter-dependence of the traits. Two QTL on chromosomes 1BL and 6BL were associated with LP and FS, and three QTL on chromosomes 1BL, 6BL and 2DS were associated with IF. Segregation of the RIL population in field experiment indicated that there were at least four and three adult plant resistance (APR) genes involved in resistance for leaf and stripe rust. Six QTL on chromosomes 3AL, 4AL, 1BL, 5BL, and 7BL were associated with APR for leaf rust, and seven QTL on chromosome 4AL, 5AL, 1BL, 2BL, 4BL, 5BL, 2DL, and 4D were associated with APR for stripe rust. Our results indicated that the major portion of genetic variability for slow-rusting resistance was additive gene action, and, to some extent, epistasis. In this research, we also explored the utility of remote sensing and geographic information systems (GIS) and analytical operations to discriminate leaf rust pustules from other parts of leaf and to accurately determine pustule size in 'Amadina' and 'Avocet S'.

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Dedication

I dedicate this dissertation to my dad and youngest brother. I wish they had lived to see me completing my PhD program, and playing a role in reduction of poverty, alleviation of hunger, and fostering of food security.

CHAPTER 1 - Literature Review

Overview

Wheat is one of the oldest and most widely grown among crop plants providing food for humans and animals. It occupies one sixth of crop acreage worldwide, feeds nearly half of the world population and provides one fifth of total food calories and protein in human nutrition (Curtis 2002). With the world population increasing and food security projected to become more critical, increasing wheat yield potential remains a high priority. Global demand for wheat in the year 2020 is forecasted to double the current production level, and to meet this demand, a step-wise annual increase in global production from the present production level becomes a necessity (Rosegrant et al. 1995; Braun et al. 1998). Global wheat breeding efforts over the past 40+ years have made significant contributions in enhancing yield potential. Even though absolute yields are determined by genetic potential, level of diseases is one of the most important determinants of actual yield.

So far, the development and widespread use of disease resistant varieties has succeeded in limiting global epidemic outbreaks. But conditions change leading to spread of existing races of a pathogen, continuous evolution of races due to mutation or recombination between different races within the same group of pathogens, sometimes with devastating effects. Consequently, specific genes that confer resistance could lose their effectiveness. One group of wheat pathogens that causes particular concern are the fungi that cause leaf and stripe rust diseases (Roelfs et al. 1992; McIntosh et al. 1995; Kolmer et al. 2007).

Crop losses due to leaf and stripe rust diseases can be high to the extent that a farmer is left little to harvest, while the seed will be mostly shriveled and of lower quality (Roelfs et al. 1992; McIntosh et al. 1995; Kolmer et al. 2007). To achieve sufficiently high resistance to these fungal pathogens is an ongoing challenge for wheat breeders. Characterization of pathogen avirulence and plant disease resistance genes, and search for new resistance sources are critical aspects for development of wheat with increased resistance to these diseases.

While wheat breeders have made significant progress in the development of resistant cultivars against the pathogens causing leaf and stripe rust diseases, major advances in the area of molecular biology and biotechnology in the last few decades have resulted in increased understanding and characterization of the disease resistant genes at the molecular level (Gupta et al. 1999; Lörz and Wenzel 2005). Tools based on molecular markers made publicly available by countless researchers around the world have provided detailed information regarding the structure of the wheat genome. Genetic linkage maps enabled researchers to estimate the number, chromosomal positions, and degrees of effects of genes conditioning resistance against the rust pathogens. Furthermore, molecular markers linked to disease resistance can be used as chromosomal landmarks to facilitate the selection of the chromosome segments that include the disease resistance trait during breeding process. Molecular markers are particularly useful for incorporating disease resistance traits that have strong interaction with the environment, and controlled by several genes with minor effects, and pyramiding of resistance genes. An additional advantage of the incorporation of marker-assisted selection into breeding programs is that very different types of traits can be manipulated using the same technology.

Slow-rusting type of resistance is currently of interest to breeders worldwide due to its race non-specificity and proven durability. This trait is partially recessive, controlled by genetic factors with moderately high heritability, and generally with additive gene action or interaction among additive genes. Although several genomic locations associated with slow rusting have been reported by various researchers, *Lr34/Yr18*, *Lr46/Yr29*, and *Sr2* are so far the only slow-rusting genes being widely utilized in breeding programs (Roelfs 1988; Singh and Rajaram 1992; Singh et al. 2001; McIntosh et al. 2003). Since new races of the rust pathogens are known to mutate rapidly and evolve new physiological races it is reasonable to assume that isolates of the pathogens with virulence to the two slow-rusting gene complexes may eventually appear and even dominate in the pathogen populations if they have selective advantage. Hence, it is prudent to identify alternative slow-rusting genes. Also tagging of slow-rusting genes with molecular markers will facilitate the incorporation of several of these additive genes

into a single background in order to obtain acceptable levels of resistance under high disease pressure.

In this dissertation, I described the mapping of genes conferring slow-rusting resistance against leaf and stripe rust diseases in wheat using a recombinant inbred line (RIL) population. I also report on the molecular dissection of the components of slow-leaf rusting, and postulation and mapping of seedling leaf rust resistant genes.

The origins of modern wheat

The evolution of wheat

Genetic studies have revealed that the polyploid wheat species constitute two evolutionary lineages (Figure 1): *Triticum turgidum* (AABB) and *T. aestivum* (AABBDD) comprise one lineage, and the other lineage is believed to have involved a hybridization of *T. timopheevi* (A^tA^tGG) and *T. monococcum* (A^mA^m) which gave rise to *T. zhukovsky* ($A^tA^tA^mA^mGG$) (http://www.k-state.edu/wgrc/).

Genetically, common bread wheat (*Triticum aestivum* L. emend. Thell) is a disomic allohexaploid (AABBDD; 2n = 42). According to Gill et al. (2004), the diploid A, B, and D genomes of the ancestral wheat species diverged from their common ancestor about 3 million years ago. Two separate amphidiploidization events were involved in the origin of the hexaploid wheat. The first event (at least 30,000 years ago) involved hybridization between the diploid *T. urartu* (AA) Tumanian ex Gandilyan and a species from section Sitopsis, a close relative to the extant *Aegilops speltoides* (genome SS) Tausch, followed by chromosome doubling to form the wild, predominantly self-pollinating emmer (*T. dicoccoides;* 2n = 4x = 28; genome AABB) (Dvorak *et al* 1993). The second event (about 9,000 years ago) involved hybridization of *Triticum turgidum* spp. *dicoccon* (AABB, n = 14), a domesticated form of the spp. *dicoccoides* (Körn.ex Asch. et Graebn.) Thell., with the diploid goat-grass, *Ae. tauschii* ssp. *strangulata* (syn. *Aegilops squarrosa* L., genomes DD, 2n =14), followed by chromosome doubling to form an early Spelt (*Triticum spelta*; 2n = 42; genome AABBDD) (McFadden and Sears 1946; Kihara 1965; Feldman 2001).

Domestication of wheat

The diploid and tetraploid wheats were domesticated mainly through selection and propagation of plants with larger kernels size that are free-threshing, and homozygous for the recessive non-shattering allele (Evans et al. 1973; Doebley et al. 2006). Humans domesticated wheat about 8,000 to 10,000 years ago in the Fertile Crescent (modern day Iraq and parts of Turkey, Syria and Iran) (Harlan 1992; Gill et al. 2004)). Einkorn (*Triticum monococcum*) and emmer (reported both as *T. araraticum* and *T. turgidum* spp. *dicoccoides*) are among the oldest known cultivated cereal grains, commonly referred to as “ancient wheats.” Recent genetic evidence indicates that einkorn wheat may have been domesticated from wild einkorn (*T. monococcum* ssp. *aegilopoides*) in the region of the Karacadag mountains in south east Turkey (Huen et al. 1997). Both wild and cultivated einkorn seed remains have been excavated in the nearby archaeological sites dating from 7500 to 6200 BC (Gill et al. 2004). Remains of cultivated emmer (*T. turgidum* spp. *dicoccum*) have been discovered at several archeological sites in Syria dating to 7500 BC (Zohary and Hopf 1993). The free-threshing form arose by mutation from primitive emmer wheats.

Bread wheat (AABBDD, 2n = 42) arose further northwest in the region between the Black Sea and Caspian Sea (Dvorak et al. 1998) which falls within the main geographical distribution of *Ae. tauschii* spp. *strangulata* (Tanaka 1983). Free-threshing, naked, bread wheat is thought to have developed soon afterwards before 4000 BC (Evans et al. 1973).

Leaf and stripe rust diseases of wheat

Leaf or brown rust and stripe or yellow rust are the most common and among the most damaging foliar diseases of wheat caused by obligate pathogens. These pathogens are of prime importance due to their geographical distribution, capacity to form new races that attack previously resistant cultivars, capacity to produce huge numbers of spores, effective long-distance dissemination, and potential to cause epidemics under optimal environmental conditions (Roelfs et al. 1992; McIntosh et al. 1995). These pathogenic fungi belong to the genus *Puccinia*, family Pucciniaceae, order Uredinales and class

Basidiomycetes. Leaf and stripe rust fungi are highly specialized plant pathogens with narrow host ranges. Resistance often follows the gene-for-gene relationship (Flor 1971).

Leaf (or brown) rust

Leaf rust, caused by the fungus *Puccinia triticina* Eriks. (formerly *P. recondita*), is a major foliar disease of wheat (*Triticum aestivum* L.) in humid and warm environments (Roelfs et al. 1992). This disease occurs more regularly and has a wider geographical distribution than stripe rust. The disease is recognized by its characteristic small to relatively large reddish-orange pustules that erupt from the upper epidermis of leaves in fall or spring. The pustules are small round lesions that contain thousands of red-colored urediniospores. Resistant cultivars are often characterized with small-sized pustules and/ or necrotic spots which do not normally develop spores. Necrosis appears around the pustule when resistance is incomplete (McIntosh et al. 1995). Moderate nights and warm days (16° – 27° C) that create long dew periods in the wheat canopy are ideal for rust development. Infection can occur in as little as four hours during favorable weather. Once established, a new generation of urediniospores may be produced every 7 to 14 days if environmental conditions are favorable. Dispersal of spores to upper leaves and between fields is favored by dry, windy conditions. As wheat nears maturity late in the season, the urediospores are replaced with teliospores (USDA-ARS Cereal Disease Laboratory: http://www.usda.gov/wps/portal/usda/usdahome).

Life cycle

Wheat leaf rust pathogen has a sexual and an asexual cycle. The sexual cycle of wheat leaf rust requires the alternate hosts, *Thalictrum speciosissimum*, *Isopyrum fumaroides*, and possibly *Anchusa* and *Clematis* species. (Knott 1989). Although *Thalictrum* and *Isopyrum* species are native to North America, *P. triticina* sexual stage is rarely found. This may be due to the fact that these species have relative resistance against basidiospore infection (Bolton et al. 2008). Saari et al. (1968) studied the reaction of 113 North American native Thalictrum species, 5 *T. alpinum* L., 4 *T. sparsiflorum* Turcs., 2 *T. dioicum* L., 36 *T. dasycarpum* Fisch, and Lall., and 66 *T. fendleri* complex comprised of plants having characteristics included in the species *T. confine* Fern., *T. jendleri* Engelm. Ex Gray, *T. occidentale* A. Gray, *T. polycarpum* (Torr) S. Wats, and *T. venulosum*

Trelease), and 21 European species, *T. alpinum* L., *T. dipterocarpum*, *T. aquilegifolium*, *T. sparsiflorum*, *T. minus* var. *adiantifolium*, 6 *T. flavum* L., and 10 *T. speciosissimum* Loefl. All species from Europe and two North American species, *T. sparsiflorum* and *T. dioicum*, were susceptible. Because the pathogen only reproduces asexually, a population of distinct races that do not cross with each other are found in North America. As a result, emergence of new races is slowed down since genetic variation is driven mainly by mutation (Kolmer 2005).

Disease epidemics

Under conditions of moderate nights and warm days (16 – 27 ^{o}C) that create long dew periods in the wheat canopy, severe rusting of susceptible varieties occurs 30 to 40 days after initial rust development. Yield losses due to leaf rust may be as high as 30 to 50% in severe epidemics (Roelfs et al. 1992; McIntosh et al. 1995). The yield losses mainly derive from premature senescence, reduced number of kernels per spike, reduced test weight, and diminished kernel quality. Yield losses depend on the crop developmental stage when the initial infections occur and the relative resistance or susceptibility of the wheat cultivar. According to Kolmer et al. 2005, greater yield losses result from infections before the jointing and tillering stages. Infections after heading, when grain filling is progressing, often cause less crop loss.

In North America, *P. triticina* was introduced with wheat cultivation in the early 17th century (Chester 1946). Currently leaf rust is the most common and widely distributed disease of wheat in the United States. Widespread and continual use of wheat cultivars with differing resistance genes has placed constant selection pressure on the *P. triticina* population, and thus has led to a highly diverse and virulent population of the pathogen (Bolton et al. 2008). Kolmer et al. (2007) reported that up to 70 different races of the leaf rust pathogen are identified in the United States each year base on virulence patterns of 20 differential lines. In Kansas, the leading wheat-producing state in the United States, losses due to leaf rust have averaged nearly 3% from 1993 to 2005, ranging from 11.0% in 1993 to trace levels of loss in 1996 and 2002 (USDA-ARS Cereal Disease Laboratory: http://www.usda.gov/wps/portal/usda/usdahome/). According to the Kansas Department of Agriculture, leaf rust caused a 14% loss in winter wheat yield in 2007 (Bolton et al. 2008).

Stripe (or yellow rust)

Stripe rust, caused by the fungal pathogen *Puccinia striiformis* Westend. f. sp. *tritici* Eriks, is one of the most damaging diseases affecting bread wheat in temperate regions (Chen 2005; Kolmer 2005). In Europe and many parts of the world, stripe rust is known as 'yellow rust' because of the distinctive coloring of the pustules. The disease is characterized by bright yellow-orange pustules arranged between the veins in stripes. Stripes are not formed on seedling leaves. Atypical symptoms for stripe rust, including yellow spotting, can occur on varieties with resistance to some strains. The disease usually appears earlier in the season than other rusts because it can develop at cooler temperatures. Dark-brown to black teliospores (sexual spores) appear as the plant matures and the temperatures rise (USDA-ARS Cereal Disease Laboratory website: http://www.usda.gov/wps/portal/usda/usdahome/).

Life cycle

Volunteer wheat and perennial grassy weeds can serve as an important reservoir of inoculum. In cooler climates it has also been known to survive as dormant mycelium under snow cover. The life history of *P. striiformis* had remained a mystery for more than a century because alternative host for the pathogen had not been identified (Stubbs 1985) and no recombination under natural conditions had been reported (Enjalbert et al. 2005). However, Jin et al. (2010) observed severe aecial infection on *Berberis chinensis*, and light infections on *B. koreana* and *B. thunbergii*. Inoculation of wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), rye (*Secale cereale*), oat (*Avena sativa*), and Kentucky blue grass (*Poa pratensis*) using aeciospores from *B. chinensis* resulted in infection only on *Poa pratensis*, producing uredinia typical of stripe rust caused by *P. striiformis*. Inoculation of *Poa pratensis* using aeciospores from *B. koreana* and 'Emerald Carousel', an interspecific hybrid between *B. koreana* and *B. thunbergii*, also produced uredinia typical of stripe rust. Telia of *P. striiformis* f. sp. *tritici* produced pycnia and aecia on *B. chinensis*, *B. holstii*, and *B. vulgaris*. Aeciospores from *B. chinensis* produced uredinia typical of stripe rust on wheat. Analysis using real-time polymerase chain reaction (RT-PCR) and DNA sequence analyses of the nuclear ribosomal internal transcribed spacer (ITS) region and the 5'–end of the large subunit confirmed the rust

fungus as *P. striiformis*. The experiments by Jin et al. (2010) unequivocally proved that *Berberis* spp. are alternate hosts of *P. striiformis* f. *sp. tritici*, the forma specialis specialized on wheat.

Disease epidemics

Stripe rust infection at early stages of plant development can result in stunted and weakened plants, leading to yield losses as high as 50% due to shriveled grain and damaged tillers (Roelfs et al. 1992). Since 2000, stripe rust has become more severe in the eastern United States, Australia, and elsewhere. (Chen 2005, 2007; Wellings 2007; Markell and Milus 2008). Recent research has shown that this coincided with the global spread of two closely related strains that have similar virulence phenotypes and amplified fragment length polymorphism patterns (Milus et al. 2009). Isolates of the pathogen causing this epidemic were new, atypical races for North America with virulence for resistance genes *Yr8* and *Yr9* that had not been detected in the United States before 2000. In spite of the fact that stripe rust development is favored by slightly cooler temperatures (13-24° C), races of the pathogen with significantly increased aggressiveness have emerged in warmer areas of the eastern USA (Chen 2005; Markell and Milus 2008; Milus et al. 2009). Isolates sampled from the eastern USA since 2000 had unusual virulence phenotypes, most similar to isolates from Western Australia (Chen 2005; Wellings 2007; Markell and Milus 2008). A similar phenotype was reported for the first time in Central and northern Europe in 2000–2001 (Hovmøller and Justesen 2007). In recent years, stripe rust also has become increasingly important in the Great Plains, particularly the south-central states. This is probably due to the development of new strains which tolerate a much broader range of temperatures, and infect a broader range of wheat varieties (Chen 2005, 2007).

Resistance to rusts

The wheat plant has developed remarkable strategies to adapt to environmental changes by using a range of constitutive or inducible biochemical and molecular mechanisms. Genetic resistance to rust diseases has been a priority of wheat breeding programs for over 100 years. Resistance genes have routinely been used in wheat cultivars as a cost-effective and environmentally sound means to control disease (Singh et al. 2001).

Different resistance genes condition characteristic resistance phenotypes or infection types (ITs) which can be clearly defined. These include hypersensitive flecks, very light flecks that are difficult to see, small uredinia surrounded by chlorosis, small uredinia surrounded by necrosis, or other combinations (McIntosh et al. 1995, 1998). Resistance against the rust pathogens may be classified as specific or non-specific.

Race-specific resistance

The commonly used race-specific resistance, typically conferred by single genes, is effective in seedling and adult plants, although in some cases it is expressed at the adult-plant stage. Race-specific resistance is usually manifested by a hypersensitive response (HR) of rapid cell death that occurs at the interface between fungal haustoria and host cells in the epidermal and mesophyll layers. This hampers infection at very early stages of the fungal development, from spore deposition to stomata recognition, resulting in reduced penetration of the fungus into the tissue (Rubiales and Niks 1992; Bolton et al. 2008).

Over 60 leaf rust and 70 stripe rust resistance genes with official or provisional symbols have been identified in either wheat or related species (McIntosh et al. 1995, 1998; Chen 2005). Most of the reported genes have been designated as seedling resistance genes, and thus interact with specific races of the pathogen to confer resistance in a gene-for-gene manner (Flor 1971). Although race-specific genes can provide highly effective resistance, they also can select rust races with the corresponding virulence, resulting in cultivars losing effective resistance within a short period of time (McIntosh et al. 1995, 1998). Wheat breeders are increasingly focusing on the identification and incorporation of race non-specific resistance genes that may provide only partial resistance but when used in combination with other genes can condition highly effective resistance.

Race non-specific resistance

The terms polygenic, non-hypersensitive, horizontal, general, minor, partial, and slow rusting all apply to this type of resistance since they all describe resistance that often behaves like a quantitative character. This type of resistance is generally complex, and primarily expressed prior to the formation of the first haustorium, with a hypersensitive

response occurring only at the relatively few infection sites at which a haustorium develops (Rubiales and Niks 1995; Heath 2004). It is often characterized by its durability, effectiveness against a wide range of pathogen races, partial resistance phenotype and optimal expression at the adult plant stage (Caldwell et al. 1970; Parlevliet 1975; McIntosh et al. 1995). The resistance is often conditioned by a few to several genes with partial or additive effects. Because race non-specific resistance is not associated with genes conferring hypersensitive response, selection for higher levels of resistance is less straightforward than for monogenically inherited traits. Slow-rusting resistance and high temperature adult plant (HTAP) resistance are examples of race non-specific resistance.

High temperature adult-plant (HTAP) resistance

Seedlings of plants with HTAP resistance are susceptible to stripe rust (high IT) at both low (6 to 21 ^{o}C) and high (13 to 32 ^{o}C) temperatures, and adult plants are susceptible at low temperatures but resistant (low IT) at high temperatures in the greenhouse (Line 2002). In the field, expression of HTAP resistance begins at stem elongation and becomes stronger at later stages of growth when the weather becomes warm. This type of resistance is effective when average night temperatures are above 10°C and day temperatures are between 25 and 30° C (Line and Chen 1995). The level of resistance conferred by HTAP resistance is usually incomplete and is affected by plant growth stage, temperature, humidity, and the inoculum load. In the Pacific Northwest of the United States, HTAP resistance has consistently proven to be durable against all *P. striiformis* f. sp. *tritici* races. For more than 40 years, there has been no evidence of race specificity for HTAP resistance in wheat. Like slow-rusting resistance, HTAP resistance is also inherited in a quantitative fashion (Line 2002). *Yr36,* first discovered in wild emmer wheat (*T. turgidum* ssp. *dicoccoides* accession FA15–3, henceforth DIC) is an example of HTAP resistance gene. The gene is located on chromosome 6BS (McIntosh et al. 2005). In controlled environments, plants with *Yr36* are resistant at relatively high temperatures (25 to 35° C) but susceptible at lower temperatures (e.g. 15° C). *Yr36* is closely linked to the grain protein content locus *Gpc-B1* (Uauy et al. 2005). *Yr36* resistance, originally discovered in adult plants, also has some effectiveness in seedlings

at high temperatures (Fu et al. 2009). Fu et al. (2009) cloned *Yr36* using map-based approach. The gene includes a kinase and a putative START lipid-binding domain. Five independent mutations and transgenic complementation confirmed that both domains are necessary to confer resistance.

Slow-rusting resistance

In slow leaf-rusting resistant cultivars, there is a delay in the appearance of uredinia on the leaf surface (or a prolonged latent period) compared to susceptible cultivars (Ohm and Shaner 1976; Shaner et al. 1978). In addition, uredinia of the fungus are often smaller (Ohm and Shaner 1976; Shaner et al. 1978), and frequency of infection is reduced (Ohm and Shaner 1976; Shaner et al. 1978; Shaner 1983). These components of resistance greatly reduce the rate of disease development in the field because *Puccinia triticina* and *P. striiformis* are polycyclic pathogens (Caldwell et al. 1970). As a result, the response to infection is essentially susceptible, with progress of disease development typically slower (Parlevliet 1975). Association between slow-rusting resistance and reduction of IT in adult plants by stripe rust has also been reported by Suenaga et al. (2003). However, Singh et al. (2005) observed that in case of potentially durable slow stripe-rusting resistance, the first uredinia to appear are moderately susceptible to susceptible. Subsequent growth of the fungal mycelium causes some chlorosis or necrosis; therefore, the final infection type is usually rated as moderately resistant to moderately susceptible (MR-MS). Varieties carrying genes for slow leaf- and stripe-rusting resistance maintain useful levels of resistance in a wide range of agro-climatic conditions and over a long period of time, showing higher infection levels when disease pressure is heavy, but not succumbing. Some yield losses may occur soon after the release of a slow-rusting resistant variety and may be greater than the losses suffered by varieties with effective race-specific resistance (Caldwell et al. 1970; Parlevliet 1975; McIntosh et al. 1995). To date, only two slow-rusting gene, i.e. *Lr34/Yr18* and *Lr46/Yr29*, have been characterized in detail, and have been incorporated into many cultivars. Although these genes may not, on their own, provide adequate resistance under high disease pressure (Singh and Huerta-Espino 1997), they can contribute to achieving acceptable levels of resistance in combination with other slow rusting genes (Singh and Rajaram 1992; Singh et al. 2001).

Slow-rusting resistance against leaf and stripe rust conferred by the pleiotropic or completely linked *Lr34* and *Yr18* genes have remained effective for more than 50 years (Singh and Rajaram 1992). Both genes have been used extensively in many wheat breeding programs across the world (McIntosh et al. 1995; Singh et al. 2001; Suenaga et al. 2003). Wheat cultivars containing *Lr34/Yr18* occupy more than 26 million ha in various developing countries alone and contribute substantially to yield savings in epidemic years (Marasas et al. 2004). Adult plants carrying *Lr34/Yr18* typically exhibit a rust pustule gradient on the flag leaf, with more and larger pustules at the leaf base and fewer and smaller pustules toward the leaf tip. The origin of *Lr34/Yr18* can be traced back to the Brazilian spring wheat (*Triticum aestivum* L., 2n = 6x = 42) *cv* 'Frontana', which was released in 1943 (Roelfs 1988; Singh and Rajaram 1992). The resistance in 'Frontana' has been reported to be based on four additive genes, one of which is *Lr34/Yr18*. It appears that *Lr12* and *Lr13,* both genes for hypersensitive resistance that is expressed progressively, in combination with *Lr34/Yr18*, provide the basis of most of this resistance (Roelfs 1988). Using monosomic analysis, Dyck (1987) mapped the *Lr34* gene to the short arm of chromosome 7D. Spielmeyer et al. (2008) fine mapped a genetic interval of less than 0.5 cM that contains *Lr34/Yr18* using interstitial deletion mutants generated through gamma irradiation of a single chromosome substitution line of 'Lalbahadur(Parula7D)'. This gene co-segregates with other traits such as leaf tip necrosis (*Ltn1*), powdery mildew [*Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal] resistant gene *Pm38*, and tolerance to barley yellow dwarf virus (*Bdv1*) (Kolmer et al. 2008). In other studies, *Lr34* was shown to both enhance the effectiveness of other leaf rust genes (German and Kolmer 1992) and permit the expression of resistance to certain stem rust races normally inhibited by a suppressor gene, thereby resulting in enhanced stem rust resistance in a backcross-derived line of 'Thatcher' (Dyck 1987; Liu and Kolmer 1998). Joshi et al. (2004) suggested that the *Lr34/Yr18* region might also be associated with resistance to spot blotch disease [*Bipolaris sorokiniana* (Sacc.) Shoemaker). These multi-pathogen resistance traits have made the *Lr34*/*Yr18* locus one of the most valuable gene regions for disease resistance breeding in wheat.

Molecular markers *SWM10* (Bossolini et al. 2006) and *csLV34* (Lagudah et al. 2006) closely linked to the *Lr34*/*Yr18* locus have been shown to provide a much wider

diagnostic ability for this multi-pathogen resistance trait in diverse wheat cultivar backgrounds. Using an insertion/deletion size variant located at the *csLV34* locus on chromosome 7D within an intron sequence of a sulfate transporter-like gene tightly linked to the *Lr34/Yr18* dual rust resistance gene to examine a global collection of wheat cultivars, landraces, and D genome-containing diploid and polyploid species of wheat relatives, Kolmer et al. (2008) found two predominant allelic size variants, *csLV34a* and *b*, among the wheat cultivars, showing disparate variation in different wheat growing zones. According to Kolmer et al. (2008), there is a strong association between the presence of *Lr34/Yr18* and the *csLV34b* allele, and wheat lines known to have *Lr34/Yr18* with the *csLV34a* allele are rare. The lineage of the *csLV34b* allele associated with *Lr34/Yr18* in modern wheat cultivars from North and South America, CIMMYT, Australia, and Russia was tracked back to the cultivars Mentana and Ardito developed in Italy by Nazareno Strampelli in the early 1900s (Kolmer et al. 2008).Recent cloning and sequence analysis revealed that the *Lr34/Yr18* protein resembles adenosine triphosphate (ATP)–binding cassette (ABC) transporters of the pleiotropic drug resistance subfamily, and that alleles of *Lr34/Yr18* conferring resistance or susceptibility differ by three genetic polymorphisms (Krattinger et al. 2009). The first one is an A/T single-nucleotide polymorphism (SNP) in intron 4, the second one is a 3-bp ins/del in exon 11, and the third one is a C/T SNP in exon 12, where an amino acid is altered. No difference within 2 kb of the putative *Lr34/Yr18* promoter region was detected between the two alleles (Krattinger et al. 2009; Cao et al. 2010). Using RIL population derived from a cross involving the leaf rust-susceptible 'Jagger' and leaf rust-resistant '2147', Cao et al. (2010) developed PCR markers distinguishing the *Lr34/Yr18* resistance allele from '2147' (*Lr34E22r*) and susceptible allele from 'Jagger' (*Lr34E22s*). Sequence analysis of the Lr34/Yr18 in 'Jagger' and '2147' revealed a G/T polymorphism in exon 22 due to a premature stop codon in the 'Jagger' susceptible allele (*Lr34E22s*) due to a point mutation.

Lr46/Yr29 is a gene identified in the spring wheat cultivar 'Pavon76' that is similar to *Lr34/Yr18* (Singh et al. 1998; William et al. 2003). 'Pavon 76', released in 1976, displayed slow-rusting or partial resistance that has remained effective in Mexico and other parts of the world where it has been released and grown. The *Lr46/Yr29* locus,

conferring resistance to both leaf rust and stripe rust, is located in the terminal portion of the long arm of wheat chromosome 1B. The chromosome location of this gene was determined through an analysis of the substitution lines for the chromosomes of the resistant cultivar 'Pavon 76' backcrossed into the susceptible spring wheat cultivar Lalbahadur (Singh et al. 1998). Subsequent quantitative trait loci (QTL) analyses have confirmed the location of a minor gene for resistance to leaf rust and stripe rust on the distal portion of chromosome 1BL in a recombinant inbred line population from the cross 'Avocet' × 'Pavon 76' (William et al. 2003). A QTL for leaf-rust resistance was reported in the same region of 1BL in a doubled haploid (DH) population from the cross of 'Fukuhokomugi' × 'Oligoculm' (Suenaga et al. 2003). Mateos-Hernandez et al. (2006) further narrowed the physical location of *Lr46/Yr29* to a submicroscopic region between the breakpoints of deletion lines 1BL-13 [fraction length (FL)=0.89-1] and 1BL-10 (FL=0.89-3)].

Although some race-specific resistance genes such as *Lr12*, *Lr22a*, and *Lr22b* are expressed only in adult plants, those involved in leaf rust resistance can be easily distinguished from genes conferring slow-rusting resistance based on IT. Race-specific adult plant resistance genes confer low IT with an avirulent race, whereas slow leaf-rusting resistance genes are associated with slow rate of disease progress (William et al. 2006). In contrast with leaf rust, durable resistance to stripe rust (Johnson 1984; Line and Chen 1995) is characterized with no clear phenotypic distinction in reaction type of cultivars that express race-specific resistance and those in which resistance was not race-specific. Low stripe rust disease severity is usually associated with some reduction in IT owing to the systemic nature of the fungus that induces chlorosis and necrosis in stripes (Singh et al. 2001). Since slow-rusting resistance is quantitatively inherited, quantitative trait loci (QTL) analysis using molecular markers has been applied to map several loci with slow-leaf-rusting, and stripe-rusting effects on all wheat chromosomes except 1A, 3D, 6B, 6D and 7A (Rosewarne et al. 2008), illustrating the diversity for these type of resistance genes in wheat germplasm.

Molecular markers and linkage mapping

Molecular markers

The availability of reliable molecular markers is of great importance for plant breeding. The ideal marker technique should generate hundreds of molecular markers that cover the entire genome in a single, simple and reliable experiment. Genome-wide molecular markers are used for germplasm characterization, assessment of genetic diversity, to accelerate introgression of traits in backcrossing programs, and for the mapping of complex traits.

Restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP markers)

RFLPs and RAPDs were the preferred types of markers for gene mapping and tagging in the early years of DNA marker applications. Time-consuming multi-step protocols for RFLP detection, low levels of polymorphisms in wheat, inability to detect single-base mutations, and the use of radioactive isotopes reduced the applicability of these markers in gene mapping. Thus the use of RFLP markers in gene mapping has been limited because of the very low level of polymorphism in wheat (Cadalen et al. 1997). Because of this limited polymorphism, gene and genome mapping has required the use of populations derived from wide crosses. However, mapping many agronomically important genes or QTL, a major goal of breeding, requires informative markers in an intraspecific context. This is particularly true when the ultimate goal is marker-assisted selection (MAS). RFLPs detected with single-copy genomic and cDNA clones are extremely powerful for comparative mapping approaches (Ahn et al. 1993) but are only of limited use for intraspecific molecular analysis of agronomic traits as less than 10% of all RFLP loci are typically polymorphic in given wheat population.

The disadvantages of RAPDs are their inability to detect heterozygous individuals, and low reproducibility of electrophoretic patterns due to their sensitivity to DNA quality, reaction conditions and PCR temperature profiles. The application of AFLPs in mapping studies was also restricted as they generally share good reproducibility of the disadvantages of RAPD with regard to multi-locus amplification and inheritance of dominance. In addition AFLPs tend to cluster in heterochromatic regions. However, some of the above mentioned problems can be overcome through

conversion of the markers into STS (sequence-tagged sites) or SCARs (sequence characterized amplified regions) based on their nucleotide sequences.

Single nucleotide polymorphism (SNP) markers

Comparison of DNA sequences from members of a species has revealed Single Nucleotide Polymorphisms (SNPs) as the most common feature underlying genetic variation within species (The Arabidopsis Genome Initiative 2000). This type of genetic variation can be screened by means of a wide variety of technologies, usually based on primer extension or on ligation of oligonucleotide ends (Jenkins and Gibson 2002). The development of these SNPs scoring technologies has led to an impressive increase in throughput capacity. A general prerequisite for these technologies is DNA sequence information. Therefore, for the majority of organisms, including agriculturally important crops such as wheat, information on SNPs is still scarce and difficult to obtain, due to limited resources and/or the complex nature of polyploid genomes. For marker-assisted breeding in such crops it is rarely cost-effective to perform SNP discovery since large numbers of markers scattered throughout the genome are needed for the identification of markers that are closely linked to major genes or QTL. Furthermore, in backcrossing programs, genome-wide markers are used to select the progeny with the maximum genetic contribution from the recurrent parent. For such purposes marker technologies that do not require SNP discovery based on sequence information may be preferred.

Simple sequence repeat (SSR) or microsatellite markers

SSRs (or microsatellites) are tandem repeats of short (2–6 bp) DNA sequences. SSRs are the markers of choice for the construction of genetic maps in wheat owing to their highly polymorphic nature, abundance, co-dominant inheritance and reproducibility (Röder et al. 1998; Somers et al. 2004; Sourdille et al. 2004). In addition, SSRs are highly informative, locus specific markers, an important feature when dealing with allopolyploid species (Röder et al. 1995, 1998). The analysis of SSRs is based on polymerase chain reaction (PCR), which is highly amenable to automation. To date, over 2000 SSR markers over all three genomes of hexaploid wheat have been developed (Ganal and Röder 2007).

Diversity array technology (DArT) markers

Although RFLP, AFLP and SSR markers have made substantial contributions to the wheat genome mapping effort, novel DNA-based markers are allowing further resolution of wheat genetic maps. Diversity Array Technology (DArT™) is an array-based platform for rapid and simultaneous identification and typing of DNA polymorphism (Jaccoud et al. 2001). The DArT genotyping method was developed originally for rice and has subsequently been applied to many other plant species, including grand eucalyptus (Lezar et al. 2004), barley (Wenzl et al. 2006), cassava (Xia et al. 2005), Arabidopsis (Wittenberg et al. 2005), pigeon pea (Yang et al. 2006), wheat (Akbari et al. 2006), and sorghum (Mace et al. 2008). DArT has been also applied to a number of animal species and microorganisms (http://www.diversityarrays.com). DArT detects primarily dominant markers, mostly resulting from single nucleotide polymorphisms at restriction sites at hundreds to thousands of arbitrary genomic loci (Wenzl et al. 2004; Wittenberg et al. 2005). The genotyping technology involves the use of methylation sensitive restriction enzymes to digest genomic DNA, thereby reducing genome complexity and enriching for low copy sequences for marker development. DNA samples enriched for low copy DNA sequences from parents and individuals of mapping populations are hybridized to a microarray panel of clones representing low copy sequences from the same plant species. Restriction site polymorphisms between individuals from mapping populations are detected through differences in hybridization signal, with clones on the microarray panel scored as dominant markers and providing for allele attribution to one or the other parental genotype. Akbari et al. (2006) typed a set of 90 doubled haploid hexaploid wheat lines for which a framework map comprising a total of 339 SSR, RFLP and AFLP markers was available, and added an equal number of DArT markers to the data set and also incorporated 71 sequence tagged SSR (STM) markers. A comparison of logarithm of the odds (LOD) scores, call rates and the degree of genome coverage indicated that the quality and information content of the DArT data set was comparable to that of the combined SSR/RFLP/AFLP data set of the hexaploid wheat framework map.

In recent years, molecular markers have become available, in both animal and plant systems, for basic and applied studies. One of the most extensive uses of molecular markers has been the development of detailed genetic and physical chromosome maps of plant species such as bread wheat. The construction of genetic maps based on molecular

markers is a prerequisite for the dissection of qualitative and quantitative genetic control of agronomically important traits in bread wheat, discovery of new genes controlling phenotypic variation and identification of markers linked to genes to tracking desirable alleles for marker-assisted selection. Genetic maps have also corroborated cytological evidence of major chromosome rearrangements in wheat (Nelson et al. 1995) and have allowed comparative mapping among related species (Börner et al. 1998).

Wheat molecular linkage map

Bread wheat has a large genome of 16 x 10^9 bp (Bennett and Leitch 1995) with an average of 810 Mb per chromosome (10 µm). The average wheat chromosome is 25-fold longer than the average rice chromosome (Argumuganathan and Earle 1991). Such a large wheat genome has resulted from polyploidy and extensive duplications (Dubcovsky et al. 1996), such that more than 80% of the haploid DNA content of bread wheat is repetitive DNA. This makes bread wheat a difficult material for genome-wide studies. Despite this, DNA markers such as RFLPs and SSRs have been used to construct detailed genetic maps (Röder et al. 1998; Pestsova et al. 2000; Sourdille et al. 2001; Gupta et al. 2002).

The genetic duplications provided by the ploidy level of common wheat have enabled extensive cytogenetical manipulation (Sears 1954). Different aneuploid stocks, such as nulli-tetrasomic, ditelosomic lines and wheat-alien addition lines, were developed in cultivar Chinese Spring of hexaploid wheat. These lines continue to be instrumental in the cytogenetic mapping of traits and DNA markers via use of nullisomic-tetrasomic and deletion lines (Endo and Gill 1996). Allocation of molecular markers, such as RFLPs and SSRs, to specific regions of wheat chromosomes enables alignment of genetic and cytogenetic maps, thus allowing a comprehensive assessment of genetic map coverage of wheat. Moreover, integration of deletion and genetic mapping facilitates the analysis of recombination in defined regions of wheat chromosomes (Gill et al. 1996).

Mapping populations

The main approaches for tagging and mapping major genes and QTL include analysis of backcross-derived lines (including near-isogenic lines, NILs), bulked segregant analysis (BSA), analysis of segregating populations, recombinant inbred lines (RILs) and double

haploid lines (DHLs), as well as single chromosome recombinant lines (SCRLs) or single chromosome recombinant-double haploid lines (SCR-DHLs).

Backcross-derived lines

Development of a population of backcrossed-derived inbred lines (BIL) involves a recurrent parent and a donor parent (Wehrhahn and Allard 1965). After the first backcross, the BC_1 plants can be used for additional backcrossing or selfing. Balanced populations, e.g. BC_1F_6, and advanced backcross populations, e.g. BC_2F_5 and BC_3F_5 are examples of typical backcross-derived populations. Generations beyond the BC_3 are likely to have too low statistical power to detect most QTLs. However, additive, dominant and over-dominant QTLs can be detected statistically in BC_2 or BC_3 generations and the plants are sufficiently similar to the recurrent parent to allow ready isolation of near-isogenic lines (NILs) which can be used to further confirm and fine map selected QTLs.

Near-isgenic lines (NILs)

NILs only differ in the genomic region of interest so that all other genes affecting the trait of interest are the same in both lines. Thus NILs are important genetic stocks for investigating the function and regulation of single genes, and precise genetic mapping of a gene, and development of tightly linked genetic markers to allow wheat breeders to track desirable alleles of a gene in different breeding pedigrees. NILs are also useful for for gene isolation through positional cloning approach (Tsujimoto 2001). For the application of NIL analysis to a self-pollinated cross such as wheat, a single elite inbred variety could be crossed to an unrelated donor line (e.g., land race or wild species). One hundred or more BC_1 progeny could be generated. Selection could be excercised on the BC_1 population to remove any individuals with obviously undesirable characteristics (e.g. sterility, seed shattering, undersirable growth habit, etc). The remaining BC_1 could be crossed again to the recurrent parent to produce BC_2 population of more than 200 individuals. If sufficiently large BC_2 population were generated, selection could be excercised again to remove obviously undesirable plants. A minimum of 200 selected BC_2 individuals could be analyzed in replicated trials. By using BC_2F_2 or BC_2F_3 families it should be possible to detect some recessive QTL donor alleles in addition to the

expected dominant and additive donor QTL alleles. BC_2 marker data would be used to search for QTL associations from the BC_2F_2 or BC_2F_3 family data. Once putatively beneficial QTLs were identified from such as analysis, whole-genome selection can be used to identify the BC_2F_2 line(s) from which NILs could be isolated. No more than two generations should be required to isolate targeted NILs and such NILs could be evaluated in replicated trials against the original elite inbred variety (Tanksley and Nelson 1996).

Bulk segregant analysis (BSA)

BSA, developed by Michelmore et al. (1991) to tag disease resistance genes in lettuce, has been successfully applied in wheat. This approach has been mostly used with RAPDs (e.g. Hartl et al. 1995 for *Pm1* and *Pm2*; Hu et al. 1997 for *Pm1*) although it is now being used with AFLPs (Goodwin et al. 1998; Hartl et al. 1998; William et al. 2003) and SSRs (Chantret et al. 2000; Adhikari et al. 2004; Li et al. 2009). Either marker technique is used to screen two bulks of DNA samples from individuals identified in the two opposite tails of a segregating population for a target trait. For a major gene, all loci in the genome should appear to be in linkage equilibrium, except in the region of the genome linked to the target gene. To overcome the problems of limited repeatability of RAPDs, and the fact that repetitive sequences are often amplified (Devos and Gale 1992). Eastwood et al. (1994) and William et al. (1997) used BSA and RAPDs on DNA enriched for low-copy sequences. In both cases, there was a noted increase in repeatability and levels of polymorphism detected compared with non-enriched DNA. The AFLP technology offers the advantage of the high number of DNA fragments amplified with one primer combination, and the problem of highly repetitive DNA is overcome by using methylation sensitive endonucleases, such as *Pst*I and *Sse*I

Recombinant inbred lines (RILs)

Recombinant inbred lines (RILs) are popular in QTL mapping because they are largely homozygous or ‘true-breeding’ lines that can be multiplied and reproduced without genetic change. This allows for the conduct of replicated trials across variable environments and years, thus having considerable potential for the identification of reliable QTL. Furthermore, seed from individual RIL lines may be transferred between different laboratories for further linkage analysis and the addition of markers to existing

maps, ensuring that all collaborators examine identical material. RIL experimental designs were shown to be more powerful than backcrosses which allow screening of fewer individuals, to cover a similarly wide spectrum of recombinants (Asíns 2002), an important economical aspect in genetic mapping.

Doubled-haploid (DH) population

In wheat, DH plants can be developed by culturing the anther or microspores (androgenesis) of F_1 plants (Craig 1974), or by pollinating the F_1 floret with maize pollen and embryo rescue to obtain haploid plants, followed by colchicine treatment (Laurie and Bennett 1988). The wheat x maize method is considered to be the more efficient of the two (Kisana et al. 1993). Brazauskas et al. (2004) used AFLPs to assay a DH population developed using the wheat x maize method and found no introgression of maize DNA in the wheat genome. A DH mapping population can be developed within about 2 years, and therefore can be generated more rapidly than RIL populations. In addition, DH lines are genetically pure immediately after doubling the chromosome number, whereas RILs usually contain some degree of heterozygosity.

According to Matzk and Mahn (1994), corn is sown ten days prior to F_1 derived from a cross involving two wheat cultivars. During heading stage, spikes from F_1 plants are emasculated and pollinated with sweet corn pollen 4–5 days later. Twenty-four hours following pollination, spikes are sprayed with 2,4–D solution (213.05 mg/l, pH = 10.36), and the spikes are collected for embryo rescue 14–16 days later. Haploid embryos from florets are rescued and placed in 25 mm diameter tubes containing MS medium with sucrose and agar (pH = 5.8) at room temperature with about 10 h light per day, which finally yields haploid plantlets. As the haploid plantlets reaches 5–7 cm, they are transferred to pots with soil in the greenhouse. At the tillering stage, the plants are removed from the pots and placed in a beaker containing 1,000 ml colchicine solution [colchicine (0.5g/l) + DMSO (20 ml/l) + GA_3 (100 mg/l) + Tween 80 (0.3 ml/l)] at room temperature in the dark for 8 h, and then rinsed with flowing water overnight and transferred back to soil. Fertile plantlets are used to form the DH population. A subset of DH lines is then randomly selected and used for genetic mapping and QTL analysis.

Single chromosome recombinant lines (SCRLs)

According to Law (1966) SCRLs are developed by crossing lines of the same background but differing for a single chromosome, back-crossing to a monosomic line for the chromosome under study, identifying the monosomic plants with a hemizygous recombinant chromosome, selfing those and detecting disomic recombinants. Despite the difficulties of producing such mapping populations, the main advantage they offer is that they allow the scoring of the phenotypic effect of the gene of interest without the confounding effects of other genes (on other chromosomes) involved in the expression of the same trait.

The International Triticeae Mapping Initiative (ITMI) population

An important genetic mapping population is the International Triticeae Mapping Initiative (ITMI) population developed by Dr. Mark Sorrells of Cornell University by crossing a synthetic (amphihexaploid) wheat (*Aegilops tauschii* [syn. *Triticum tauschii*] x 'Altar 84' durum) to a spring bread wheat cultivar 'Opata 85' (van Deynze et al. 1995). The use of such a non-intervarietal cross resulted in a very dense map due to the higher polymorphism level. However, the effective application of the ITMI population and its genetic map to breeding studies is limited by the lack of trait variation of commercial relevance. Genetic maps developed from populations derived from intervarietal crosses are therefore being developed for genetic analysis and marker-trait associations relevant to breeding programs, despite low levels of DNA polymorphism in cultivated wheat accessions. Comparison of genetic maps between studies can identify ambiguities, and combining single population genetic maps into a consensus map can resolve disagreements in marker order. This approach was taken by Somers et al. (2004) who joined four independent genetic maps developed from populations derived from three intervarietal crosses involving Canadian wheat varieties and ITMI population, into a single consensus map. Similarly, other consensus maps have been developed using other intervarietal populations (Komugi integrated database of wheat website: http://www.shigen.nig.ac.jp/wheat/komugi/about/about.jsp). This work resulted in a comprehensive analysis of marker order and distance of DNA markers for use in molecular breeding. The consensus maps provide a new tool for wheat breeding and genomics research. These maps are particularly useful references for targeting additional markers in specific chromosomal regions for fine mapping of QTL.

The ITMI map has been used to map some important traits in addition to several major genes. Known genes include vernalization (*Vrn1* and *Vrn3*), red-coleoptile (*Rc1*), kernel hardness (*Ha*) and powdery mildew (*Pm1* and *Pm2*) genes (Nelson et al. 1995), as well as genes conferring and suppressing leaf rust resistance (Nelson et al. 1997). QTL mapped for kernel hardness (Sourdille et al. 1996), resistance to tan spot (Faris et al. 1997) and Karnal bunt resistance (Nelson et al. 1998)

Hypothesis of the dissertation

Breeding approaches to achieve more durable resistance against leaf rust and stripe rust can be greatly enhanced if molecular markers with tight linkages to genes conferring this type of resistance are available. Although two slow-rusting gene complexes are currently being used extensively in many wheat breeding programs across the world, several genomic locations associated with other possible slow-rusting resistance genes against leaf and stripe rust have been reported. Scientists at the International Maize and Wheat Improvement Center (CIMMYT) believe that at least 10 to 12 slow rusting genes are involved in the APR of CIMMYT wheat materials.

Slow-rusting resistance is expressed by slower development of disease symptoms and epidemics in the field. Hence, identifying slow-rusting can be time consuming and prone to error. Recently a population of RILs derived from the cross ‘Avocet S’ x ‘Amadina’ was developed by Dr. Ravi Singh of CIMMYT. This population segregates for slow rusting resistance to leaf and stripe rust diseases. ‘Amadina’ has expressed a high level of resistance over a period of time against various races of *P. triticina* and *P. striiformis* pathogens across Mexico. From previous genetic studies, Singh et al. (2004) hypothesized that ‘Amadina’ carries a minimum of four additive genes for resistance to leaf rust and a minimum of three additive genes for resistance to stripe rust in addition to the *Lr46/Yr29* gene from ‘Pavon 76’. On the other hand ‘Avocet S’ is highly susceptible to the two rust diseases. The hypothesis is that molecular markers can be used to dissect the resistance in ‘Amadina’ into discrete components. In turn, these markers would be valuable for improving the precision and efficiency of selecting for non-specific rust resistance. Segregation of markers will indicate the number and chromosomal locations of linkage groups associated with the slow-rusting resistance expressed in the field.

Associations with linkage groups identified from these studies will be useful to design additional studies that will provide more effective selection by molecular markers. Although much work has been done on slow-rusting resistance, little work has been done on characterization of the genomic locations conditioning the components of slow-rusting. Furthermore, knowledge of the identity of seedling rust resistance genes present in ‘Amadina’ will aid breeders in the identification of possible major gene interactions and provide detailed information on the genetic basis of the slow-rusting resistance in ‘Amadina’.

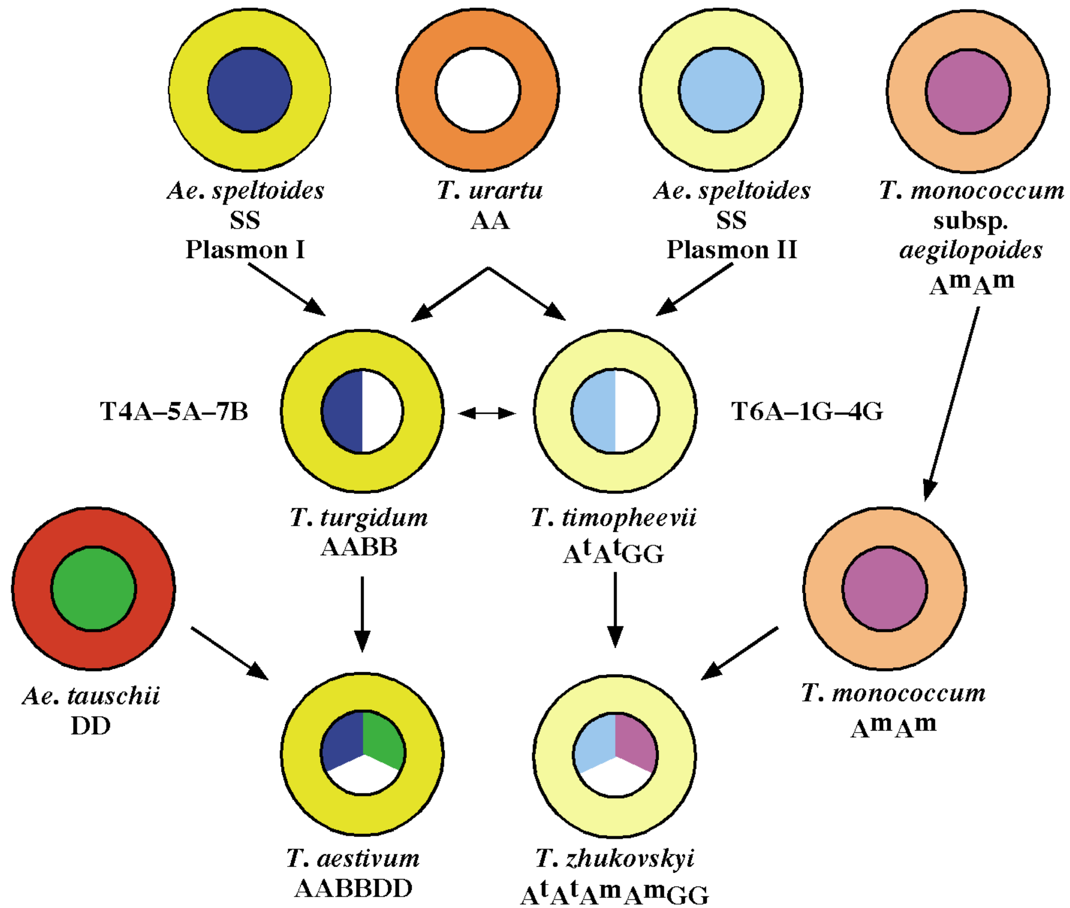
Objectives to test hypothesis

The primary objective is to increase understanding of the mechanism of slow-rusting resistance in ‘Amadina’. Specific objectives are to:

1. Utilize full linkage mapping to determine the number and genomic locations of genes conferring slow-rusting resistance against stripe rust
2. Utilize full linkage mapping to determine the number and genomic locations of genes conferring slow-rusting resistance against leaf rust
3. Utilize full linkage mapping to identify the genomic locations specifically conditioning prolonged latent period, low receptivity, and reduced uredial size
4. Examine the genetic base of seedling leaf rust resistance in ‘Amadina’ and ‘Avocet S’

Figures and Tables

Figure 1.1 Evolutionary lineages of wheat



Species	Genome	Nucleus	Cytoplasm
Aegilops speltoides (Plasmon I)	SS		
Triticum urartu	AA		
Aegilops speltoides Plasmon II)	SS		
Triticum monococcum ssp. *aegilopoides*	A^mA^m		
Aegilops tauschii	DD		

Source: Wheat Genetic and Genomic Resource Group (WGGRC), Kansas State University (http://www.k-state.edu/wgrc/)

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CHAPTER 2 - Seedling resistance to leaf rust in the spring wheat ‘Amadina’

Abstract

Leaf rust caused by the fungus *Puccinia triticina* Eriks. is one of the most important diseases of hexaploid wheat (*Triticum aestivum* L.) worldwide and can be controlled through the use of genetic resistance. Characterization of breeding lines greatly facilitates the effective utilization of host resistance genes. The objective of this study was to identify and map seedling resistance genes in the CIMMYT breeding line ‘Amadina’. Seedling leaf rust resistance genes in ‘Amadina’ were postulated based on their reaction to 15 isolates of *Puccinia triticina*. Two known *Lr* genes, *Lr23*, and *Lr26* were postulated to be present in ‘Amadina’. In the case of *Lr26*, the result was corroborated through pedigree, cytogenetic, and PCR analyses. Based on the gene postulation, and in some cases PCR analysis, resistance genes *Lr2a*, *Lr2c*, *Lr9*, *Lr16*, *Lr24*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *Lr18*, *Lr21*, *Lr28*, *Lr39*, *Lr42*, *Lr47*, *Lr50*, *Lr52*, and *Lr19* are absent in ‘Amadina’. Presence or absence of resistance genes *Lr1*, *Lr3a*, *LrB*, *Lr10*, *Lr14a* could not be postulated in ‘Amadina’ because the isolates used in this study lacked sufficient diversity in combinations of avirulence and virulence genes. A gene on the short arm of chromosome 2D, and an unknown gene associated with seedling resistance in the centromeric region of the long arm of chromosome 1B were mapped using recombinant inbred line population derived from ‘Avocet S’ x ‘Amadina’ cross. Although presence of *Lr23* was postulated in ‘Amadina’, the gene could not be detected in the RIL population due to presence of a suppressor gene in ‘Avocet S’. *Lr13*, known to express better in adult plants but can be recognizable at warmer temperatures in the seedling stage was also mapped in ‘Avocet S’. Additive x additive, and additive x non-additive epistatic gene interactions between the seedling genes were detected. This study demonstrates the utility of gene postulation, genetic mapping, and cytogenetic analysis for identification of seedling leaf rust resistance genes in wheat.

Introduction

Leaf rust caused by the fungus *Puccinia triticina* Eriks. is one of the most important foliar diseases of wheat (*Triticum aestivum* L.) worldwide. Use of resistant wheat varieties is the most economical and environmentally friendly method of controlling the disease (Singh et al. 2001). Resistance gene pyramiding is of paramount importance since the combined effects of several genes give the cultivar a wider base of disease resistance (Roelfs et al. 1992), thereby helping to avoid the release of cultivars that are genetically uniform (Statler 1984; McVeh and Long 1993; Kolmer 2003). Identification of different sources of resistance is a prerequisite to pyramiding of several resistance genes into a single genetic background. The probable identity of seedling leaf rust resistance genes (*Lr* genes) can be quickly determined if the pathogen possesses diverse combinations of avirulence and virulence genes using the gene postulation approach which follows the principle of gene-for-gene interaction (Flor 1971) between the host line and *P. triticina* genotype. Presence of seedling resistance genes can be postulated on the basis of phenotypic expressions in the form of infection types (ITs) as the wheat lines are infected with a series of pathogen races (Kolmer 2003). ITs produced by a diverse group of *P. triticina* races on standard set of differential lines that differ for single *Lr* genes are the basis for comparing the ITs of wheat cultivars with unidentified or unknown genes for leaf rust resistance. Leaf rust races that produce distinct low ITs on specific *Lr* genes in the differential series will also produce low ITs on those lines that have the same resistance genes.

The strength of the gene postulation method is that it allows the identification of new resistance genes or unknown gene combinations in host lines, which can then be further characterized, if necessary, by genetic analysis. This method has been used successfully by many researchers for identifying *Lr* genes in a group of wheat genotypes. For instance, Statler (1984) identified genes *Lr1*, *Lr2a*, *Lr2c*, *Lr10*, *Lr17*, and *Lr18* in 25 hard red spring wheats; Singh (1993) postulated *Lr1*, *Lr3a*, *Lr13*, *Lr16*, *Lr17*, *Lr23*, *Lr26*, and *Lr34* in 26 Mexican cultivars released between 1950 and 1989; McVey and Long (1993) postulated the presence of genes *Lr1*, *Lr2a*, *Lr3a*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a*, *Lr16*, *Lr17*, *Lr18*, *Lr24*, *Lr26*, and *Lr30* in 86 hard red winter wheat lines; Singh et al. (2001) postulated *Lr1*, *Lr3a*, *Lr9*, *Lr10*, *Lr17*, *Lr19*, *Lr23*, and *Lr27+31* in *30* Japanese wheat cultivars and 7 supplementary differentials; Singh et al. (2001) postulated *Lr1*,

Lr3a, *Lr10*, *Lr13*, *Lr17b*, *Lr20*, *Lr26*, and *Lr37* in 70 wheat cultivars grown in the United Kingdom; Kolmer (2003) postulated *Lr1*, *Lr2a*, *Lr9*, *Lr10*, *Lr11*, *Lr18*, and *Lr26* in a group of 35 soft red winter wheat cultivars and 17 breeding lines; Wamishe and Milus (2004) postulated genes *Lr1*, *Lr2a*, *Lr2c*, *Lr3a*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a*, *Lr18*, *Lr20*, *Lr23*, *Lr24*, and *Lr26* to be present in 116 North American wheat lines; and Mebrate et al. (2008) postulated *Lr1*, *Lr2c*, *Lr3a*, *Lr3ka*, *Lr9*, *Lr10*, *Lr14a*, *Lr14b*, *Lr13*, *Lr16*, *Lr18*, *Lr21*, *Lr23*, *Lr27+31*, *Lr30*, *Lr37*, and *Lr44* in 23 selected Ethiopian wheat cultivars, and *Lr9*, *Lr20*, and *Lr21* in 13 selected German wheat cultivars.

Genetic diversity for effective leaf rust resistance is currently one of the practical methods of maintaining acceptable levels of leaf rust resistance in commonly grown wheat cultivars. Selection for virulence in *P. triticina* populations is less likely to affect the resistance of a number of wheat cultivars if the cultivars differ for effective resistance genes. Genetic uniformity of resistance genes can result in different wheat cultivars being vulnerable to the increase and spread of a few *P. triticina* phenotypes with virulence to the common resistance genes. Information on the identity of the leaf rust resistance genes in commonly grown cultivars is a pre-requisite for diversification of leaf rust resistance in breeding programs. Thus, the objectives of this study were to identify probable leaf rust seedling resistance genes, and DNA markers linked to seedling resistance genes in the CIMMYT spring wheat breeding line 'Amadina' which is an important parent in the rust-resistance breeding programs of CIMMYT and Kansas State University due to its high level of resistance against a wide array of *P. triticina* races across environments over a long period of time.

Materials and methods

Plant materials

The plant material comprised a population of 148 $F_{5:7}$ recombinant inbred lines (RILs) derived from the cross of 'Avocet S' (WW-119/WW-15//Egret) with 'Amadina' (Bobwhite/Crow//Buckbuck/Pavon 76/3/Veery#10), 'Avocet S', 'Amadina', 18 'Thatcher' NILs, and other 6 differentials (Table 2.1). The $F_{5:7}$ RIL population was developed at the International Center for Maize and Wheat Improvement (CIMMYT).

Pathogen isolates

Fifteen isolates of *P. triticina*, (PRTUS 60, PRTUS 55, PRTUS 54, PRTUS 45, PRTUS 35, PRTUS 25, PT1, PT2, PT3, PT4, PT5, PT6, PT7, and PT8), shown in Table 2.4 were used to postulate the presence of seedling leaf rust resistance genes in 'Amadina'. The isolates included in the study were collected from the United States, and were chosen on the basis of low or high IT to particular leaf rust resistance gene in the series of leaf rust differentials. The isolates were assigned five-letter race designations shown in Table 2.3 based on high and low ITs to the host sets 1–5 in Table 2.2 (Long and Kolmer 1989).

Testing procedure

Fifteen independent experiments were conducted in completely randomized design (CRD) comprising two replications. An average of five seeds of each of the $F_{5:7}$ RILs plus the two parents were planted in clumps in an arrangement of 5 rows with 2.5 cm and 6 columns with 3 cm between each entry in 20 cm x 20 cm aluminum flats that were filled with Metro Mix 360 containing 35–45% Canadian Sphagnum peat moss, horticultural grade vermiculite, bark ash, composited pine bark, Dolomitic limestone for pH adjustment of materials in the soilless mix and a wetting agent (Sun Gro Horticulture Co., Bellevue, WA). The 18 'Thatcher' wheat with single resistance genes *Lr1*, *Lr2a*, *Lr2c*, *Lr3a*, *Lr9*, *Lr16*, *Lr24*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *LrB*, *Lr10*, *Lr14a*, *Lr18*, *Lr28*, and *Lr23*, 6 differentials with single resistance genes *Lr21*, *Lr39*, *Lr42*, *Lr50*, *Lr52*, and *Lr19*, and the leaf rust susceptible 'Thatcher' listed in Table 2.1 were seeded in a separate 20 cm x 20 cm tray. The seeded flats were placed in a growth chamber (Conviron, Manitoba, Canada) with day/night regime of 16 h light (supplied by 65W fluorescent tubes) and 8 h of darkness at a constant temperature of 20 ^{o}C with 50% relative humidity. Seedlings were well-watered on a daily basis. The gibberellin inhibitor, cycocel [(2-chloroethyl)trimethylammonium chloride], (OHP, Mainland, PA) was applied twice (4 and 7 days after germination) to the seedlings at a concentration of 1500 ppm to slow down the rate of growth. When the seedlings were 11 days old (primary leaf fully expanded), flats of the seedlings were inoculated with each test isolate of *P. triticina* by spraying the seedlings with an atomized suspension of urediniospores mixed with isoparaffinic light oil (Soltrol 170) (Chevron Phillips Chemical Company, The

Woodlands, TX). After 10 minutes to allow the oil to evaporate, the inoculated seedling flats were placed in a dark mist chamber (Percival Scientific, INC, Perry, IA) for 16 h at 20 ^{o}C where the relative humidity was maintained near 100%. Afterwards, plants were returned to the growth chamber. To validate postulation of *Lr23*, the RILs, along with 'Amadina', 'Avocet S', 'Thatcher', and all the differentials were tested at 25° C growth chamber temperature with isolate(s) displaying intermediate ITs on *Lr23* but high ITs on all other seedling genes postulated in 'Amadina' since the gene is more effective at high temperatures (Dyck and Johnson 1983).

IT scoring and seedling resistance genes postulation

ITs were scored 8–10 days after inoculation when the rust was fully developed on the susceptible check, 'Thatcher', based on the scale described by Long and Kolmer (1989): 0 = no hypersensitive flecks, necrosis, or uredinia; 0; = faint hypersensitive flecks; ; = distinct hypersensitive flecks; 1 = small uredinia surrounded by distinct necrosis; 2 = small uredinia surrounded by distinct chlorosis; 3 = moderate size uredinia with or without chlorosis; 4 = very large uredinia without chlorosis or necrosis. Designations + and – were added as superscripts to the 0 to 4 IT to indicate larger and smaller uredinia than normal, respectively. Designations C and N were also added to indicate more than usual degrees of chlorosis and necrosis, respectively. Generally, ITs of 0 to 2^{+} indicated host resistance to the race and were classified as low, and ITs of 3 to 4 indicated host susceptibility to the race and were classified as high. ITs combining different numbers and symbols indicated a range of ITs present on the same leaf. The RILs that had only low ITs were classified as homozygous resistant, RILs with only high ITs were classified as homozygous susceptible, and RILs that had plants with low and high ITs were as classified as segregating. The numbers of homozygous resistant, homozygous susceptible and segregating RILs were used in determining the numbers of seedling genes that conditioned resistance to each race. The reaction of each RIL and the two parents to specific *P. triticina* race was postulated on the basis of comparing the IT displayed by the set of 18 'Thatcher' NILs and 6 other differential lines (Table 2.1) possessing known resistance genes. Individuals exhibiting the same reaction pattern as a specific differential line were postulated to carry that respective *Lr* gene. Chi-square analyses were performed

to test the goodness of fit of the observed ratios with those expected for the hypothesized number of resistance genes

C-banding analysis of wheat-rye translocations

'Amadina' was known to carry the 1RS.1BL translocation. To determine the status of this chromosome in 'Amadina' and 'Avocet S', C-banding was carried out according to the procedure of the Wheat Genetic and Genomic Resources Center of Kansas State University (www.k-state.edu/wgrc/Protocols/Cytogenetics/cbanding.html) described by Gill et al. (1991). Seeds of 'Amadina' and 'Avocet S' were germinated on wet filter paper in a Petri dish at 25° C for about 3–4 days. Roots were excised when 1.5–2.5 cm long, and pretreated with 0.05% colchicine for 3 h at room temperature. The colchicine pretreatment results in a lower mitotic index compared with ice water pretreatment but gives better chromosome morphology and band contrast. The excised roots were fixed in Carnoy's I for at least 1 h at room temperature. After fixation, roots were pretreated with 45% acetic acid for 2–3 min to soften the tissues. The very tip of the roots were removed with a razor blade, the meristematic tissue was squeezed out with a scalpel, and squashed in 45% acetic acid using gentle heat. Quality of preparations was checked under a phase contrast microscope. Cover-glasses were removed by freezing the slides, and the slides were immediately placed in 99% ethanol for 12–16 h. Slides were air-dried for 7 min, incubated for 1 min in 0.2 N HCl in a water bath at 60° C. Then the slides were washed briefly in distilled water, incubated for 7 min in a saturated $Ba(OH)_2$ at room temperature, washed briefly in distilled water, incubated for 30 min in 2 X SSC in a water bath at 60° C. The slides were moved directly from the 2 X SSC into a 10% staining solution of Geisma (Gallard-Schlesinger Industries, Inc.; Carle Place, NY) in Soerensen phosphate buffer (pH 7.2). Slides were individually checked every few minutes for best contrast. After staining, the slides were rinsed with distilled water, and air-dried. Identification of chromosomes was based on the standard karyotypes of wheat (Gill and Kimber 1974a) and rye (Gill and Kimber 1974b; Mukai et al. 1992).

DNA extraction

Genomic DNA was extracted using a modified CTAB method (Murray and Thompson 1980). For each 150-200 mg sample in a 2 ml centrifuge tube, 1000 µl 2 x CTAB

extraction buffer containing 2% 2-Mercaptoethanol (Sigma, St. Louis, MO) and 100 μg/ml proteinase K (Roche Diagnostics, USA) was added. Tubes were incubated for 60 min at 60 ^{o}C in a water bath, followed by 10 min cooling at room temperature. Chloroform:isoamyl alcohol (24:1) extraction was performed twice. DNA in aqueous phase was precipitated by adding approximately two-third volume of isopropanol and placing the tubes at -20 ^{o}C overnight. RNA was removed from DNA re-dissolved in 500 μl 1 X TE pH 8.0 by RNAse A (Roche Diagnostics, USA) treatment at 37 ^{o}C for 30 min. To extract the DNA, chloroform:isoamyl alcohol (24:1) extraction was performed twice. Intact high quality genomic DNA was precipitated using half volume of 7.5 M ammonium acetate (Sigma-Aldrich, Steinheim, Germany) and 2 volumes of 100% ethanol (Pharmco, CT).

SSR assay, and PCR amplification with *Lr* specific primers

The two parents were screened with a total of 1600 SSRs (SSRs) for polymorphism. These markers consisted primarily of Gatersleben Wheat SSRs (GWM) and markers from Wheat SSR Consortium (WMC), INRA Clermont-Ferrand (CFA and CFD), Beltsville Agriculture Research Center (BARC), and Kansas State University (KSUM). SSR primer sequences of GWM, WMC, CFA, CFD, BARC, and KSUM primer's sets are publicly available on the GrainGenes Triticeae database (http://wheat.pw.usda.gov/). For each polymerase chain reaction (PCR) reaction, 100 ng genomic DNA was used in a 25 μl solution containing 250 μM of each dNTP, 1 X PCR buffer, 0.4 pmol of each primer with 2.5 mM $MgCl_2$ and approximately 0.1 U *Taq* polymerase. An MJ Research PTC-200 thermal cycler (Watertown, MA) was programmed as follows for BARC: 'hot start' 95° C for 1 min, followed by 40 cycles of 40 sec at 94° C, 40 sec of annealing, and 1 min of extension at 72° C, with a final extension at 72° C for 10 min. For the remaining markers the program was: 'hot start' at 94° C for 5 min, followed by 45 cycles of 1 min at 94° C, 1 min of annealing, and 2 min of extension at 72° C, with a final extension at 72° C for 10 min. PCR products were size-separated on 6% polyacrylamide gels and silver stained. Visual allele identification followed a conservative approach, i.e. only clearly different bands were accepted as to be different. In case of doubt, e.g. null alleles, experiments were repeated.

Capillary fragment analysis

For the entire RIL population genotyping, PCR products were analyzed by capillary electrophoresis on an ABI3730 (Applied Biosystems, Foster City, CA). Each forward primer was 5'-tailed with the M13 forward consensus sequence. The M13-tailed forward primers were then used in combination with a standard M13 primer dye-labeled (VIG, NED, FAM, PET) at its 5'-end (Boutin-Ganache et al. 2001). Samples were prepared by pooling 3 µl of PCR product from separate primer sets, each with a different fluorescent dye. The DNA pool was mixed and centrifuged. One microliter of the pooled DNA was added to a mixture of 6 µl of Hi-Di formamide (Applied Biosystems, Foster City, CA), 0.25 µl of Genescan 500-LIZ size standard (Applied Biosystems, Foster City, CA), and 3 µl of water. The samples were again mixed well and centrifuged. The 96-well plate was placed on an MJ Research PTC-200 thermal cycler for 5 min at 95°C and then on an ice slurry for 5 min. Raw data files from the ABI3730 were imported into GeneMarker v1.1 (SoftGenetics, State College, PA) for fragment analysis.

DArT assay

The development of the DArT marker set used in this study is described by Akbari et al. (2006) to provide additional genomic coverage. DArT genotyping of wheat is offered as a commercial service by Triticarte Pty. Ltd. Yarralumla, ACT, Australia (www.triticarte.com.au) who conducted the analyses for this study. Briefly, a genomic representation of a mixture of the entire population was produced with *Pst*I-*Taq*I digestion, spotted on microarray slides, and the individual genotypes were screened for polymorphism based on fluorescence signals. DNA samples from the parents were first screened for polymorphism and then the individual RILs were genotyped. A total of 501 polymorphic loci were scored as present or absent. Names of loci that were previously mapped by Triticarte Pty. Ltd include the prefix "wPt" (followed by numbers corresponding to a particular clone); loci that were mapped for the first time on the current map were presented by clone ID number. DArT technology is protected by patent No. WO 01/73119.

The Primer3 Software ver. 0.40 (Rozen and Skaletsky 2000) was used for designing PCR-based DArT primers (Table 2.10) from DNA sequences of DArT probes

available online at http://www.diversityarrays.com/sequences.html with amplicon sizes ranging from 200 to 400 bp.

Molecular markers for *Lr1*, *Lr3*, *Lr9*, *Lr10*, *Lr13*, *Lr24*, *Lr26*, and *Lr47*

The presence of known *Lr* markers were also tested in 'Amadina', 'Avocet S', and the RIL population with specific PCR primers which are listed in Table 2.8. Markers pTAG621 was located at about 0.53 cM from *Lr1* (Feuillet et al. 1995), and Xmwg798, J13, Xbarc183, Lr24/Sr24#50, SCM9, and PS10 co-segregate with *Lr3* (Sacco et al. 1998), *Lr9* (Schachermayr 1994), *Lr13* (Cakir et al.), *Lr24/Sr24 Agropyron elongatum* segment (Mago et al. 2005), *Lr26 Secale cereal* segment (Saal and Wricke 1999), and *Lr47 Aegilops speltoides* segment (Helguera et al. 2000). Primers were synthesized by Integrated DNA Technologies (IA, USA). The cycle conditions for each primer set are listed in Table 2.9. After amplification, the specific PCR products were separated on 2.5% high resolution agarose (ISC BIOEXPRESS, UT, USA)-containing 1.875×10^{-5} volume of 10 mg/ml ethidium bromide (Sigma-Aldrich, MO, USA) gel in 1 X TBE buffer (0.178 M Tris-borate, 0.178 M boric acid, 0.004 M EDTA). The 25 µl PCR products were combined with 3 µl of loading buffer (0.25% bromophenol blue, 30% glycerol), which was added to prepare samples for agarose-gel electrophoresis. A 100 bp DNA HyperLadder IV (BIOLINE, MA, USA) was used to estimate the size of each amplified DNA fragment. Amplification products were electrophoresed at 50 V for about 5 h, and bands were visualized and photographed using Gene Flash UV visualizer (SYNGENE, MD, USA).

Linkage map construction

The scores of all polymorphic DArT and SSR markers were converted into genotype codes ('A', 'B') according to the scores of the parents; heterozygotes were recorded as missing data. Genetic linkage maps were constructed using the computer program MAPMAKER v3.0 (Lander et al. 1987). Centimorgan (cM) values were calculated according to Haldane mapping function (Haldane 1919). Linkage groups were identified using a minimum logarithm of the odds (LOD) threshold value of 3.0 after preliminary analysis using LOD scores ranging from 3.0 to 20.0. Pair-wise, three-point and multi-point analyses were used in order to determine the best order of marker loci within the

linkage groups. Loci whose locations were ambiguous were placed in the interval in which they were best fitted using the "try" command. Markers from multi-locus primers or those that were different from the reported locus were distinguished with a suffix a, b, or c, with the suffix "a" given to the first mapped locus. SSR markers were used as anchors in map construction and their relative order was compared with the reference wheat maps (Somers et al. 2004; Sourdille et al. 2004; Song et al. 2005). DArT markers were referred to the bread wheat maps published by Akbari et al. 2006, Semagn et al. 2006, Crossa et al. 2007, Huynh et al. 2008, Mantovani et al. 2008, Francki et al. 2009, and Neumann et al. 2010.

Genetic and statistical analysis

Analysis of leaf rust seedling resistance genes was undertaken using the composite interval mapping (CIM) from QTL Cartographer v2.5 (Wang et al. 2005), and the mixed linear model (MLM)-based CIM in QTLNetwork v2.0. (Yang et al. 2005). Disease scores were treated as qualitative data. ITs 0 to 2^+, were assigned the value "1", and ITs of 3 to 4 were assigned the value of "0" for gene mapping. For the QTL Cartographer the parameter set-up of "model 6 standard analysis" was used; for both programs, walk speed 1 cM step, "forward and backward" regression for the selection of the markers to control the genetic background (control markers or cofactors) with a probability into and out of the model of 0.05, and a blocked window size of 10 cM to prevent tightly linked markers tagging a single gene from being included in the model. Significant thresholds for seedling resistance gene detection were calculated for each dataset using 1,000 permutations and a genome-wide LOD threshold (experiment-wise $P \leq 0.05$). In the mixed linear model CIM, genes are fixed variables while molecular markers are random variables. Thus estimates of the gene will not depend upon a particular fixed set of markers being in the model. The genetic model, using QTLNetwork v2.0, also incorporates significant additive effects and epistatic effects as well as their environment interaction (Wang et al. 1999).

According to Wang et al. (1999), the MLM for simultaneous search of interacting gene (Q_i between flanking markers M_{i-} and M_{i+}, and Q_j between flanking markers M_{j-} and M_{j+}), under the assumption of presence of gene x environment interaction (QE) was:

$$y_{hk} = \mu + a_i x_{Aik} + a_j x_{Ajk} + aa_{ij} x_{AAijk} + u_{Ehk} e_{Eh} + u_{AiEhk} e_{AiEh} + u_{AjEhk} e_{AjEh} + u_{AAijEhk} e_{AAijEh} + \sum_{f(h)} u_{Mfk(h)} e_{Mf(h)} + \sum_{l(h)} u_{MMlk(h)} e_{MMl(h)} + \varepsilon_{hk}$$

where y_{hk} is the phenotypic value of the k–th RIL in environment h; μ is the population mean; a_i and a_j are the additive effects (fixed) of two putative genes (Q_i and Q_j), respectively; aa_{ij} is the additive x additive epistatic effect (fixed) between Q_i and Q_j; x_{Aik}, x_{Ajk} and x_{AAijk} coefficients of gene effects derived according to the observed genotypes of the markers (M_{i-}, M_{i+} and M_{j-}, M_{j+}) and the test positions ($r_{Mi\text{-}Qi}$ and $r_{Mj\text{-}Qj}$); e_{Eh} is the random effect of environment h with coefficient u_{Ehk}; e_{AiEh} (or e_{AjEh}) is the additive x environment interaction effect with coefficient u_{AiEhk} (or u_{AjEhk}) for Q_i (or Q_j); e_{AAijEh} is the epistasis x environment interaction effect with coefficient $u_{AAijEhk}$; $e_{Mf(h)}$ is the effect of marker f nested within the h-th environment with coefficient $u_{Mfk(h)}$; $e_{MMl(h)}$ is the effect of marker x marker interaction nested within the h–th environment with coefficient $u_{MMlk(h)}$; and ε_{hk} is the residual effect. Additive effects were negative if the allele of 'Amadina' or 'Avocet S' reduced IT score and positive if the 'Amadina' or 'Avocet S' allele increased IT score.

'Chinese Spring' nullitetrasomic and ditelosomic genetic stocks (Sears 1954; Endo and Gill 1996) Nulli–1A/Tetra-1D (abbreviated as N1A–T1D), N3A–T3D, N4A–T4D, N5A–T5B, N6A–6D, N7A–T7D, N1B–T1D, N2B–T2A, N3B–T3D, N4B–T4D, N5B–T5D, N6B–T6D, N7B–T7D, N1D–T1B, N2D–T2B, N3D–T3A, N4D–T4B, N5D–T5A, N6D–T6A, N7D–T7B, Ditelo1AS (abbreviated as DT1AS), DT1AL, DT2AS, DT3AS, DT3AL, DT4AL, DT5AL, DT6AS, DT6AL, DT7AS, DT7AL, DT1BS, DT1BL, DT2BL, DT3BS, DT3BL, DT4BS, DT5BL, DT6BS, DT6BL, DT7BS, DT7BL, DT1DS, DT1DL, DT2DS, DT2DL, DT3DS, DT3DL, DT4DS, DT4DL, DT5DL, DT6DS, DT6DL, DT7DS, and DT7DL, were used to verify the chromosomal location of markers.

Results

Postulation of seedling resistance genes

The *P. triticina* isolates PRTUS 60, PRTUS 55, PRTUS 54, PRTUS 50, PRTUS 45, PRTUS 35, and PRTUS 25 were assigned the five-letter race designations (Table 2.3), MRDSD, TKLSQ, MTPTB, MKPSG, TTRSD, TNRSD, and MFBJG, based on high and low ITs (Long and Kolmer 1989) to host sets 1 to 5 shown in Table 2.2. The *P. triticina* isolates PT1, PT2, PT3 PT4, PT5, PT6, PT7, and PT8 were also assigned the five-letter race desginations, MGNTQ, PSMTJ, TFGSB, TDRSH, TTBSG, LBGTG, MBRTG, and BBBPB, based on high and low ITs to the five host sets shown in Table 2.2.

Seedling ITs for the 15 *P. triticina* isolates observed on the differential lines, 'Thatcher', 'Avocet S' and 'Amadina' are shown in Table 2.5. Isolates MRDSD, TKLSQ, and TFGSB produced high IT of 3^+ to 'Amadina'. Therefore, the genes *Lr2a*, *Lr2c*, *Lr9*, *Lr16*, *Lr24*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *Lr18*, *Lr21*, *Lr28*, *Lr39*, *Lr42*, *Lr50*, *Lr52*, and *Lr19*, which confer resistance to MRDSD, TKLSQ, and/or TFGSB (Figures 2.1, 2.2, and 2.10), cannot be present in 'Amadina'. All isolates except BBBPB produced high ITs of 3 to 3^+ to 'Avocet S' (Table 2.5). Therefore, the genes *Lr2a*, *Lr2c*, *Lr3*, *Lr9*, *Lr16*, *Lr24*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *LrB*, *Lr18*, *Lr21*, *Lr28*, *Lr39*, *Lr42*, *Lr50*, *Lr52*, *Lr19*, and *Lr23*, confering resistance to isolates MRDSD, TKLSQ, MTPTB, MKPSG, TTRSD, TNRSD, MFBJG, MGNTQ, PSMTJ, TFGSB, TDRSH, TTBSG, LBGTG, and MBRTG cannot be present in 'Avocet S'.

The 'Thatcher' NILs RL6003-*Lr1*, RL6002-*Lr3a*, RL6078-*Lr26*, RL6004-*Lr10*, and RL6012-*Lr23* conditioned ITs ;, ;, ;1, 2^+, and 2^+3C to isolate BBBPB. In this study, both 'Amadina' and 'Avocet S' displayed low IT of 0; to BBBPB (Table 2.5; Figure 2.14), slightly different from the ITs displayed by RL 6003-*Lr1*, RL6002-*Lr3a*, and RL 6078-*Lr26*. Isolate MTPTB (Table 2.5; Figure 2.3) was virulent on *Lr1* (IT = 3^+), *Lr3* (IT = 3), *Lr10* (IT = 3), and *Lr26* (IT = 3). Isolate TNRSD (Table 2.5; Figure 2.6) was virulent (IT ranges from 3^+ to 4) on *Lr1*, *Lr3*, *Lr10*, and *Lr23*, but avirulent on *Lr26* (IT = 2^+N). *Lr26* further conditioned a low IT of ;1 to PSMTJ and TDRSH (Table 2.5; Figures 2.9, 2.11), whereas genes *Lr1*, *Lr3*, *Lr10*, and *Lr23* conditioned high ITs ranging from 3 to 3^+. The 'Avocet S' x 'Amadina' RILs segregation for both PSMTJ and TDRSH was a good fit to 1R:3S ratio, with resistant plants having low ITs ranging from 0; to ;1N and 0; to ;2 to PSMTJ and TDRSH, respectively. Based on the segregation data, it is likely that

two complementary recessive genes were involved in the resistance to PSMTJ and TDRSH. However, a single recessive gene and a dominant inhibitor could also explain the results. Since ‘Amadina’ displayed a low IT to both PSMTJ and TDRSH, slightly similar to that of RL6078-*Lr26*, the resistance expressed by ‘Amadina’ could be due to the presence of *Lr26* and another seedling gene. High association ($P < 0.0001$) of the the RILs segregating for resistance to PSMTJ with the RILs segregating for resistance to TDRSH (Table 2.7), further confirmed that resistance to both PSMTJ and TDRSH was conditioned by the same genes in ‘Amadina’. Presence of *Lr26* in ‘Amadina’ was further validated by PCR amplification of the characteristic 206 bp product of the translocated rye fragment using the diagnostic marker SCM9 (Saal and Wricke 1999), and by C-banding detection (Gill et al. 1991) of the 1RS.1BL wheat-rye translocation karyotype (Gill et al. 1974a, b). The RILs that segregated for resistance to both PMSTJ and TDRSH were also highly associated ($P < 0.001$) with the RILs segregating for resistance to isolates MKPSG, TTRSD, TNRSD, MFBJG, and MGNTQ. This indicated that the same genes conditioning resistance to PSMTJ and TDRSH also conditioned resistance to MKPSG, TTRSD, TNRSD, MFBJG, and MGNTQ (Table 2.7).

A 560 bp PCR product of the pTAG621 STS marker for resistance gene *Lr1* (Feuillet et al. 1995) was amplified from the DNA of both ‘Amadina’ and ‘Avocet S’. However, amplification of the *Lr1* marker does not indicate presence of the gene because applicability of the marker in practical breeding program differs widely (Feuillet et al. 1995). Due to lack of diverse combinations of avirulence and virulence in the *P. triticina* isolates for *Lr1* and *Lr3a* in this study, we could not postulate the absence or presence of these two genes. However, *Lr3a* conditioned a low IT of ;1 to LBGTG similar to that of ‘Amadina’. Kolmer et al. (2005) reported that nearly all isolates in North America have virulence on *Lr3a*. Singh and Rajaram (1991) previously postulated *Lr10* and *Lr13* in ‘Avocet S’. According McIntosh et al. (1995) low ITs of *Lr13* ranges from ;1 through X, i.e. resistant host response characterized with heterogenous uredinia, similarly distributed over the leaves. Therefore, the resistance in ‘Avocet S’ to BBBPB could also be attributed to the presence of either *Lr10* or *Lr13*, or combination of both genes.

Isolate MFBJG was virulent on *Lr1* (IT = 3^{+}), *Lr10* (IT = 3), *Lr26* (IT = 3^{-}), and *Lr14a* (IT = 3). The RILs segregated in a 1R:3S ratio for MFBJG (Table 2.6), indicating

the presence of either two complementary recessive genes, or a recessive gene and a gene with dominant suppressive effect. Since the low IT 1^+N displayed by 'Amadina' in response to isolate MFBJG (Table 2.5; Figure 2.7) was distinct from the intermediate IT 2^+ displayed by RL6051-*LrB*, the resistance in 'Amadina' could be due to genes different from *LrB*. The presence or absence of *Lr14a* could not be determined in 'Amadina' because all the isolates in this study were virulent on *Lr14a*. *Lr23* conditioned an intermediate IT of 2^+, 23^-, and 2^+3 to isolates MTPTB, MKPSG, and MGNTQ at 20° C. To confirm the presence of *Lr23*, 'Amadina', 'Avocet S', the RILs derived from the 'Avocet S' x 'Amadina' cross, RL6012-*Lr23*, and the susceptible check 'Thatcher' were tested with isolates MTPTB, MKPSG, MGNTQ, and LBGTG at 25° C. RL6012-*Lr23* displayed a low IT of ;1 to MTPTB, MKPSG, and MGNTQ at 25° C. The RILs of 'Avocet S' x 'Amadina' segregated in a 1R:3S ratio to isolates MTPTB and MKPSG, and 1:1 ratio to isolate MGNTQ at both 20° and 25° C. The resistant plants had ITs ranging from 1 to 2^+N for MTPTB, 0; to ;2^+N for MKPSG, and 0; to 2^+N for MGNTQ at 20° C. The resistant plants displayed ITs ranging from 0; to 2^+N for MTPTB, ;1 to 2^+N for MKPSG, and ; to 2^+ for MGNTQ at 25° C. The ITs of 2^+ and ;1 displayed by RL6012-*Lr23* at 20° C and 25° C for MTPTB, respectively, were similar to that of 'Amadina'. Since MTPTB was virulent on *Lr1*, *Lr3a*, *Lr26*, *LrB*, *Lr10*, *Lr14a* at both temperatures, the resistance to MTPTB in 'Amadina' was most likley due to the presence of *Lr23*. The 1R:3S ratio of the RILs for MTPTB at both temperatures indicated the involvement of either a second complementary recessive gene in addition to *Lr23*, or a second gene with a dominant suppresive effect on the expression of *Lr23* in the segregating population. *Lr23* also conditioned resistance to isolates MKPSG, TTRSD, MFBJG, MGNTQ, PSMTJ, TDRSH, TTBSG, and LBGTG because of the association (Table 2.7) of the RILs segregating for resistance to MTPTB with the RILs segregating for resistance to MKPSG ($P < 0.0001$), TTRSD ($P < 0.01$), MFBJG ($P < 0.05$), MGNTQ ($P < 0.0001$), PSMTJ ($P < 0.05$), TTBSG ($P < 0.0001$), and LBGTG ($P < 0.001$).

LBGTG displayed virulence on *Lr1* and *Lr10* (Figure 2.13). The intermediate IT 2^+N displayed by RL6078-*Lr26* and RL6012-*Lr23* at 20° C in response to LBGTG was not similar to the low IT of ;1 displayed by 'Amadina'. At 25° C, *Lr23* conditioned a low IT of ;1 to LBGTG similar to that of 'Amadina'. The 1R:1S and 1R:3S segregation ratios

of the RILs for LBGTG at both 20 and 25° C indicated the inovolvment of either two complementary recessive genes, or a recessive gene and a gene with dominant suppressive effect. The RILs segregating for resistance to LBGTG were highly associated ($P < 0.001$) with the RILs segregating for resistance to MTPTB at 20° C (Table 2.7). Therefore, *Lr23*, which conditioned resistance MTPTB could possibly be one of the two genes conditioning resistance in 'Amadina' to LBGTG. However, the low IT of ;1 expressed by 'Amadina' to LBGTG at 20° C could not be attributed to *Lr23* because RL6012-*Lr23* displayed an intermdiate IT of 2^{+}N.

The low IT of 'Amadina' to TTRSD and TTBSG cannot be explained by the presence of *Lr1*, *Lr3*, *Lr10*, *Lr23*, and *Lr26* because both TTRSD and TTBSG were virulent on all these genes (Figures 2.5; 2.12). However, it is possible that the presence of other seedling gene(s) in 'Amadina' may be responsible for the observed low to intermediate ITs.

Map construction

Among the 1600 SSR markers tested on 'Avocet S' and 'Amadina', 103 SSRs were polymorphic between the two parents. These SSR markers were supplemented with 437 polymorphic DArT markers. The loci of the linkage map constructed with the RIL population were grouped into 35 linkage groups. The total distance covered was 1878 cM. The average genetic distance was 5.26 cM with at least 2 marker loci per linkage group. Linkage groups were assigned to chromosomes by comparing positions of SSR and DArT markers to previously published hexaploid wheat maps (Neumann et al. 2010; Francki et al. 2009; Singh et al. 2009; Huynh et al. 2008; Mantovani et al. 2008; Crossa et al. 2007; Akbari et al. 2006; Semagn et al. 2006; Song et al. 2005; Somers et al. 2004; Sourdille et al. 2004). Final mapping was done by combining 2 or more linkage groups that belong to the same chromosome. The map position of most of the marker loci for 'Avocet' x 'Amadina' RIL population showed generally good consistency to the reference maps.

Gene mapping and cytogenetic analysis of markers flanking putative loci

PCR-based SSR and DArT primers (shown in Table 2.10) were used to assign markers flanking putative loci to specific chromosome arms. In cases where sequence information

of marker was not available, primers were designed for the nearest markers to the markers whose sequence information was not available.

CIM of the phenotypic leaf rust response and marker data using QTL Cartographer and QTLNetwork v2.0. detected loci at the intervals *XwPt3753–XwPt4434*, *XwPt1781–XwPt8267*, and *XwPt1684–XwPt1781* in response to the *P. triticina* isolates MKPSG, TNRSD, and LBGTG, and *XwPt3451–XwPt7094* and *XwPt8949–XwPt3852* in response to isolates MFBJG, MGNTQ, PSMTJ, TDRSH, and TTBSG. Based on the genetic distances of markers in the linkage group, we believe the loci detected at intervals *XwPt3753–XwPt4434*, *XwPt1781–XwPt8267*, and *XwPt1684–XwPt1781* correspond to the same locus, and the loci detected at intervals *XwPt3451–XwPt7094* and *XwPt8949–XwPt3852* are also the same locus. Differences in the intervals were likely due to shifts in the gene position as a result of race-specificity. Sequence information was not available for all markers flanking the putative loci. Using the cytogenetic stocks, we were able to assign *XwPt6442* and *XwPt8986*, which were the closest markers to the first locus, and *XwPt1560* which was the closest marker to the second locus on chromosome 1BL (Figure 2.15). A third locus with non-additive effect was also detected at the *XwPt359–XwPt6434* interval in response to MGNTQ (Figure 2.32). The X*wPt359–XwPt6434* interval was located 4.0 cM from the first locus and 16.0 cM from the second locus on 1BL (Figure 2.15). Thus, a total of three loci, two with additive main effect and one with non-additive effect, were identified on chromosome 1BL.

CIM detected loci at *XwPt615-Xbarc183*, *XwPt6932–XwPt8492*, and *XwPt6477–Xbarc7* intervals in linkage group 2B in response to MTPTB, MKPSG, TNRSD, MGNTQ, and TDRSH. Based on previous published genetic maps, *XwPt615*, *Xbarc183*, *XwPt6932*, *XwPt6477*, and *XwPt8492* mapped to the short arm of chromosome 2B (Somers et al. 2004; Huynh et al. 2008; Mantovani et al. 2008; Singh et al. 2009). The loci at the *XwPt615–Xbarc183*, *XwPt6932–XwPt8492*, and *XwPt6477–Xbarc7* intervals correspond to same locus based on the genetic distances in the linkage group. CIM also detected loci at *XwPt6419–XwPt6200*, *XwPt9780–XwPt6419*, and *XwPt4144–XwPt9780* intervals in response to isolates MTPTB, TNRSD, and MGNTQ. Shift in gene position was also evident in response to the three isolates. The DArT marker *XwPt4144* was previously mapped to chromosome 2DS (Crossa et al. 2007; Neumann et al. 2010).

Estimation of additive, non-additive, and epistatic effects

Additive (A), dominance (D), and epistatic effects categorized as additive x additive effects (AA), additive x non-additive effects (AD), non-additive x additive effects (DA), and non-additive x non-additive effects (DD) (Table 2.12) were estimated using QTLNetwork v2.0. (Yang et al. 2005).

MTPTB isolate

We identified two genes at intervals *XwPt615–Xbarc183* and *XwPt6419–XwPt6200* on chromosomes 2BS and 2DS (Figures 2.18 and 2.28) with significant ($P < 0.0001$) additive effects of -0.2466 and -0.1860 that accounted for 20.61 and 21.55% of the phenotypic variance, respectively (Table 2.11). Interaction between the two genes significantly ($P < 0.0001$) reduced IT, with additive x additive interaction explaining 16.63% of the observed phenotypic variance (Table 2.12).

MKPSG isolate

Two genes with additive effects were identified at *XwPt3753–XwPt4434* and *XwPt6932–XwPt8492* intervals on chromosomes 1BL and 2BS (Figures 2.19 and 2.29) explaining 25.62% and 8.31% of the phenotypic variance due to significant ($P > 0.0001$) additive effects of -0.2030 and -0.1584, respectively (Table 2.11). Epistatic interaction between the two genes significantly ($P < 0.01$) reduced IT, with additive x additive interaction explaining 3.78% of the observed phenotypic variance (Table 2.12).

TNRSD isolate

Two genes were identified at *XwPt1781–XwPt8267* and *XwPt9780–XwPt6419* intervals on chromosomes 1BL and 2DS (Figures 2.21 and 2.30) with significant ($P < 0.0001$) additive effects of -0.5105 and +0.1007 that accounted for 48.27 and 9.98% of the observed phenotypic variance, respectively (Table 2.11). Interaction between the two genes significantly ($P < 0.05$) reduced IT, with additive x additive interaction explaining 1.46% of the phenotypic variance (Table 2.12).

MFBJG isolate

A single gene was identified at *XwPt3451X–wPt7094* interval on the long arm of chromosome 1B (Figures 2.22 and 2.31) with significant ($P < 0.0001$) additive effect of -0.4164 that accounted for 58.84% of the observed phenotypic variance (Table 2.11).

MGNTQ isolate

Three genes were identified on chromosomes 1BL (*XwPt8949–XwPt3852* and *XwPt359–XwPt6434*), 2BS (*XwPt6477–Xbarc7*), and 2DS (*XwPt4144–XwPt9780*) (Figures 2.23 and 2.32). The genes at *XwPt8949–XwPt3852*, *XwPt6477–Xbarc7*, and *XwPt4144–XwPt9780* intervals on 1BL, 2BS and 2DS had significant ($P < 0.0001$) additive effects of -0.3311, -0.1595, and -0.0974, accounting for 32.21, 10.02, and 8.08% of the observed phenotypic variation (Table 2.11). Three interactions for response to MGNTQ were detected (Table 2.12). Additive x additive interaction between genes at interval *XwPt8949–XwPt3852* on 1BL and *XwPt6477–Xbarc7* on 2BS significantly ($P < 0.01$) increased IT, with the interaction explaining 3.12% of the phenotypic variance. Additive x additive interaction between genes at *XwPt8949–XwPt3852* interval on 2BS and *XwPt6477–Xbarc7* interval on 2DS significantly ($P < 0.0001$) reduced IT, with the interaction explaining 5.66% of the observed phenotypic variance. Non-additive x additive interaction, involving a gene at *XwPt359–XwPt5067* interval on 1BL and the gene on 2DS, was also detected, with interaction significantly ($P < 0.0001$) increasing IT and explaining 4.90% of the observed phenotypic variance. The region at the *XwPt359–XwPt5067* interval was located 46.8 cM from the gene at *XwPt8949–XwPt3852* interval on 1BL and approximately 4.0 cM (Figures 2.15 and 2.32) from the gene detected on 1BL in response to isolates, MTPTB, TNRSD and LBGTG

LBGTG isolate

A single gene with a significant ($P < 0.0001$) additive main effect of -0.4032 at the *XwPt1684–XwPt1781* interval on the long arm of chromosome 1B (Figures 2.27 and 2.33) accounted for 49.96% of the observed phenotypic variation (Table 2.11).

Discussion

Seedling resistance genes

Variation in ITs of the known *Lr* gene-carrying differential lines, inoculated with 15 *P. triticina* isolates (Table 5), indicated the possibility of the identification of 24 resistance genes (*Lr1*, *Lr2a*, *Lr2c*, *Lr3*, *Lr9*, *Lr16*, *Lr24*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *LrB*, *Lr10*, *Lr14a*, *Lr18*, *Lr21*, *Lr28*, *Lr39*, *Lr42*, *Lr50*, *Lr52*, *Lr19*, and *Lr23*). To corroborate the postulated genes, the pedigrees (where available) were examined, cytogenetic analysis was carried out, and chromosomal locations of mapped seedling resistance genes were taken into account in some cases. 'Amadina' was determined to have leaf rust resistance genes *Lr23* and *Lr26*. 'Amadina' was highly susceptible to isolates MRDSD and TKLSQ at the seedling stage. Thus, *Lr23* and *Lr26* were not effective against the isolates MRDSD and TKLSQ used in this study.

The resistance of *Lr23* is best expressed at temperatures above 25° C (Dyck and Johnson 1983). With isolates MTPTB, MKPSG, MGNTQ, and LBGTG, ITs to RL 6012-*Lr23* ranged from 2^{+} to $2^{+}3$ at 20° C. 'Amadina' displayed an intermediate IT of 2N to MTPTB at 20° C, slightly similar to that of RL 6012-Lr23. Kolmer and Oelke (2006) reported that RL 6012-*Lr23* can produce ITs varying from 2 to 3 at normal greenhouse temperatures of 18–22° C with the same leaf rust isolate, depending on ambient temperature and lighting conditions. At 25° C, RL 6012-*Lr23* displayed low IT ;1 to MTPTB, MKPSG, MGNTQ, and LBTG, similar to that of 'Amadina'. The RILs segregation patterns in the 'Avocet S' x 'Amadina cross indicated two complementary recessive genes for resistance to isolates MTPTB, MKPSG, and LBGTG. Since complementary leaf rust resistance genes are rare (Singh and McIntosh 1984) the possibility of a suppressor of *Lr23* (McIntosh and Dyck 1972; Oelke and Kolmer 2005) was considered. Involvement of a recessive resistance gene and a dominant suppressor would give the same 1R:3S ratio as explained by two complementary recessive genes. In this study, it is highly likely the expression of *Lr23* in the segregating population was suppressed by a gene in 'Avocet S' since we failed to map the gene even at 25° C. If the suppressor gene had been present in 'Amadina', in combination with *Lr23*, we would not have observed the low IT ;1 in seedling tests of 'Amadina' to the isolates MTPTB, MKPSG, MGNTQ and LBGTG that have low ITs to RL6012-*Lr23*. Genes suppressing rust resistance have been identified in wheat, including suppressors of resistance of stripe

rust (Kema et al. 1995), stem rust (Kerber and Aung 1999), and leaf rust (McIntosh and Dyck 1972; Dyck 1982; Nelson et al. 1997). A gene in 'Thatcher' that suppressed the expression of *Lr23* had previously been reported (McIntosh and Dyck 1972; Dyck 1982). The suppressor gene in 'Thatcher' that inhibited the expression of *Lr23* when tested with isolates of *P. triticina* from Canada acted as a partial suppressor when tested with isolates from Australia (McIntosh and Dyck 1972). McIntosh and Dyck (1972) further reported that *Lr23* was recessive in some crosses, and partially dominant in other crosses. Nelson et al. (1997) described an interaction between the *Lr23* region on chromosome 2B of the durum wheat 'Altar C84' and a region on chromosome 2D known to possess the suppressor *SuLr23* using a cross between a synthetic hexaploid developed from 'Altar C84' and a *T. tauschii* and the bread wheat *cv* 'Opata'. Singh et al. (1996) found that suppressors for leaf and stem rust resistance in *Triticum* interspecific crosses occur at all ploidy levels and can be accession-specific. Accessions of both AA and DD species can be donors of either the resistance or the suppressor genes in combination with various AABB *T. durum* accessions.

According to Dyck (1982), the dominance effect of *Lr23* increased at higher temperatures. Temperature-sensitivity and isolate-dependency of *Lr23* makes it difficult to reliably detect the gene in segregating populations. 'Amadina' (Bobwhite/Crow//Buckbuck/Pavon 76/3/Veery#10) likely inherited *Lr23* from 'Bobwhite' and 'Veery #10' which carry the 1BL.1RS translocation derived from the Russian cultivar 'Kavkaz' respectively (Zeller and Hsam 1984; http://wheat.pw.usda.gov/ggpages/awn/47/Textfiles/UKRAINE.html). This gene is no longer effective in most geographic areas except Europe (McIntosh et al. 1995; Singh 1993). Virulence for *Lr23* is common in Australia (Park and Wellings 1992) and Mexico (Singh 1991). Pathogenicity for *Lr23* is not routinely surveyed in North America due to variable ITs that a single isolate can produce on a RL 6012-*Lr23* (Oelke and Kolmer 2005; Kolmer et al. 2007). In most areas pathogenicity for *Lr23* shows wide range of variation over space and time. The RILs segregating for resistance to MTPTB were highly associated with RILs segregating for resistance to MKPSG ($P < 0.0001$), TTRSD ($P < 0.01$), MGNTQ ($P < 0.0001$), TTBSG ($P < 0.0001$) and LBGTG ($P < 0.001$), indicating that the same genes conditioning resistance to MTPTB also conditioned

resistance to MKPSG, TTRSD, MGNTQ, TTBSG, and LBGTG. However, the low IT displayed by 'Amadina' at 20° C in response to MKPSG (IT = ;1), TTRSD (IT = 1$^+$), MGNTQ (IT = ;1), TTBSG (IT = ;1), and LBGTG (IT = ;1) could not be due to *Lr23* since the gene conditioned intermediate ITs ranging from 2$^+$ to 2$^+$3 at 20° C. Isolates MKPSG, TTRSD, and TTBSG were virulent on *Lr1*, *Lr3a*, *LrB*, *Lr10*, and *Lr14a*. Isolate MGNTQ was virulenct on *Lr1*, *Lr3a*, *LrB*, *Lr10*, and *Lr14a*, but aviurlent on *Lr26*. Isolate LBGTG was virulent on *Lr1*, *LrB*, *Lr10*, and *Lr14a*, but aviurlent on *Lr3a* and *Lr26*. The resistance displayed by 'Amadina' to both MGNTQ and LBGTG could also not be due to *Lr26* since the low IT ;1 displayed by 'Amadina' to both isolates was different from the intermediate ITs displayed by RL6078-*Lr26*. It is possible that the presence of other seedling gene(s) in 'Amadina' may be responsible for the observed low ITs. Segregation for isolates MKPSG and MGNTQ conformed to a 1R:1S ratio, a good fit for a genetic model implying the presence of one dominant gene in 'Amadina', whereas segregation for isolates TTRSD, TTBSG, and LBGTG conformed to a 1R:3S ratio in the RIL population, a good fit for a genetic model implying the presence of a recessive gene and a gene with a dominant suppressive effect.

Isolate LBGTG was virulent on *Lr1*. RL6078-*Lr26* and RL6012-*Lr23* conditioned intermediate ITs of 2$^+$N to LBGTG, whereas *Lr3a* conditioned a low IT of ; to LBGTG at 20° C. 'Amadina' conditioned a low IT of ;1 different from that of the RL6078-*Lr26* and RL6012-*Lr23*. At 25° C, 'Amadina' displayed a low IT of ;1 similar to that of RL6012-*Lr23*. LBGTG was virulent on 'Avocet S' at both 20 and 25° C. Sacco et al. (1998) identified an RFLP marker *Xmwg798* co-segregating with the allele *Lr3a*, which is located on 6BL of the common wheat 'Sinvalocho MA'. This RFLP marker has been converted to a PCR-based STS marker (Künzel et al., 2000). The STS version of the marker *Xmwg798*, reported to co-segregate with *Lr3a* in the common wheat cultivar Sinvalocho MA (Sacco et al., 1998), and Storlom (Herrera-Foessel et al. 2007) was present in 'Amadina', 'Avocet S' and all the RILs derived from 'Avocet S' x 'Amadina' cross, indicating that the low IT of ;1 displayed by 'Amadina' in response to LBGTG was due to a gene different from *Lr3a* since 'Avocet S' and some of the RILs displayed susceptibility to the isolate. Kolmer et al. (2005) previously reported that nearly every current leaf rust isolate in North America is virulent to *Lr3a*.

DNA amplification product corresponding to STS marker of *Lr1* was identified in both 'Amadina' and 'Avocet'. 'Pavon 76', which is prominent in the pedigree of 'Amadina', and 'Avocet S' was postulated to carry *Lr1* (Singh and Rajaram et al. 1991). However, the presence or absence of *Lr1* could not be postulated in this study due to the limited number and lack of diversity in the isolates used. Feuillet et al. (1995) reported that the marker for the *Lr1* resistance gene, pTAG621, can only be used in half of the breeding material because 50% of the breeding lines carry the same marker allele as the lines with *Lr1* but do not contain this resistance gene. Chełkowski et al. (2003) screened a set of 40 Thatcher NILs with the pTAG621 marker, and sucessfully amplified the characteristic marker 560 bp product in 'Thatcher' and all differentials lacking the *Lr1* resistance gene. As a result of the allele nonspecificty of the pTAG621 STS marker, its application in pratical breeding programs can differ widely. Thus, sequencing of the PCR product and comparison of the sequence with the original sequences (included in the NCBI database: www.ncbi.nlm.gov) of the two alleles coresponding to susceptible 'Thatcher' and 'Thatcher' differential RL6003-*Lr1* (Feuillet et al. 1995) could have clearly validated the absence or presence of the resistance gene in both 'Amadina' and 'Avocet S'. Resistance gene *Lr1* was first described in cultivar Malakoff (Mains et al. 1926) and reported as *Lr1* in 1946 (Ausemus et al. 1946). Feuillet et al. (1995) mapped *Lr1* to the distal end of chromosome 5DL close to markers pTAG621 and Xpsr567. *Lr1* is putatively orthologous to a gene in *Ae. tauschii* which confers the same phenotype to a number of rust races, suggesting that the gene originally evolved in diploid goat grass and was introgressed into the wheat D genome during or after domestication of hexaploid wheat (Ling et al. 2004). Map based cloning and sequencing of the *Lr1* gene revealed coiled coil (CC), nucleotide-binding-site (NBS), and leucine-rich-repeat (LRR) motifs (Cloutier et al. 2007).

All isolates except BBBPB were virulent on *Lr10*. The resistance in 'Amadina' and 'Avocet S' to isolate BBBPB was not due to *Lr10* since both 'Amadina' and 'Avocet S' displayed a low IT of 0;, while *Lr10* displayed an intermediate IT of 2^+. A 310 bp PCR product corresponding to STS marker for *Lr10* was detected only in 'Avocet S'. This is consistent with the previous postulation of *Lr10* in 'Avocet S' by Singh and Rajaram et al. (1991). Low ITs expressed by cultivars carrying *Lr10* range from 0; to 12 depending

on the pathogen culture, environmental conditions, and host genetic background (McIntosh et al. 1995). *Lr10*, located on chromosome 1AS (Feuillet et al. 1997) was also recently cloned in hexaploid wheat through map based cloning (Feuillet et al. 2003). Sequencing analysis of the gene revealed coiled coil (CC), nucleotide-binding-site (NBS), and leucine-rich-repeat (LRR) motifs (Feuillet et al. 2003). Using a combination of subgenome map-based cloning and haplotype studies in the genus, Feuillet et al. (2003) isolated *Lr10*. The gene encodes a coiled coil (CC), nucleotide-binding-site (NBS)-leucine-rich-repeat (LRR) type of protein with an N-terminal domain, which is under diversifying selection. *Lr10* has similarities to *RPM1* in *Arabidopsis thaliana* (Grant et al. 1995) and to resistance gene analogs in rice and barley, but is not closely related to other wheat *Lr* genes based on Southern analysis.

Resistance genes *Lr1*, *Lr3a*, *Lr10*, and *Lr14a* are completely ineffective even though they may be common in wheat germplasm because nearly every *P. triticina* isolate in the north central region of the United States and other wheat growing areas (Kolmer et al. 2003; Kolmer and Oelke 2005) has virulence to these genes.

In this study, the Thatcher NIL, RL6078-*Lr26*, expressed a low IT of ;1 in response to the isolates PSMTJ and TDRSH, and 'Amadina' expressed a low IT of 0; in response to both isolates which is similar to the characteristic IT of 0; for *Lr26* (McIntosh et al. 1995). The presence of *Lr26* in 'Amadina' was corroborated by the pedigree data from CIMMYT website. 'Bobwhite' and 'Veery #10' in the pedigree of 'Amadina' are the likely sources of the 1BL.1RS translocation. Both 'Bobwhite', and 'Veery #10' carry the 1BL.1RS translocation derived from the Russian cultivars 'Aurora', and 'Kavkaz' respectively (Zeller and Hsam 1984). The SCM9 marker diagnostic for the 1RS.1BL wheat-rye translocation (Saal and Wricke 1999), coupled with the C-banding detection of the 1RS.1BL wheat-rye translocation karyotype in 'Amadina' further validates the presence of *Lr26* in 'Amadina'. *Lr26* is completely linked with *Sr31*, and *Yr9*. Several wheat cultivars and CIMMYT lines carry this translocation (Braun et al. 1998; Singh and Rajaram 1991). Virulence on *Lr26* has been reported in many wheat growing areas, including the United States in 2004 (Long and Kolmer 1989; Singh 1993; McIntosh et al. 1995 Kolmer et al. 2006).

Composite interval mapping of seedling genes

CIM of the phenotypic leaf rust response and marker data detected four genomic regions: two on the long arm of chromosome 1B in response to *P. triticina* isolates MKPSG, TNRSD, MFBJG, MGNTQ, and LBGTG, the third gene on 2BS in response to MTPTB, MKPSG, and MGNTQ, and the fourth gene on 2DS in response to MTPTB, TNRSD, and MGNTQ. All regions except the one detected at interval *XwPt9780–XwPt6419* in response to isolate TNRSD reduced IT significantly (Table 2.11). The two regions on chromosome 1B were 50.9 cM apart. SSR *Xwmc128* was 10.5 cM from the first region on chromosome 1B, and *Xwmc216* was 19.6 cM from the second gene on 1B. Based on the Consensus and ITMI maps, and the location of the *Xwmc128* and *Xwmc216* loci (Somers et al. 2004; Song et al. 2005), both genes on 1B should map to C–1BL6–0.32 bin, the centromeric region of the long arm of chromosome 1B. Althoug the genes are located 50.9 cM apart on chromosome 1BL, we cannot unambigously declare them to be distinct given the biased mapping results from CIM because of the simulateous estimation of QTL and background effects in the implementation algorithm (Li et al. 2007). To the best of our knowledge, no leaf rust resistance seedling genes on chromosome 1BL have been reported.

We mapped a gene on chromosome 2D. The SSR marker *Xcfd116* was 25.1 cM from the gene. Referring to the ITMI and Consensus maps and the location of the *Xcfd116* locus, the gene at the interval *wPt4144–wPt6200* should map between the 2DS1–0.33–0.47 and 2DS1–0.33 bins of the linkage group 2D. Our gene postulation study indicated the absence of *Lr2a* and *Lr2c* (which are located on 2DS) in 'Amadina'. Thus, the gene detected on 2DS can either be *Lr2b* or *Lr15* (McIntosh et al. 1995). However, McIntosh et al. (1995) reported that *Lr2b* would seem to have no advantage over *Lr2a*. Though *Lr15* remains as the only likely gene detected on 2DS by the CIM, a further study on the response of differentials carrying *Lr2b* and *Lr15* to the isolates of *P. triticina* is required for proper identification of the gene on 2DS.

Cakir et al. (personal communication) reported tight linkage of Xbarc183 molecular marker with the leaf rust adult plant resistance gene *Lr13* on 2BS using 'Leichardt' x 'WAWHT2071' F_2 and F_3 mapping populations. The gene mapped on chromosome 2BS in response to the isolate MKPT in this study was flanked by *XwPt615*

and *Xbarc183* markers. Singh et al. (2009) previously reported linkage between the *Lr13* locus and *XwPt8492* which was 1.1 cM from *XwPt615* in this study. We therefore concluded that the gene on 2BS in this study was *Lr13*. If we refer to the ITMI and Consensus maps and the location of the *Xbarc183* locus, *Lr13* should map to the 2BS1–0.53–0.75 bin of linkage group 2B. Presence of *Lr13* in 'Avocet S' (McIntosh et al. 1995; Singh and Rajaram 1991) validates its presence in the population. Although *Lr13* was originally reported as a gene for adult plant resistance, it was always clear that the onset of resistance occurred at a relatively early growth stage (McIntosh et al. 1995). *Lr13* was shown previously to interact with other leaf rust resistance genes in cultivars and NILs to condition a higher level of resistance than expressed with the single genes (Samborski and Dyck 1982; Kolmer 1992). *Lr13* enhances the effect of certain seedling resistance genes that by themselves confer an intermediate IT (Kolmer 1992). Furthermore, German and Kolmer (1992) reported enhancement of leaf rust resistance conferred by unlinked race-specific genes when combined with the slow-rusting adult plant resistance gene *Lr34*. However, Kolmer and Oelke (2006) reported that the adult-plant gene *Lr13*, present in many spring wheat cultivars, no longer conditions effective resistance in the northern Great Plains region. Since the tests in this study were conducted with two-leaf stage seedling plants, the effect of the *Lr13* adult-plant IT for each isolate on the degree of interaction for resistance was not determined.

Epistatic interactions

Three possible epistatic interactions, as suggested by Marwede et al. (2005), can be (type I) interactions between two genes with additive effect, (type II) interactions between a gene with additive effect and a "background" locus without additive effect, or (type III) interactions between two loci showing epistatic effects only. In this study, type I and type II interactions were detected, suggesting that the interaction between the seedling genes and some of the isolates in the present study involved more than a single gene in the plant and a single gene in the isolates. Epistatic interaction involving *Lr13* and the seedling resistance gene on 2DS enhanced resistance, whereas interaction between *Lr13* and the gene on 1BL enhanced resistance as well as susceptibility. Non-additive x additive interaction between a gene, with no main effect, on 1BL and the gene on 2DS was also

detected, with the interaction having a very small but significant effect on IT. Our finding is not consistent with Sidhu (1984) who argued that successful application of gene-for-gene concept in agriculture occurs under conditions in which a crop is subject to attack mostly by one particular parasite or pathogen species. Gene-for-gene interactions involving the formal analysis of at least a few genes for resistance and avirulence have been determined in plant-pathogen associations (Singh and McIntosh 1984; Singh et al. 2001; Kolmer 2003; Mebrate et al. 2008). In all of these associations, although resistance and avirulence are largely inherited as single genes in plant-pathogen associations, additional complications have occasionally been detected. Singh and McIntosh (1984) reported that resistance in wheat to *P. triticina* strain 10-1,2,3,4 requires both *Lr27* on chromosome 3BS and *Lr31* on chromosome 4BS. Salmeron et al. (1994) found that mutations in either of two closely linked tomato genes (*PRF* and *PTO*) result in the loss of recognition of *P. syringae* pv. tomato strains that express *avrPto*. Mutations in these genes also blocked the recognition of an unidentified second avirulence gene, as an avirulent strain that lacked *avrPto* also became virulent on the mutant tomato lines. Thus both *PRF* and *PTO* are required for the recognition of two different *P. syringae* avirulence genes. A screen for mutants in tomato revealed that resistance to race 9 of the fungal pathogen *Cladosporium fulvum* requires three genes, the classically defined R gene *Cf9*, and two previously unidentified loci designated *Rcr1* and *Rcr2* (Hammond-Kosack et al. 1994). Mutations in *Rcr1* and *Rcr2* produce a partially susceptible phenotype, suggesting that their functions may be partially redundant to each other, or to other unidentified genes. Jørgensen (1988) and Freialdenhoven et al. (1994) reported similar results in barley for resistance to powdery mildew (*Erysiphe graminis*); resistance mediated by the *Mla12* gene requires two additional genes, *Nar1* and *Nar2*. Our results suggest that additive effects (and not dominance effects) play a major role in the genetic basis of seedling leaf resistance, and epistasis played a minor role.

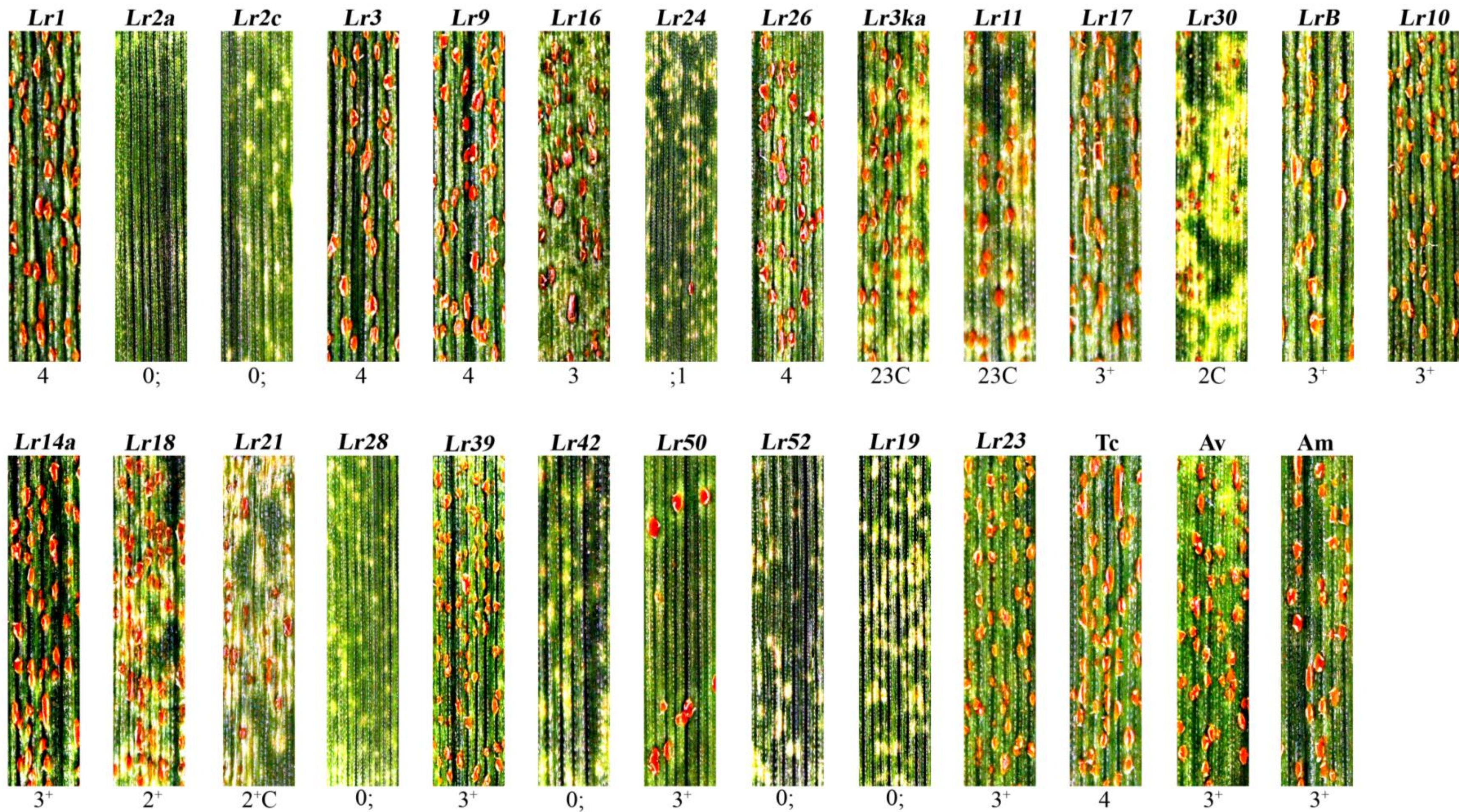
Conclusion

Identification of leaf rust resistance genes in current breeding materials, and continued monitoring and identification of leaf rust virulence phenotypes can aid in the development of wheat cultivars with effective leaf rust resistance. We were able to map a

total of three genes on chromosomes 1BL, 2BS, and 2DS. There is no report that we are aware of seedling leaf rust resistance genes residing in the centromeric region of the long arm of chromosome 1B; therefore the gene on 1BL can be a potentially new unexploited gene for leaf rust resistance in wheat. The identities of the seedling resistance genes *Lr23*, and *Lr26* in 'Amadina' were postulated on the basis of gene-for-gene specificity, and in the case of *Lr26*, we corroborated the result through PCR and cytogenetic analyses. The resistance of *Lr23* is best expressed at temperatures above 25° C (Dyck and Johnson 1983). Presence of a suppressor gene in 'Avocet S' further complicated the expression of *Lr23* in the 'Avocet S' x 'Amadina' RIL population. We postulated the absence of *Lr2a*, *Lr2c*, *Lr9*, *Lr16*, *Lr24*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *Lr18*, *Lr21*, *Lr28*, *Lr39*, *Lr42*, *Lr50*, *Lr52*, and *Lr19* in 'Amadina', and in some cases the results were verified through molecular markers. The characteristic 282 bp PCR product of the diagnostic PS10 marker for *Lr47* was not amplified from the DNA of 'Amadina' and 'Avocet S'. The resistance gene *Lr47*, derived from *Ae. speltoides*, is effective on all isolates of leaf rust in Mexico. Observed susceptibility of both 'Amadina' and 'Avocet S' to some isolates in Mexico validates the absence of *Lr47*. There are obvious limitations to the gene-for-gene approach for analysis of *Lr* genes in wheat. The *P. triticina* isolates that were used in this study were not adequate to identify all of the seedling *Lr* genes that were present in 'Amadina'. For example, we could not postulate the absence or presence of *Lr1*, *Lr3a*, *LrB*, *Lr10* and *Lr14a* in 'Amadina' due to lack of diverse combinations of avirulence and virulence genes in the isolates used in this study. A more diverse collection of isolates may have allowed the postulation of all the leaf rust *Lr* genes in 'Amadina'. Thus, further genetic analysis is required to determine the number and identity of leaf rust resistance genes in 'Amadina'.

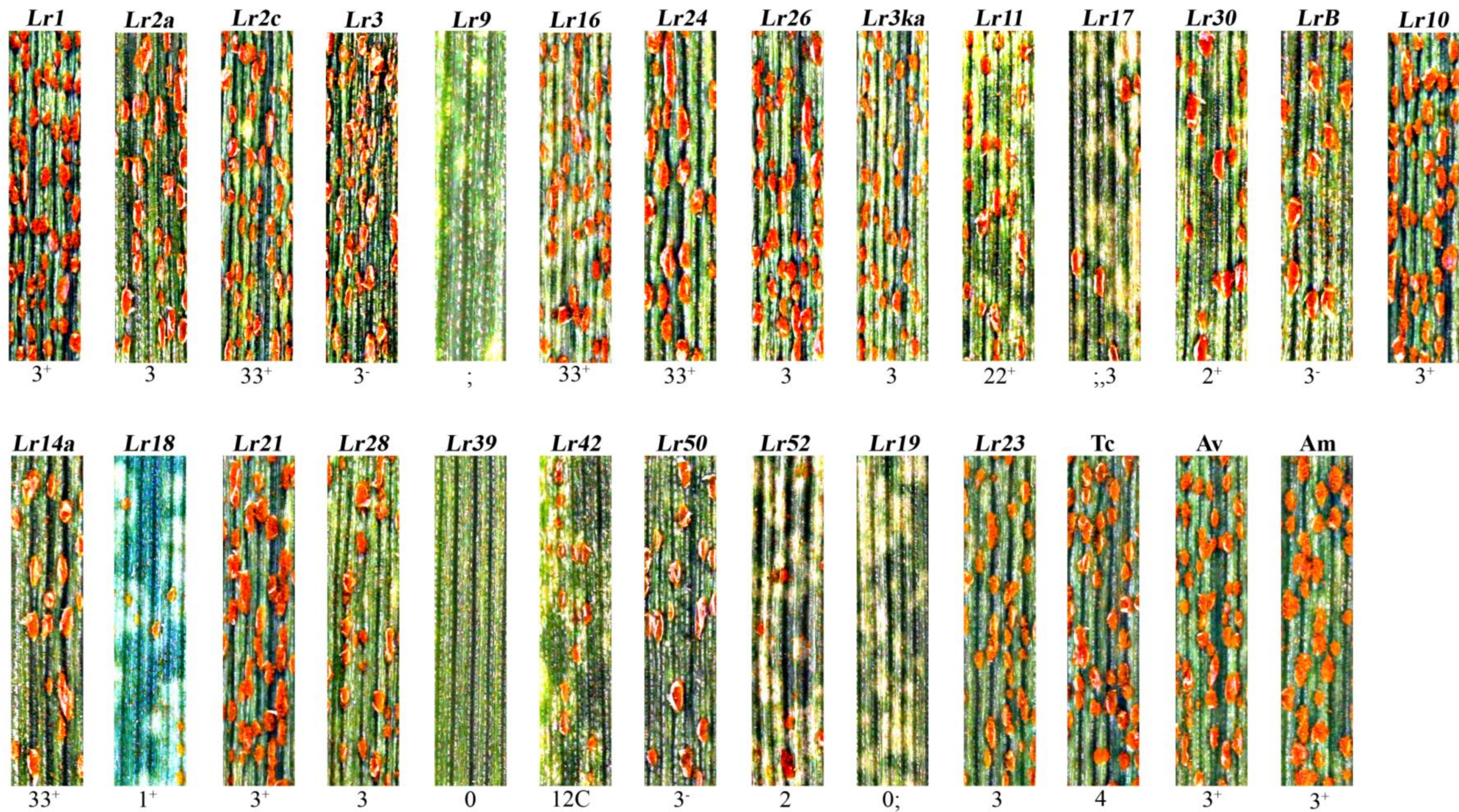
Figures and Tables

Figure 2.1 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S '(Av), and 'Amadina' (Am) inoculated with isolate MRDSD (PRTUS 60)



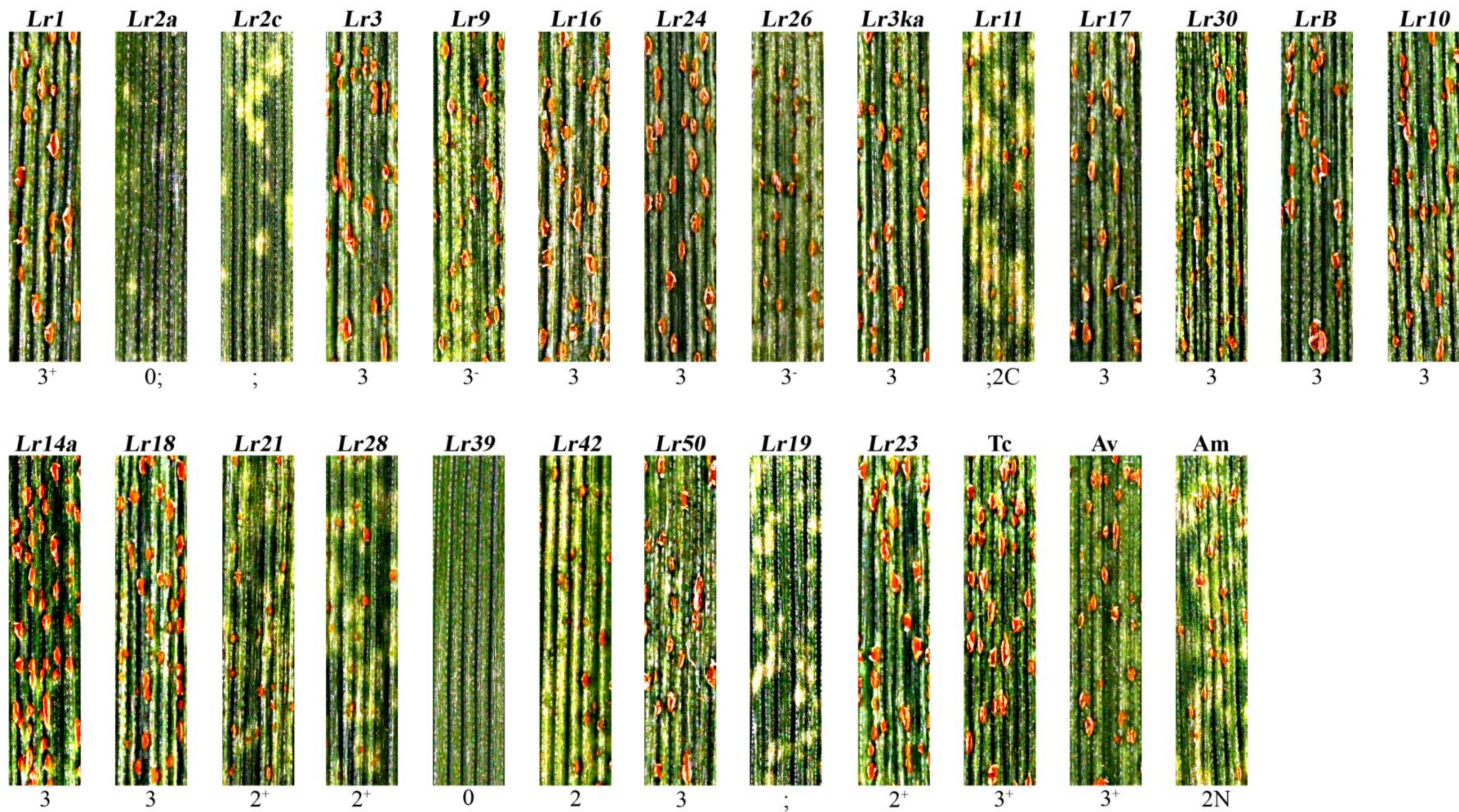
‡**Virulence/avirulence formula:** *Lr1*, *3*, *9*, *16*, *26*, *17*, *B*, *10*, *14a*, *39*, *50*, *23* / *Lr2a*, *2c*, *24*, *3ka*, *11*, *30*, *18*, *21*, *28*, *42*, *52*, *19*

Figure 2.2 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S' (Av), and 'Amadina' (Am) inoculated with isolate TKLSQ (PRTUS 55)



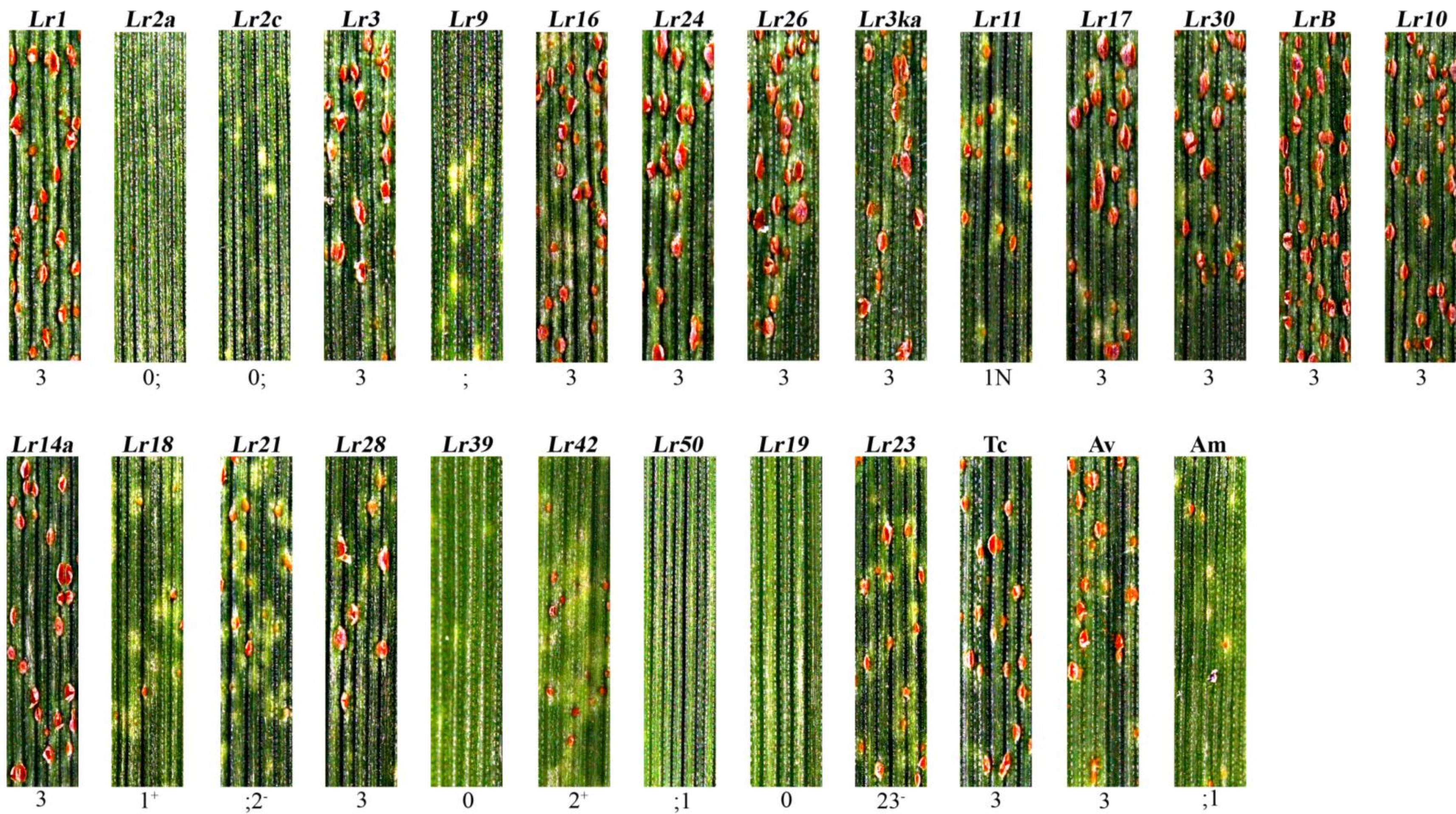
‡**Virulence/avirulence formula:** *Lr1*, *2a*, *2c*, *3*, *16*, *24*, *26*, *3ka*, *B*, *10*, *14a*, *21*, *28*, *50*, *23* / *Lr9*, *11*, *30*, *18*, *39*, *42*, *52*, *19*

Figure 2.3 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S' (Av), and 'Amadina' (Am) inoculated with isolate MTPTB (PRTUS 54)



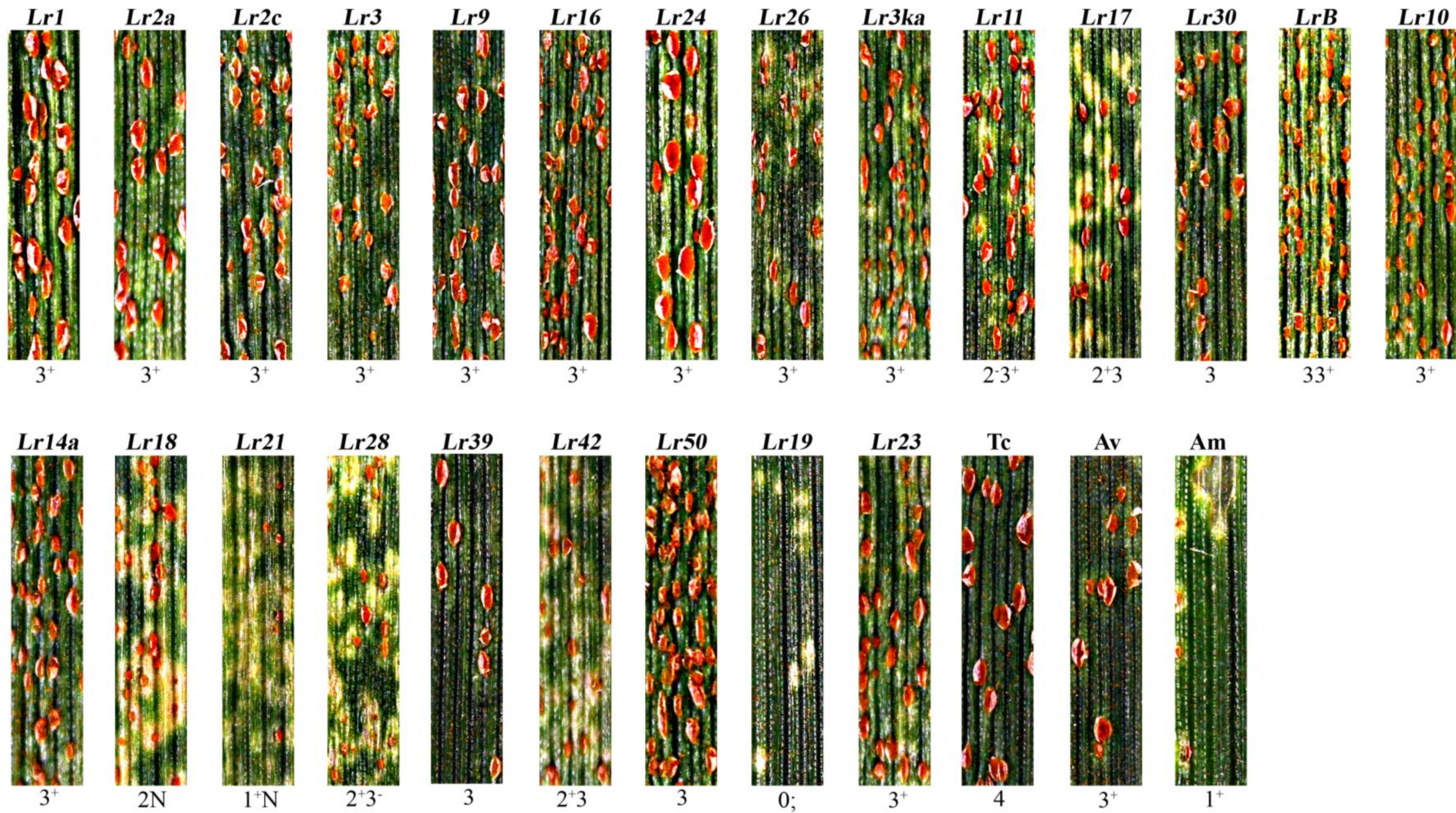
‡**Virulence/avirulence formula:** *Lr1*, *3*, *9*, *16*, *24*, *26*, *3ka*, *17*, *30*, *B*, *10*, *14a*, *18*, *50*, *23* / *Lr2a*, *2c*, *11*, *21*, *28*, *39*, *42*, *19*, *23*

Figure 2.4 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S' (Av), and 'Amadina' (Am) inoculated with isolate MKPSG (PRTUS 50)



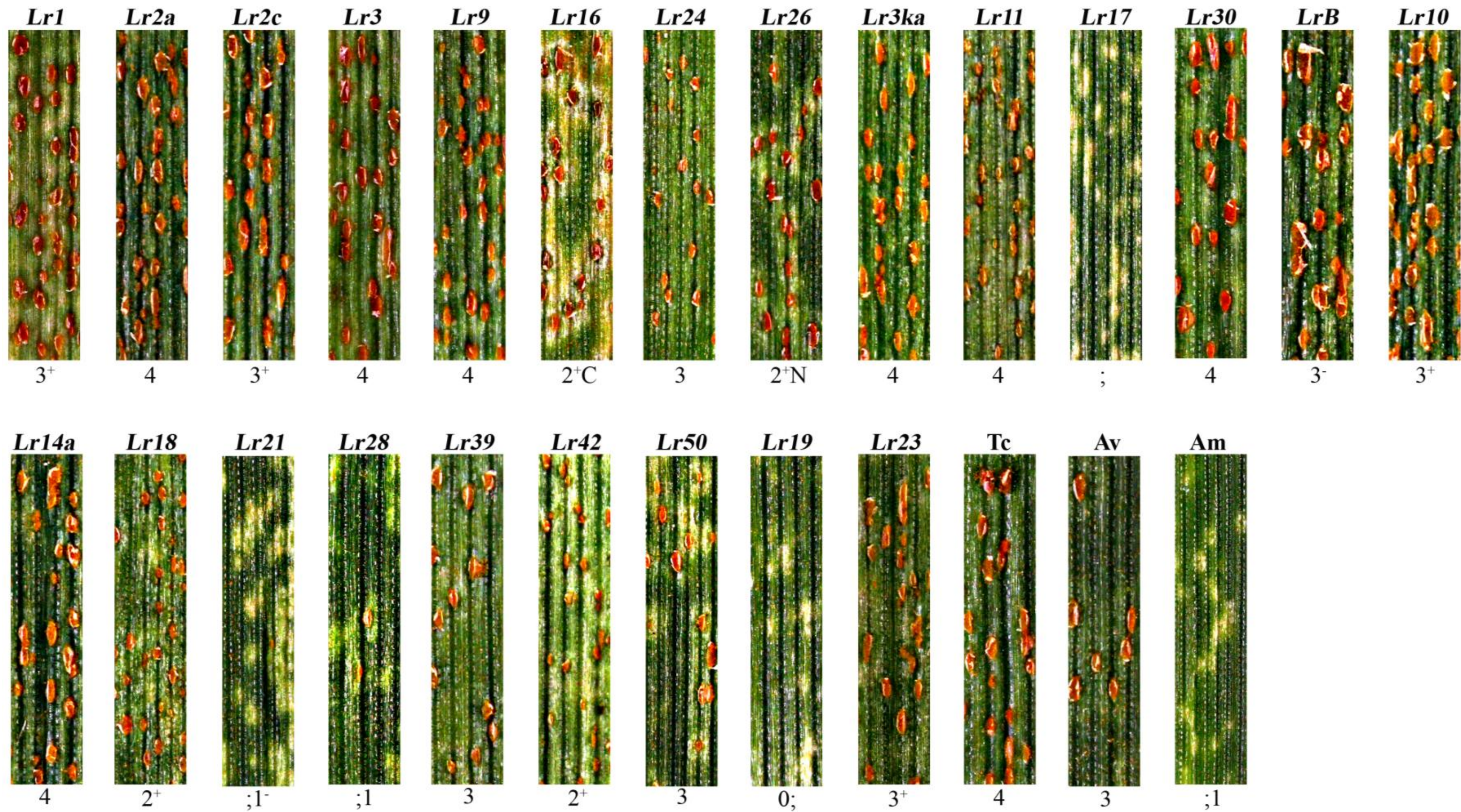
‡**Virulence/avirulence formula:** *Lr1*, *3*, *16*, *24*, *26*, *3ka*, *17*, *30*, *B*, *10*, *14a*, *28*, / *Lr2a*, *2c*, *9*, *11*, *18*, *21*, *39*, *42*, *50*, *19*

Figure 2.5 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S' (Av), and 'Amadina' (Am) inoculated with isolate TTRSD (PRTUS 45)



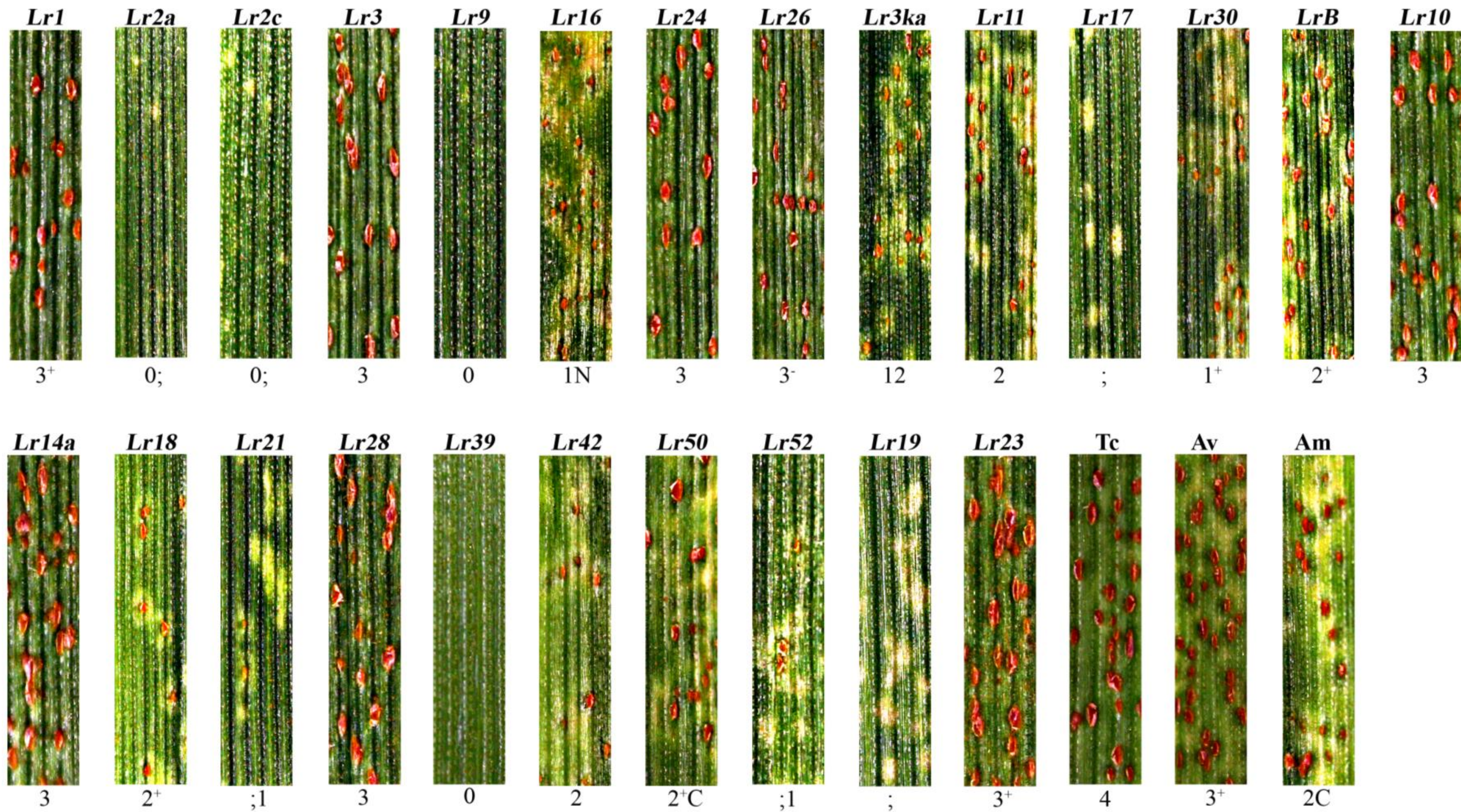
[‡]**Virulence/avirulence formula:** *Lr1*, *2a*, *2c*, *3*, *9*, *16*, *24*, *26*, *3ka*, *11*, *30*, *B*, *10*, *14a*, *39, 50*, *23* / *Lr18*, *Lr21*, *Lr19*

Figure 2.6 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S' (Av), and 'Amadina' (Am) inoculated with isolate TNRSD (PRTUS 35)



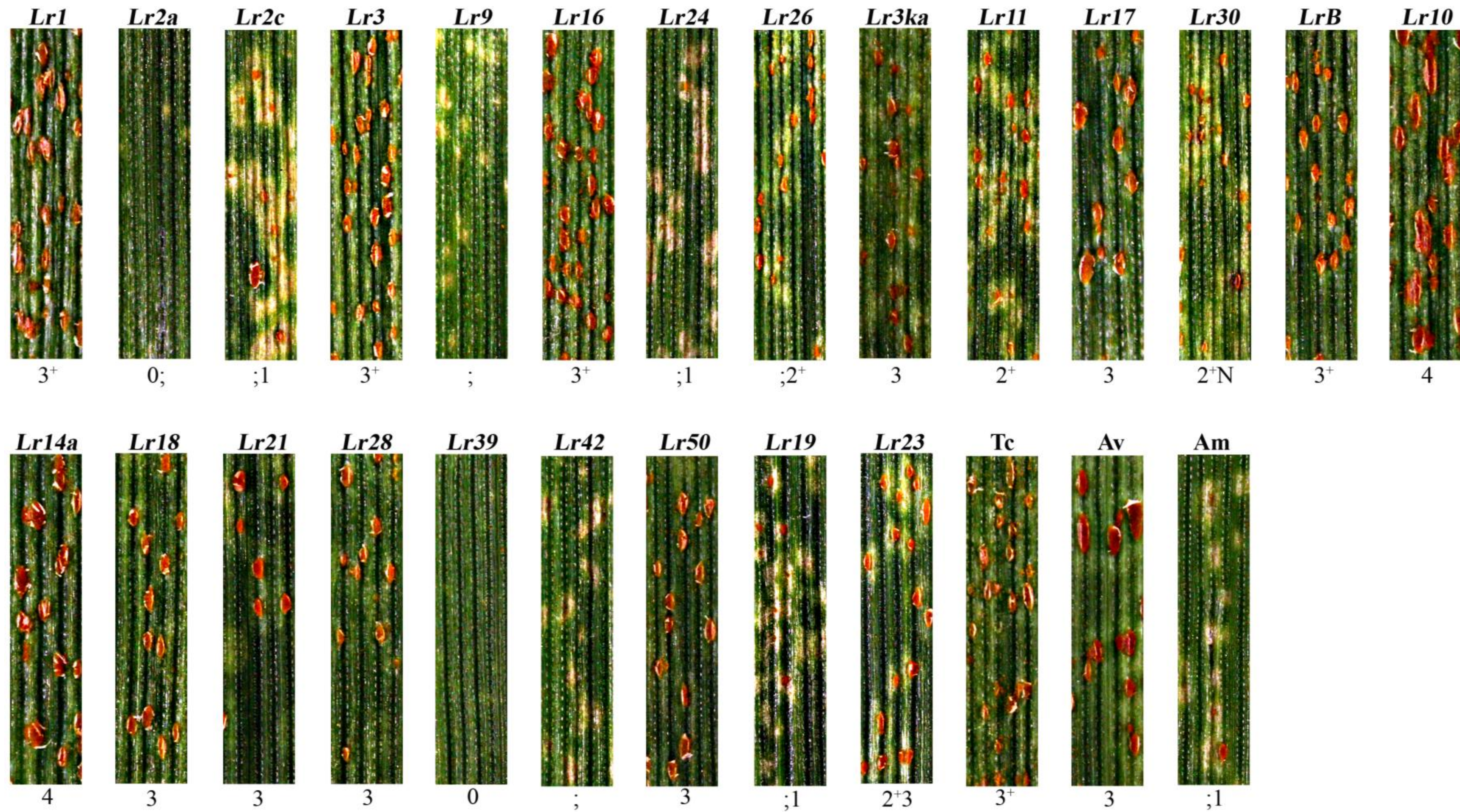
‡**Virulence/avirulence formula:** *Lr1*, *2a*, *2c*, *3*, *9*, *24*, *3ka*, *11*, *30*, *B*, *10*, *14a*, *39*, *50*, *23* / *Lr16*, *26*, *17*, *18*, *21*, *28*, *42*, *19*

Figure 2.7 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S' (Av), and 'Amadina' (Am) inoculated with isolate MFBJG (PRTUS 25)



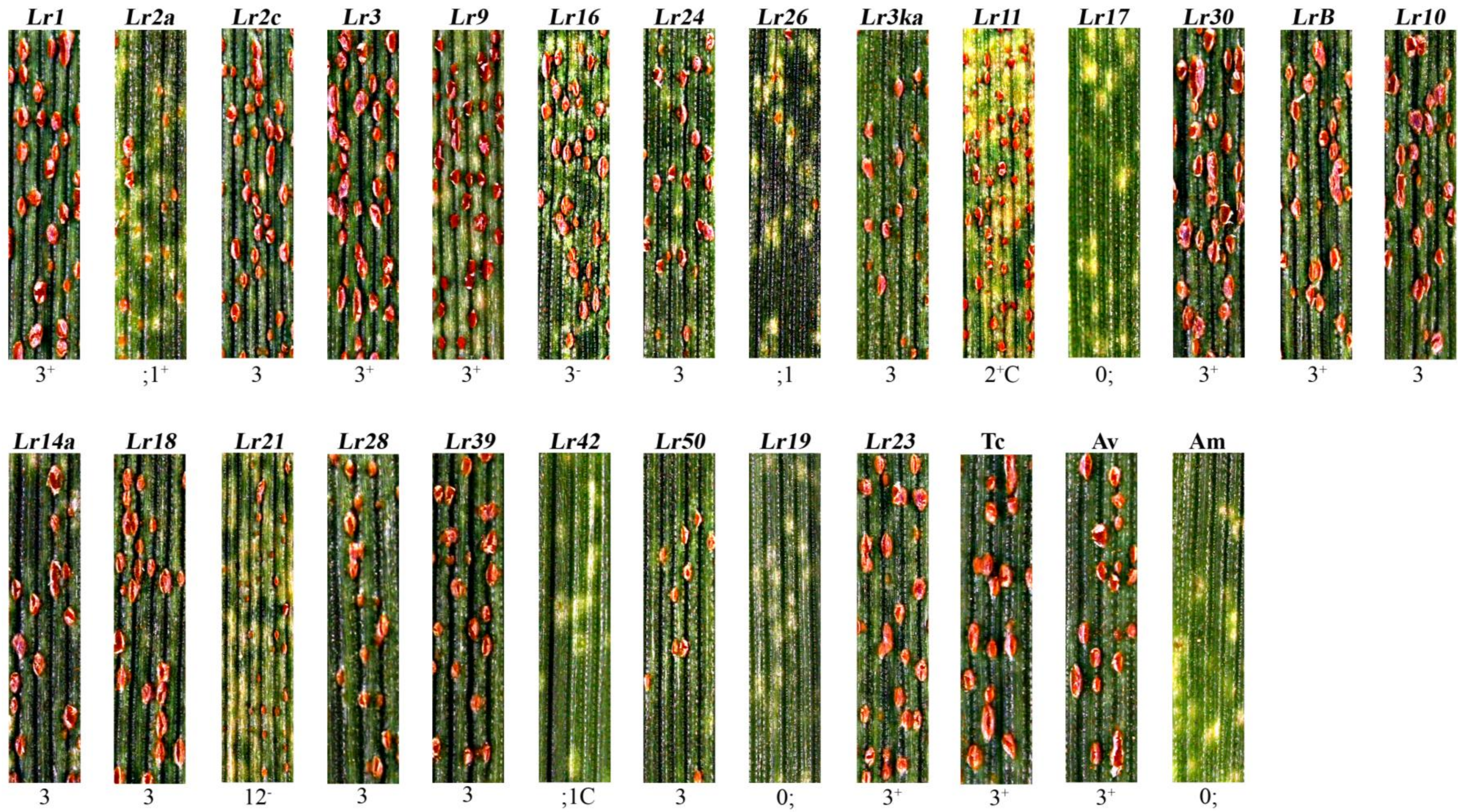
‡**Virulence/avirulence formula:** *Lr1*, *3*, *24*, *26*, *10*, *14a*, *28*, *23* / *Lr2a*, *2c*, *9*, *16*, *3ka*, *11*, *17*, *30*, *B*, *18*, *21*, *39*, *42*, *50*, *52*, *19*

Figure 2.8 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), ‘Avocet S’ (Av), and ‘Amadina’ (Am) inoculated with isolate MGNTQ (PT1)



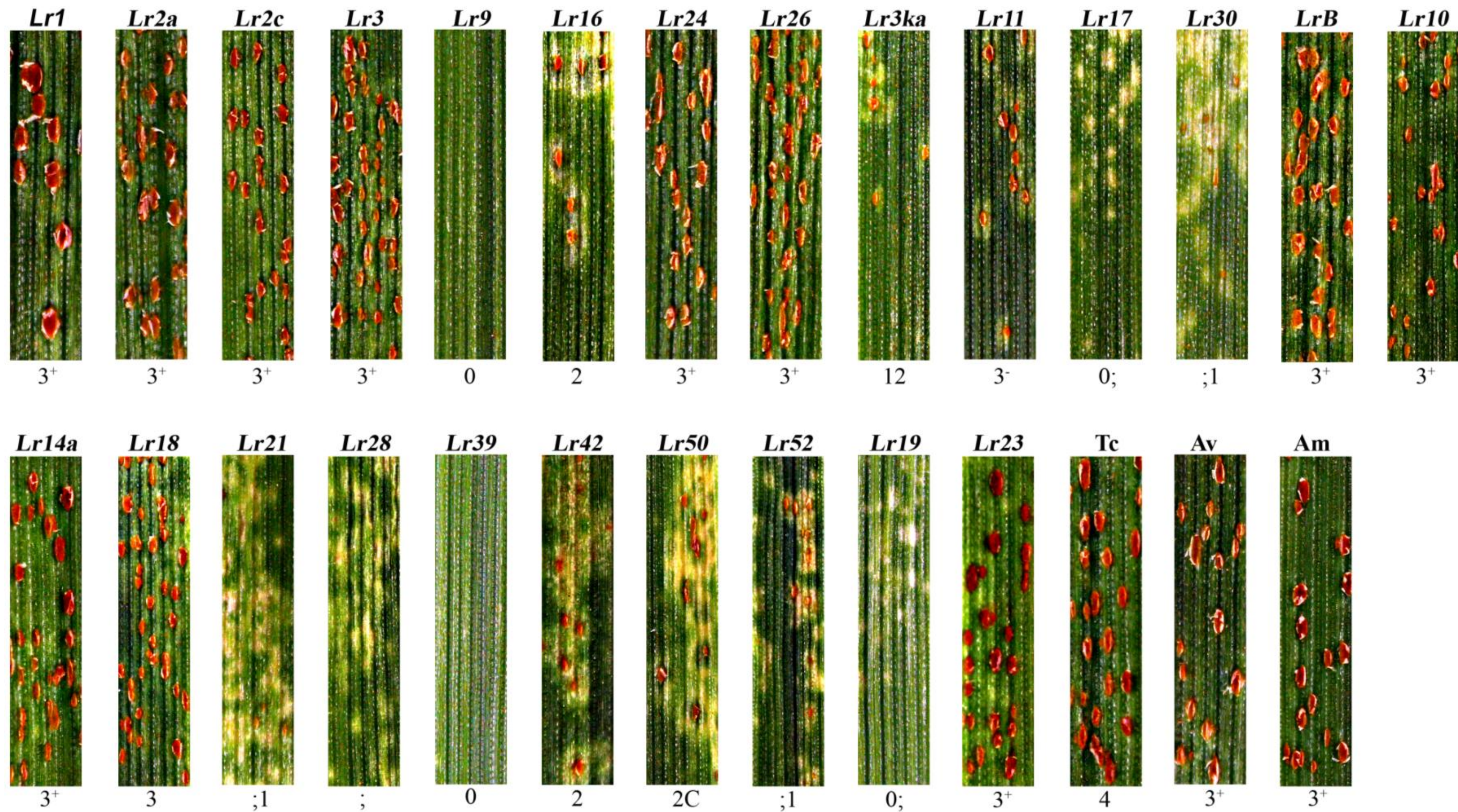
‡**Virulence/avirulence formula:** *Lr1*, *3*, *16*, *3ka*, *17*, *B*, *10*, *14a*, *18*, *21*, *28*, *50* / *Lr2a*, *2c*, *9*, *24*, *26*, *11*, *30*, *39*, *42*, *19*

Figure 2.9 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), ‘Avocet S’ (Av), and ‘Amadina’ (Am) inoculated with isolate PSMTJ (PT2)



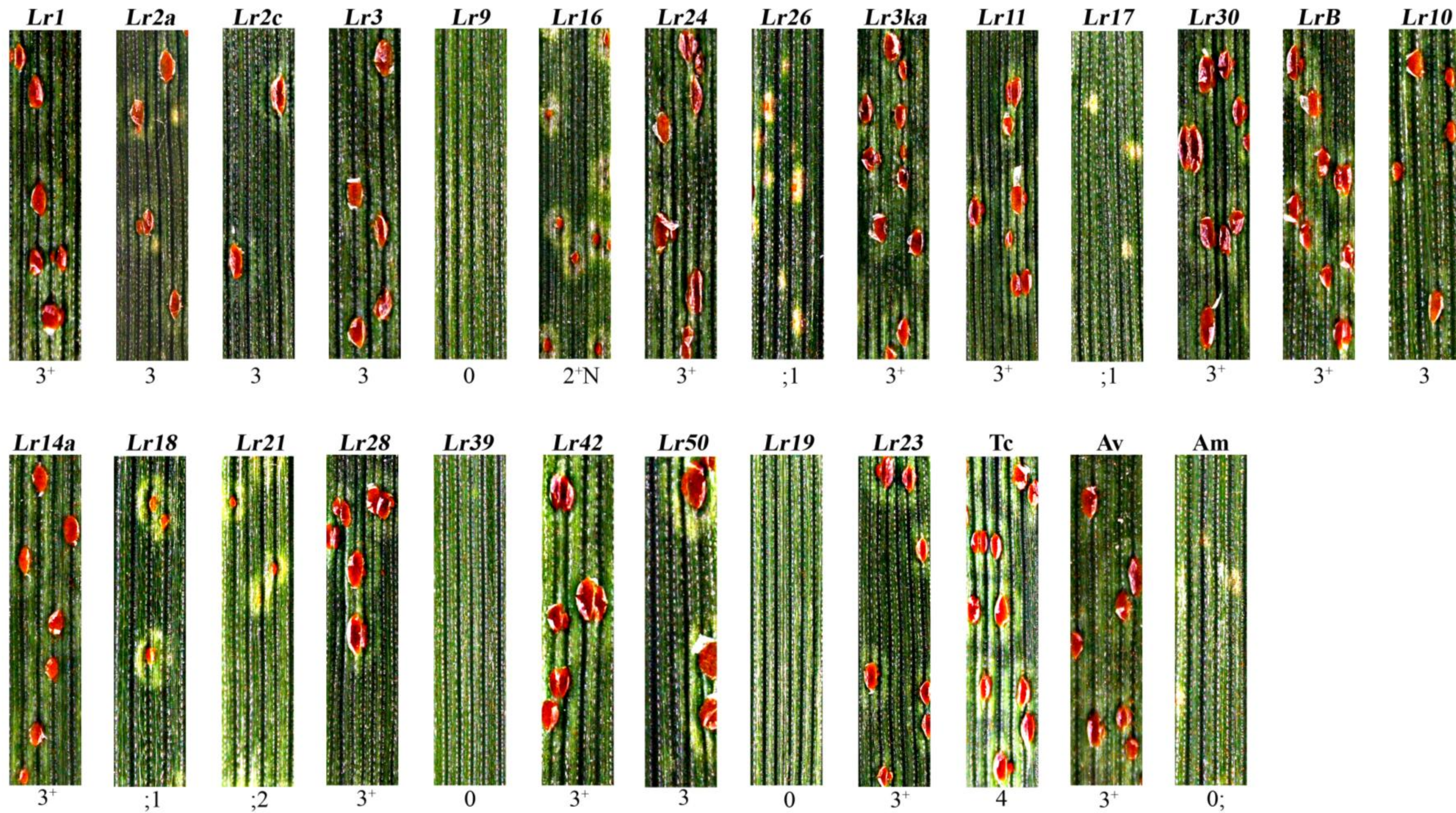
[‡]**Virulence/avirulence formula:** *Lr1*, *2c*, *3*, *9*, *16*, *24*, *3ka*, *30*, *B*, *10*, *14a*, *18*, *28*, *39*, *50*, *23* / *Lr2a*, *26*, *11*, *17*, *21*, *42*, *19*

Figure 2.10 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S' (Av), and 'Amadina' (Am) inoculated with isolate TFGSB (PT3)



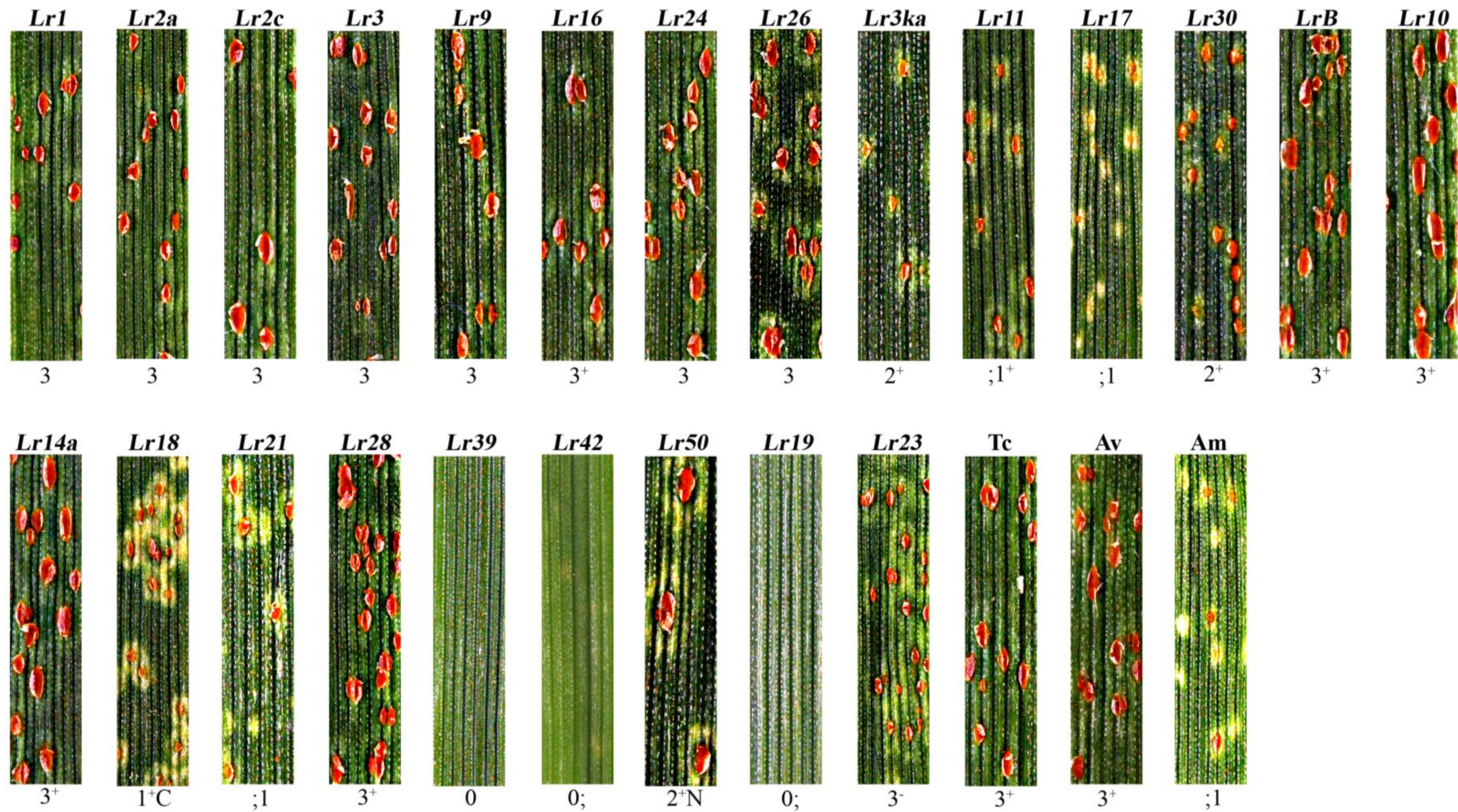
‡**Virulence/avirulence formula:** *Lr1*, *2a*, *2c*, *3*, *24*, *26*, *11*, *B*, *10*, *14a*, *18*, *23* / *Lr9*, *16*, *3ka*, *17*, *30*, *21*, *28*, *39, 42*, *50*, *52*, *19*

Figure 2.11 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S' (Av), and 'Amadina' (Am) inoculated with isolate TDRSH (PT4)



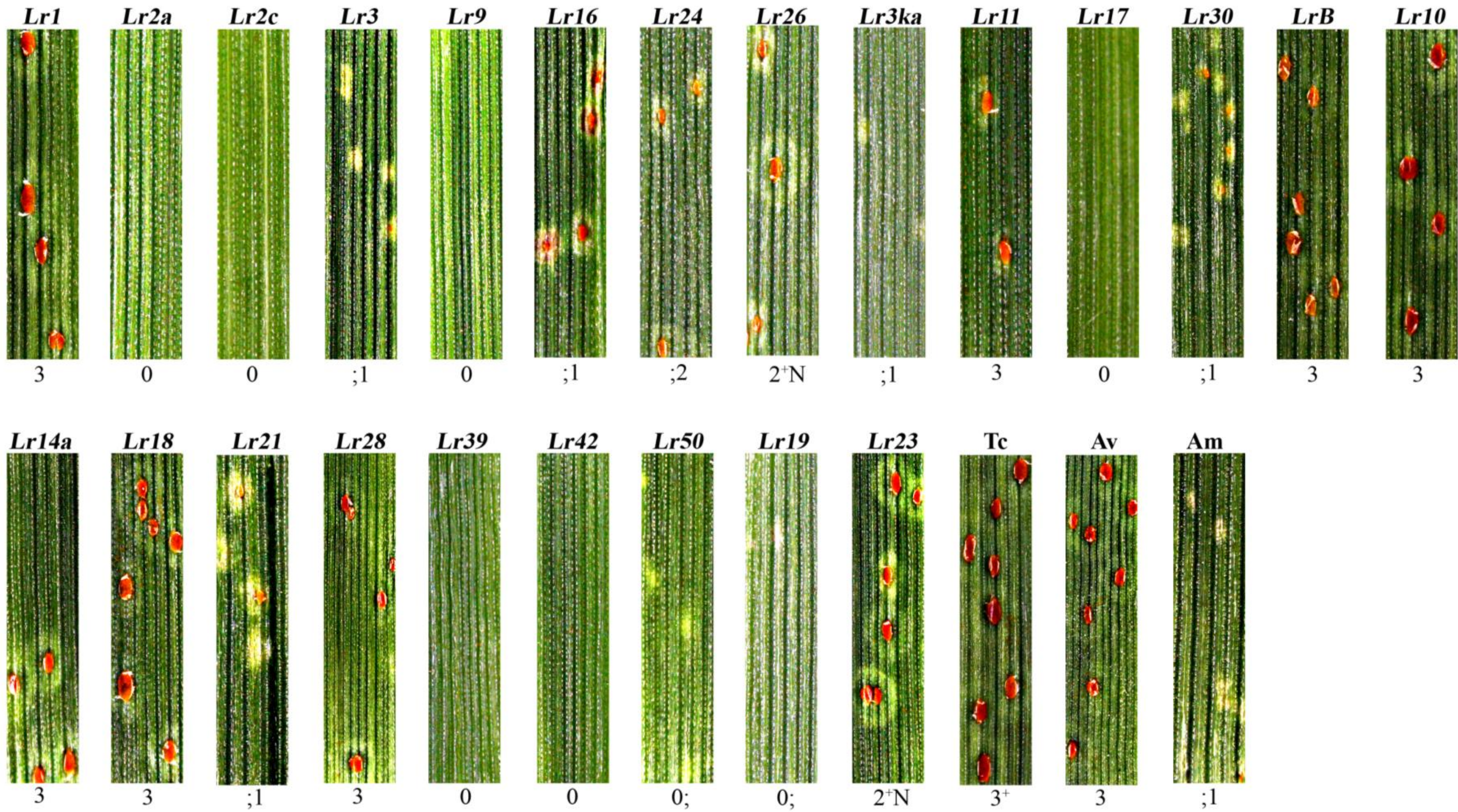
‡**Virulence/avirulence formula:** *Lr1*, *2a*, *2c*, *3*, *24*, *3ka*, *11*, *30*, *B*, *10*, *14a*, *28*, *42*, *50*, *23* / *Lr9*, *16*, *26*, *17*, *18*, *21*, *39*, *19*

Figure 2.12 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S' (Av), and 'Amadina' (Am) inoculated with isolate TTBSG (PT5)



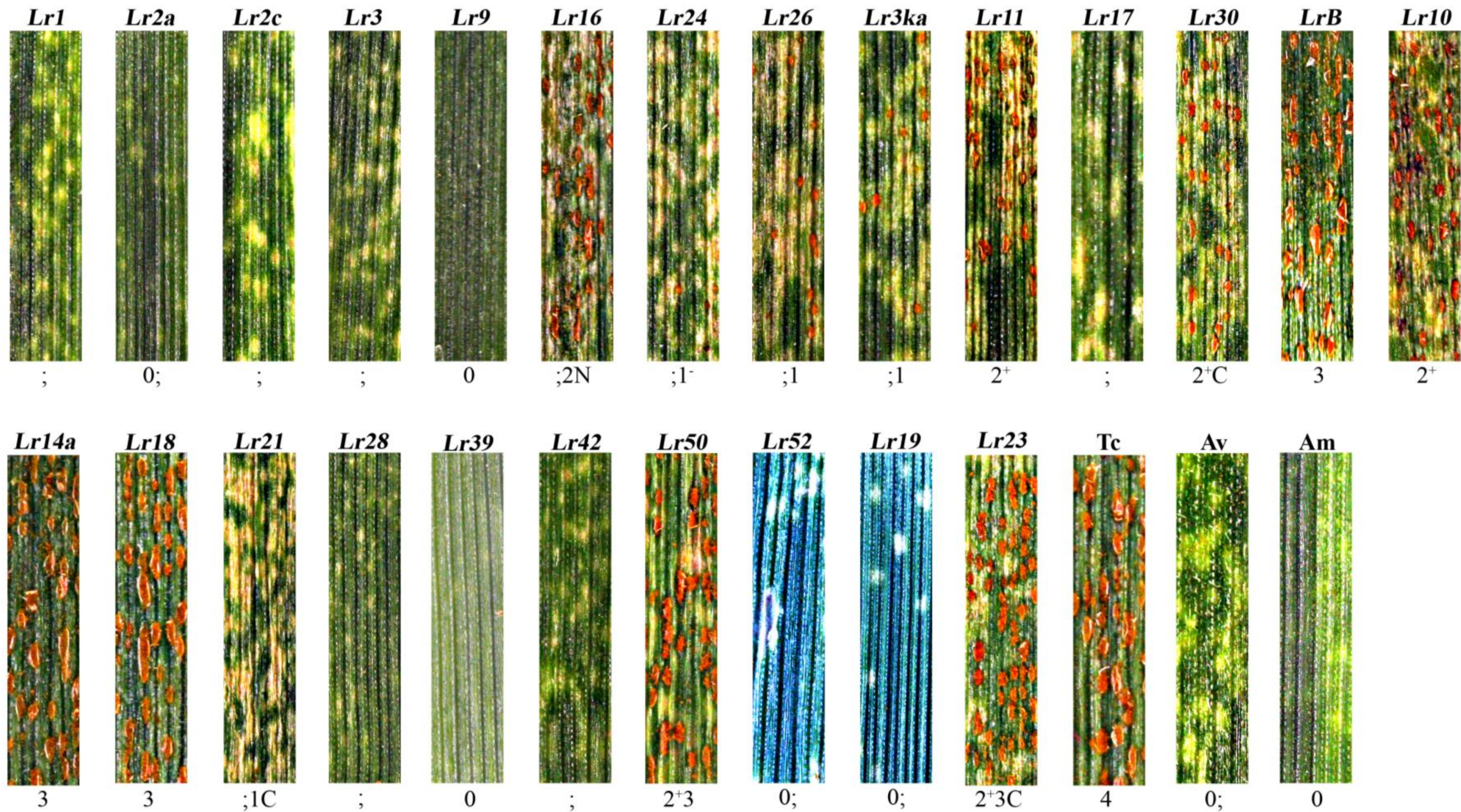
‡**Virulence/avirulence formula:** *Lr1*, *2a*, *2c*, *3*, *9*, *16*, *24*, *26*, *30*, *B*, *10*, *14a*, *28*, *23* / *L11*, *17*, *30*, *18*, *21*, *39*, *42*, *50*, *19*

Figure 2.13 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S' (Av), and 'Amadina' (Am) inoculated with isolate LBGTG (PT6)



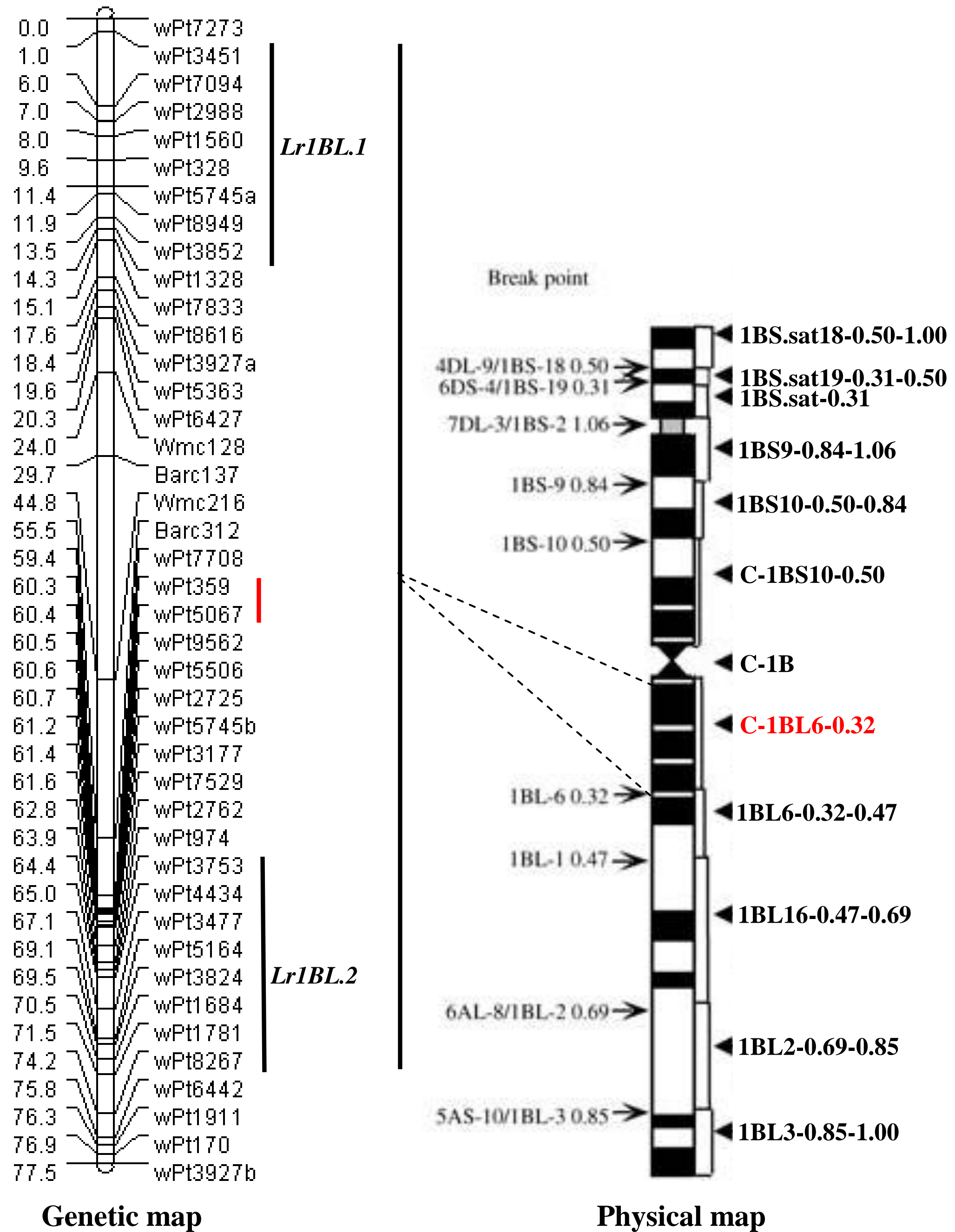
‡**Virulence/avirulence formula:** *Lr1*, *11*, *B*, *10*, *14a*, *18*, *28* / *Lr2a*, *2c*, *3*, *9*, *16*, *24*, *26*, *3ka*, *17*, *30*, *21*, *39*, *42*, *50*, *19*, *23*

Figure 2.14 Seedling infection types (ITs) of Thatcher NILs, Thatcher (Tc), 'Avocet S' (Av), and 'Amadina' (Am) inoculated with isolate BBBPB (PT8)



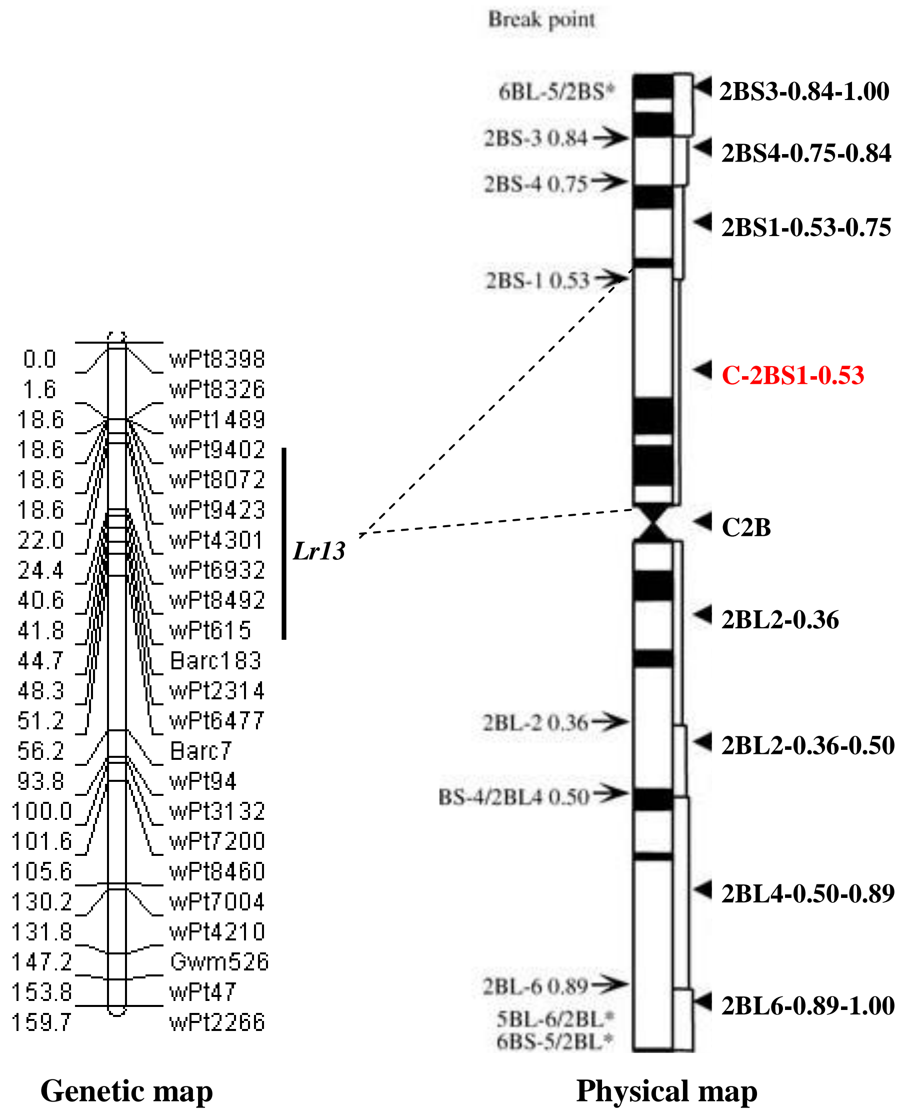
‡**Virulence/avirulence formula:** *Lr14a*, *18* / *Lr1*, *2a*, *2c*, *3*, *9*, *16*, *24*, *26*, *3ka*, *11*, *17*, *30*, *10*, *21*, *28*, *39*, *42*, *52*, *19*

Figure 2.15 Partial Linkage map of 'Amadina' x 'Avocet S' showing likely genomic location of *Lr* genes (black bar), and genomic locations with no main effect involved in epistatic interactions (red bar) on chromosome 1BL



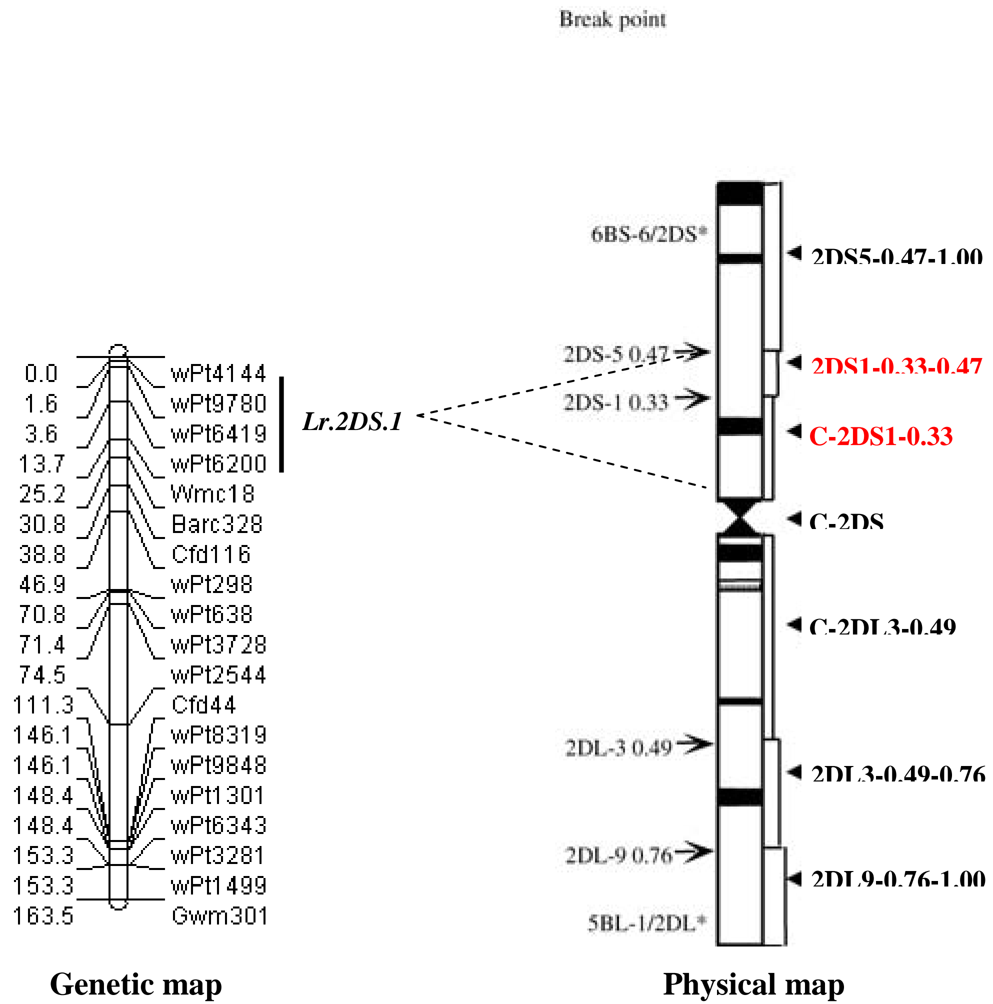
[†]The resistance allele was derived from 'Amadina'. Both *Lr1BL.1* and *Lr1BL.2* map to the centromeric region, C-1BL6-0.32 bin, of the long arm of chromosome 1B.

Figure 2.16 Partial Linkage map of 'Amadina' x 'Avocet S' showing likely genomic location of *Lr* genes (black bar) on chromosome 2BS



[†]The resistance gene *Lr13* was derived from the susceptible parent 'Avocet S'. *Lr13* maps to the centromeric region, C-2BS1-0.53 bin, of the short arm of chromosome 2B.

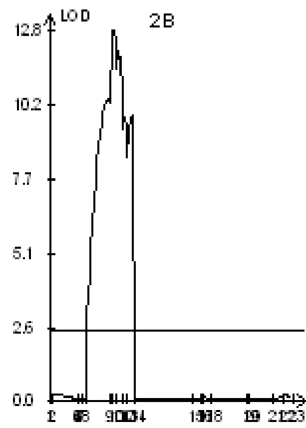
Figure 2.17 Partial Linkage map of 'Amadina' x 'Avocet S' showing likely genomic location of *Lr* genes (black bar) on chromosome 2DS



†Resistance allele for *Lr2DS.1* was derived from 'Amadina'. *Lr2DS.1* maps in the region between the C-2DS1-0.33 and 2DS1-0.33-0.47 bins.

Figure 2.18 Likelihood ratio of major genes associated with seedling infection type in the ‘Avocet S’ x ‘Amadina’ RIL population in response to *P. triticina* isolate MTPTB (PRTUS 54)

Chromosome 2BS



Chromosome 2DS

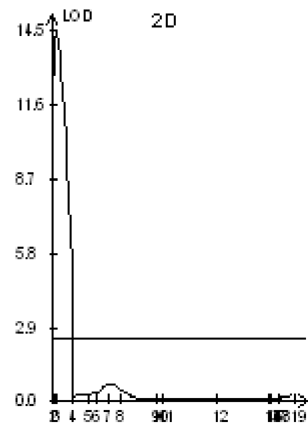


Figure 2.19 Composite interval mapping (CIM) for seedling resistance in the 'Avocet S' x 'Amadina' RIL population in response to *P. triticina* isolate MKPSG (PRTUS 50)

LOD score is shown on the *y-axis*, and marker number and scaled distance are shown on the *x-axis*.

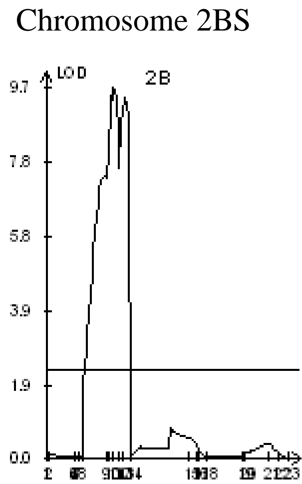


Figure 2.20 Composite interval mapping (CIM) for seedling resistance in the 'Avocet S' x 'Amadina' RIL population in response to *P. triticina* isolate TTRSD (PRTUS 45)

LOD score is shown on the *y-axis*, and marker number and scaled distance are shown on the *x-axis*.

Chromosome 1BL

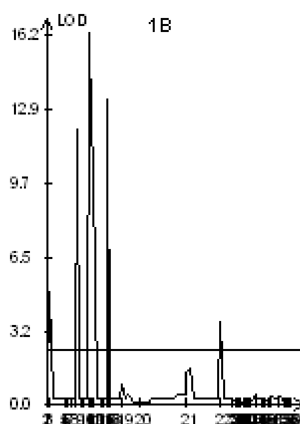


Figure 2.21 Composite interval mapping (CIM) for seedling resistance in the 'Avocet S' x 'Amadina' RIL population in response to *P. triticina* isolate TNRSD (PRTUS 35)

LOD score is shown on the *y-axis*, and marker number and scaled distance are shown on the *x-axis*.

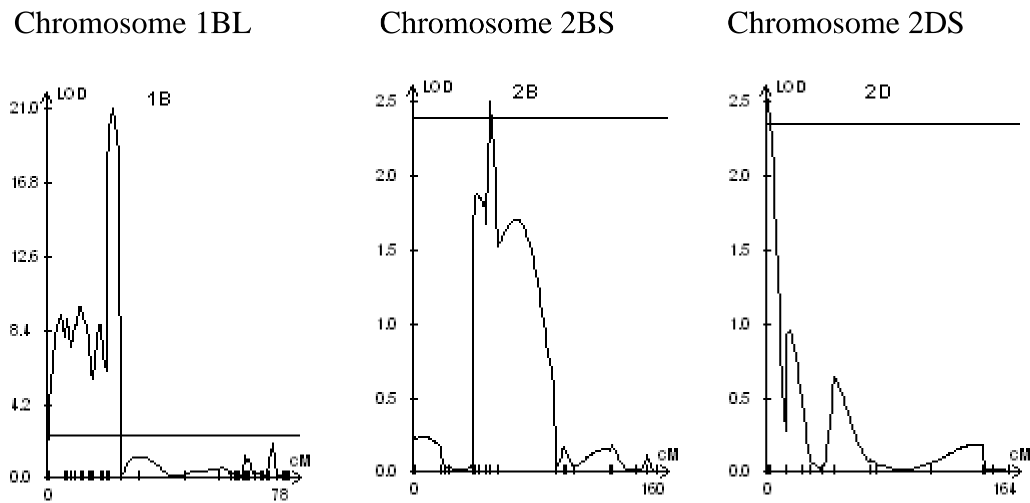


Figure 2.22 Composite interval mapping (CIM) in the 'Avocet S' x 'Amadina' RIL population in response to *P. triticina* isolate MFBJG (PRTUS 25)

LOD score is shown on the *y-axis*, and marker number and scaled distance are shown on the *x-axis*.

Chromosome 1BL

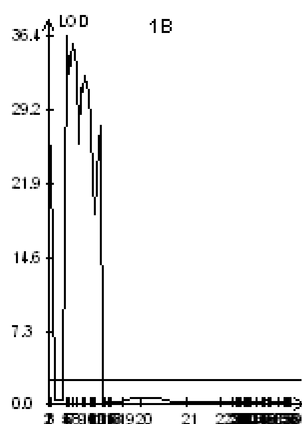
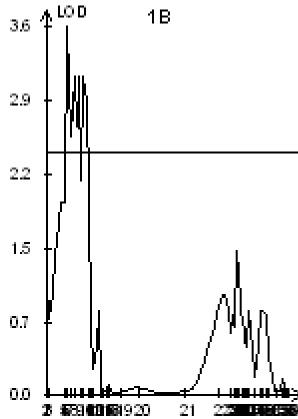


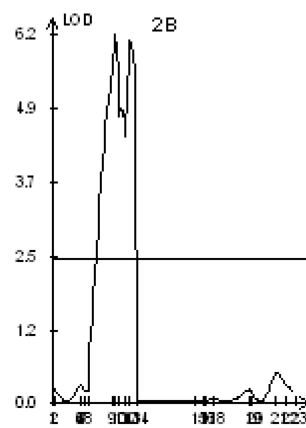
Figure 2.23 Composite interval mapping (CIM) in the 'Avocet S' x 'Amadina' RIL population in response to *P. triticina* isolate MGNTQ (PT1)

LOD score is shown on the *y-axis*, and marker number and scaled distance are shown on the *x-axis*.

Chromosome 1BL



Chromosome 2BS



Chromosome 2DS

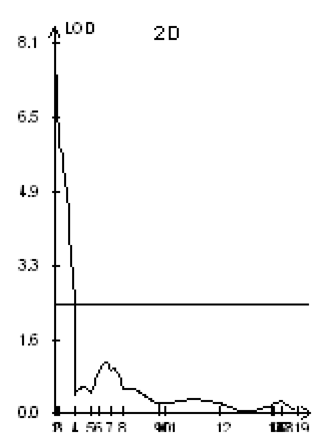


Figure 2.24 Composite interval mapping in the 'Avocet S' x 'Amadina' RIL population in response to *P. triticina* isolate PSMTJ (PT2)

LOD score is shown on the *y-axis*, and marker number and scaled distance are shown on the *x-axis*.

Chromosome 1BL

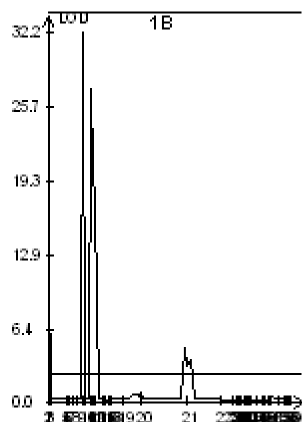
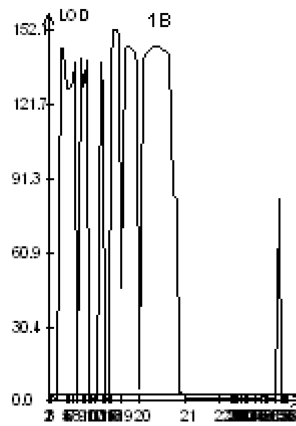


Figure 2.25 Composite interval mapping (CIM) in the 'Avocet S' x 'Amadina' RIL population in response to *P. triticina* isolate TDRSH (PT4)

LOD score is shown on the *y-axis*, and marker number and scaled distance are shown on the *x-axis*.

Chromosome 1BL



Chromosome 2BS

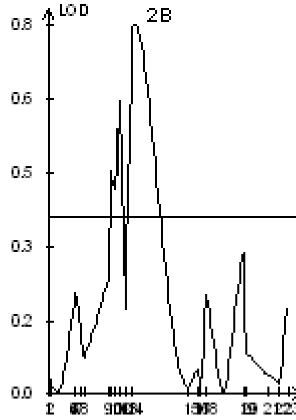
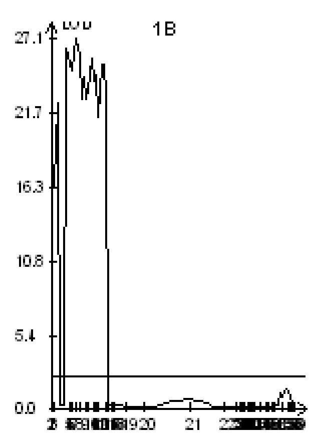


Figure 2.26 Composite interval mapping (CIM) in the 'Avocet S' x 'Amadina' RIL population in response to *P. triticina* isolate TTBSG (PT5)

LOD score is shown on the *y-axis*, and marker number and scaled distance are shown on the *x-axis*.

Chromosome 1BL



Chromosome 2DS

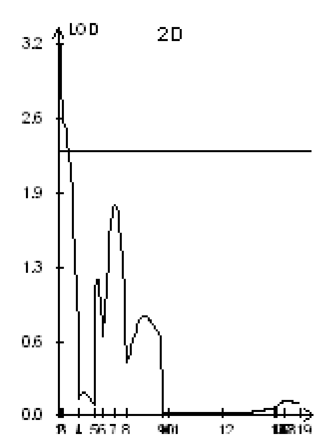


Figure 2.27 Composite interval mapping (CIM) in the 'Avocet S' x 'Amadina' RIL population in response to *P. triticina* isolate LBGTG (PT6)

LOD score is shown on the *y-axis*, and marker number and scaled distance are shown on the *x-axis*.

Chromosome 1BL

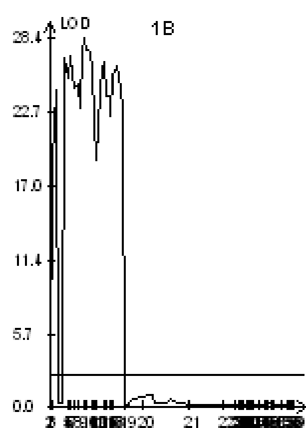
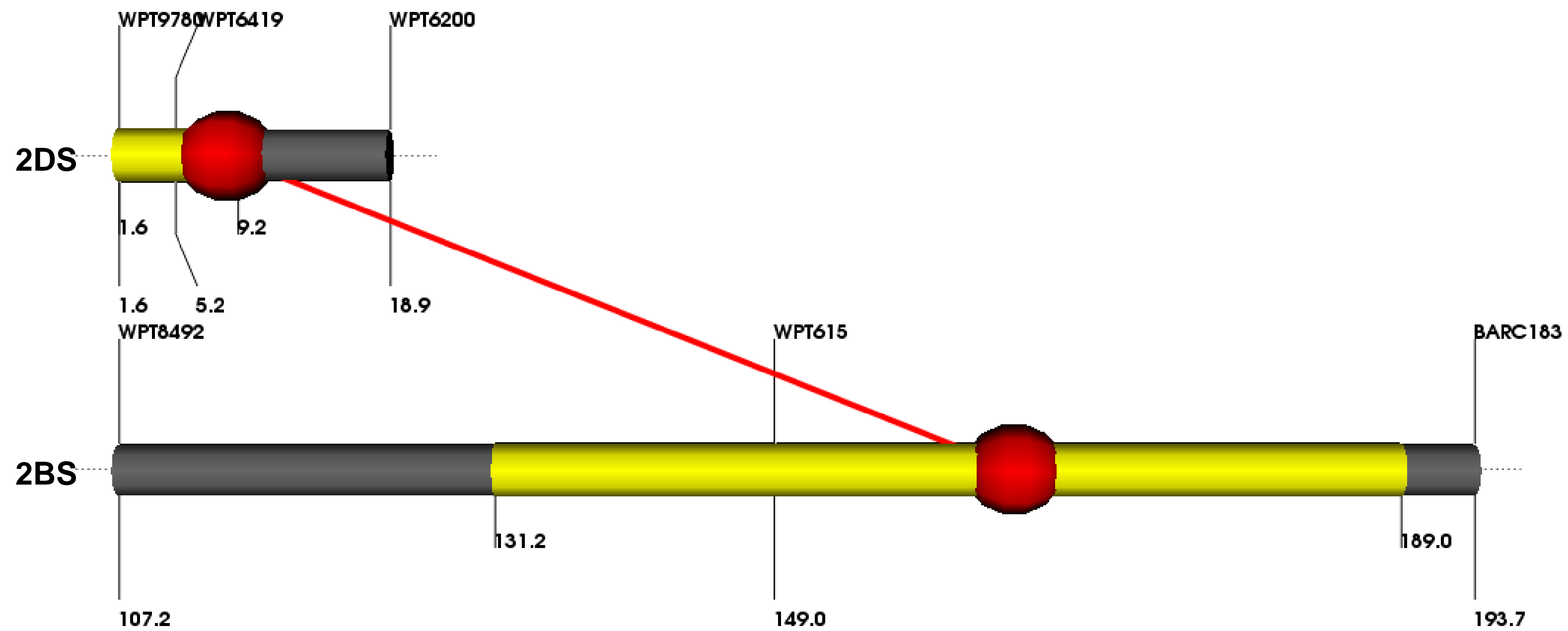


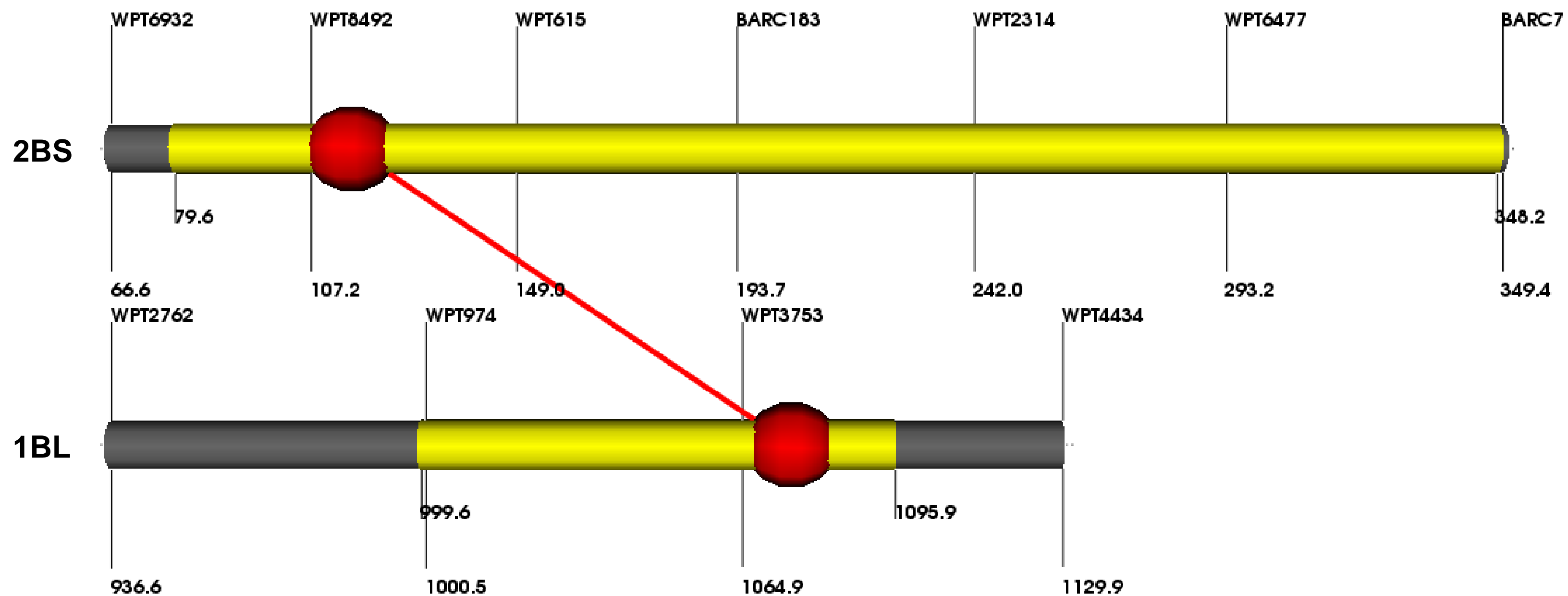
Figure 2.28 The genetic architecture of leaf rust resistance genes generated with QTLNetwork 2.0 in response to isolate MTPTB (PRTUS 54)



† - No additive main effect; - Additive main effect; - Epistatic interaction; - QTL support interval.

‡Main effect QTL detected on chromosomes 2DS and 2BS.The yellow shade indicates the support interval of QTL position. Epistatic interaction between 2DS and 2BS was detected.

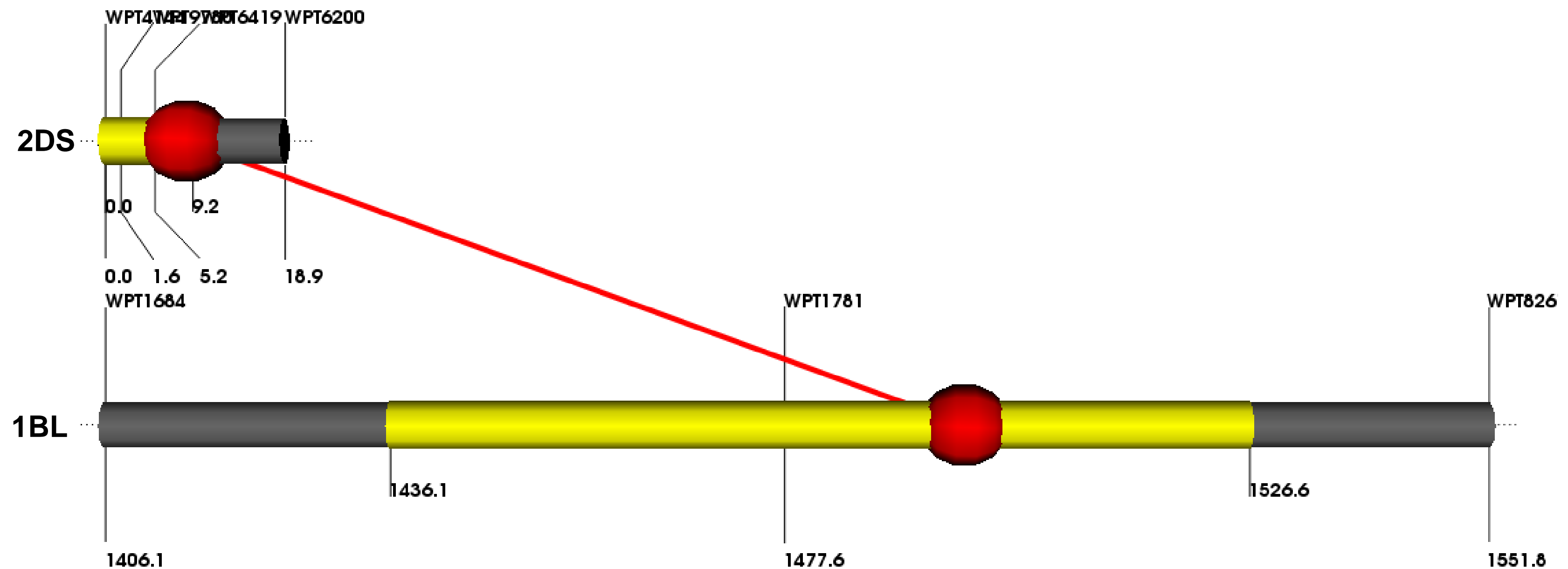
Figure 2.29 The genetic architecture of leaf rust resistance genes generated with QTLNetwork 2.0 in response to isolate MKPSG (PRTUS 50)



† ⬤ - No additive main effect; ⬤ - Additive main effect; ▬▬ - Epistatic interaction; ▬▬ - QTL support interval.

‡Main effect QTL detected on chromosomes 2DS and 1BL.The yellow shade indicates the support interval of QTL position. Epistatic interaction between 2DS and 1BL was detected.

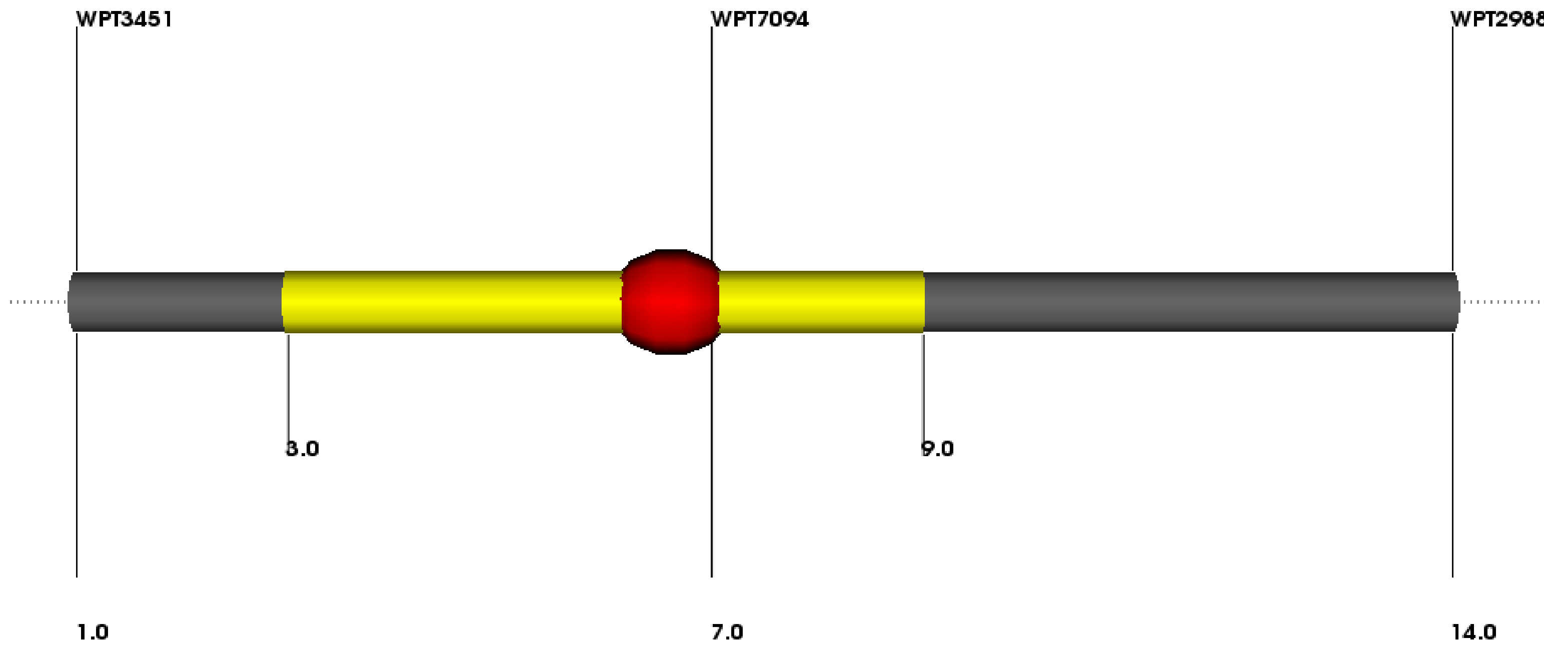
Figure 2.30 The genetic architecture of leaf rust resistance genes generated with QTLNetwork 2.0 in response to isolate TNRSD (PRTUS 35)



[†] ⬤ - No additive main effect; ⬤ - Additive main effect; ▬▬ - Epistatic interaction; ▬▬ - QTL support interval.

[‡]Main effect QTL detected on chromosomes 2DS and 1BL.The yellow shade indicates the support interval of QTL position. Red line indicates epistatic interaction between 2DS and 1BL.

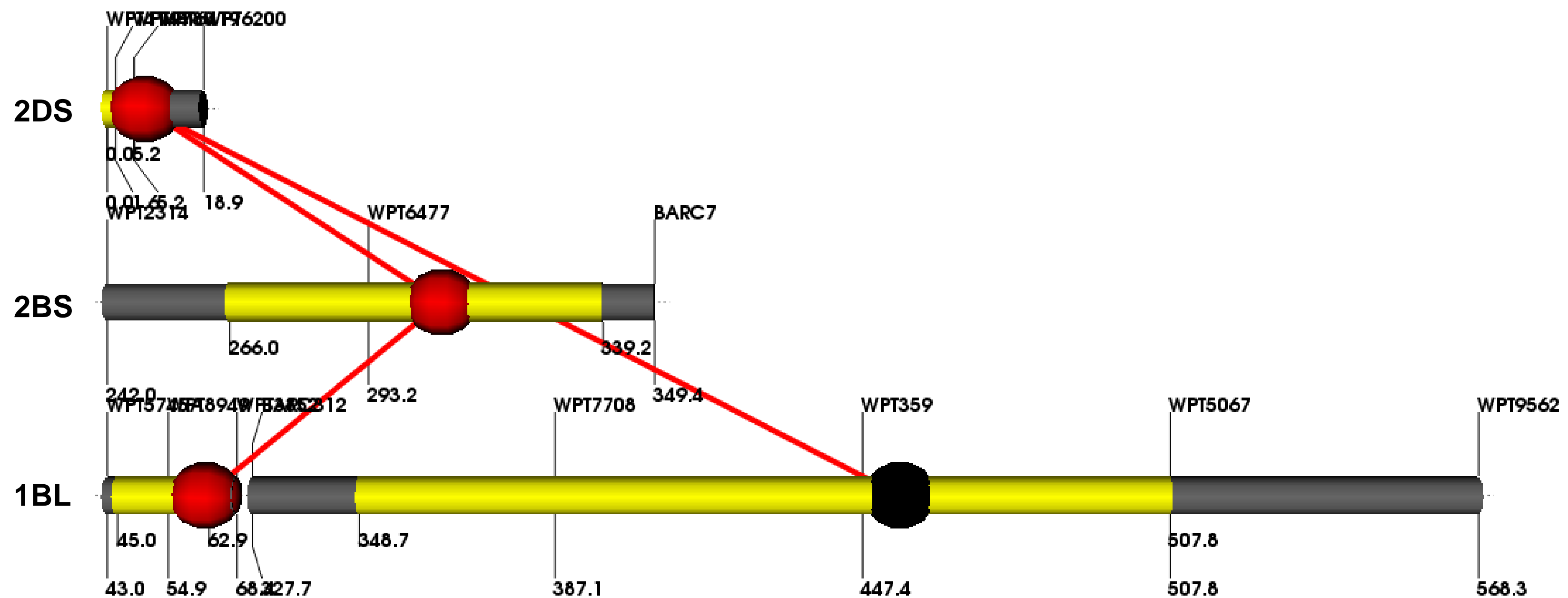
Figure 2.31 The genetic architecture of leaf rust resistance genes generated with QTLNetwork 2.0 in response to isolate MFBJG (PRTUS 25)



[†] - No additive main effect; - Additive main effect; - Epistatic interaction; - QTL support interval.

[‡]Main effect QTL detected on short arm of chromosome 1B.The yellow shade indicates the support interval of QTL position.

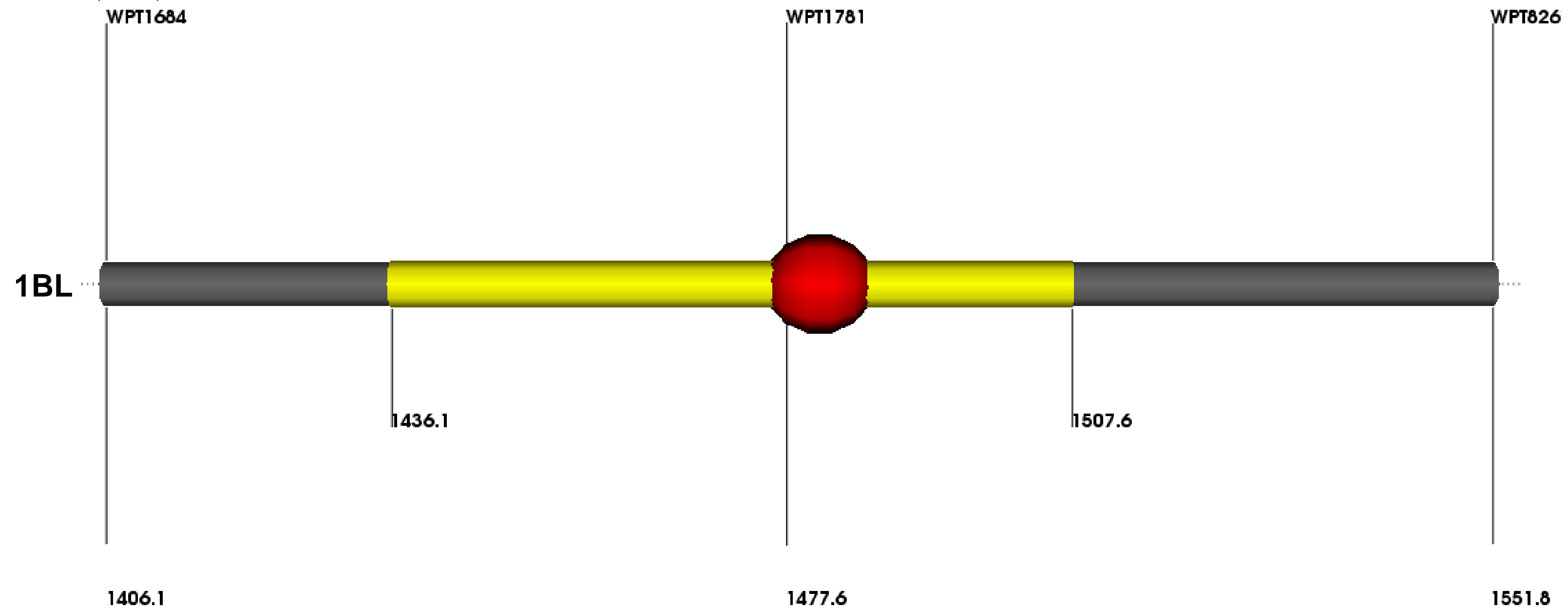
Figure 2.32 The genetic architecture of leaf rust resistance genes generated with QTLNetwork 2.0 in response to isolate MGNTQ (PT1)



† ⬤ - No additive main effect; ⬤ - Additive main effect; ▬▬ - Epistatic interaction; ▬▬ - QTL support interval.

‡Main effect QTL detected on chromosomes 2DS, 2BS and 1BL.The yellow shade indicates the support interval of QTL position. Epistatic interactions between 2DS and 2BS, 2BS and 1BL, and 2BS and 1BL were detected.

Figure 2.33 The genetic architecture of leaf rust resistance genes generated with QTLNetwork 2.0 in response to isolate LBGTG (PT6)



† ⬤ - No additive main effect; ⬤ - Additive main effect; ▬ - Epistatic interaction; ▬ - QTL support interval.

‡Main effect QTL detected on chromosome 1BL.The yellow shade indicates the support interval of QTL position.

Table 2.1 List of Thatcher-derived near-isogenic lines (NILs) and differentials used for virulence analysis and gene postulation studies

Differential	Resistance gene	Infection type	Chrom. location	Pedigree
RL6003	*Lr1*	0;	5DL	Tc*6/Centenario
RL6016	*Lr2a*	0;, ;1	2DS	Tc*6/Webster
RL6047	*Lr2c*	;1, ;1^+	2DS	Tc*6/Loros
RL6002	*Lr3a*	;C, 2	3BL	Tc*6/Democrat
RL6010	*Lr9*	0;	6BL	Transfer/Tc*6
RL6005	*Lr16*	;1N	2BS	Tc*6/Exchange
RL6064	*Lr24*	0;	3DL	Tc*6/Agent
RL6078	*Lr26*	0;, ;1	1RS.1BL	Tc*6/St-1-25
RL6007	*Lr3ka*	;C, 12C	6BL	Tc*6/Klein Aniversario
RL6053	*Lr11*	Y	2A	Tc*2/Hussar
RL6008	*Lr17*	;1^+, 0;	2AS	K.Lucero/Tc*6
RL6049	*Lr30*	;1, 23	4BL	Tc*6/Terenzio
RL6051	*LrB*	2, ;	5D	Tc*6/Carina
RL6004	*Lr10*	;, 2	1AS	Tc*6/Exchange
RL6013	*Lr14a*	X	7BL	Selkirk/Tc*6
RL6009	*Lr18*	2^+2^-	5BL	Tc*7/Africa43
KS89WGRC07	*Lr21*	0;, 12^-	1DS	Wichita/TA1649(*Ae. tauschii*)/2*Wichita
RL6079	*Lr28*	0;, 1^+ to 2^+	4AL	Tc*6/C-77-1
Overley	*Lr39*	0;	2DS	U1275 1-4-2-2/Heyne 'S'//Jagger
KS91WGRC11	*Lr42*	2	1DS	Century*3/TA2450 (*Ae. tauschii*)
KS96WGRC36	*Lr50*	N/A	2BL	TAM 107*3 / TA2460 (*Ae. tauschii*)
Iran landrace	*Lr52*	N/A	5BS	PI 245487
Morocco	*Lr19*	N/A	7DL	PI 431591
RL6012	*Lr23*	; to 3	2BS	Lee 310/Tc*6
Thatcher	*LrTc*	N/A		Marquis/lumillo/2Marquis/Kanred

N/A, not available

Table 2.2 Code for North American differential hosts of *Puccinia triticina* in ordered sets of five

	Infection (IT) produced on near-isogenic *Lr* lines‡			
Host set 1	*Lr1*	*Lr2a*	*Lr2c*	*Lr3a*
Host set 2	*Lr9*	*Lr16*	*Lr24*	*Lr26*
Host set 3	*Lr3ka*	*Lr11*	*Lr17*	*Lr30*
Host set 4	*LrB*	*Lr10*	*Lr14a*	*Lr18*
Host set 5	*Lr21*	*Lr28*	*Lr39*	*Lr42*

‡*Puccinia triticina* code consists of the designation for set 1 followed by that for sets 2, 3, and 4.

Table 2.3 Race codes[‡]

B	L	L	L	L
C	L	L	L	H
D	L	L	H	L
F	L	L	H	H
G	L	H	L	L
H	L	H	L	H
J	L	H	H	L
K	L	H	H	H
L	H	L	L	L
M	H	L	L	H
N	H	L	H	L
P	H	L	H	H
Q	H	H	L	L
R	H	H	L	H
S	H	H	H	L
T	H	H	H	H

[‡]L = low infection type (avirulent pathogen); H = high infection type (virulent pathogen).

Source: USDA Cereal Disease Laboratory website

Table 2.4 Avirulence/virulence formulae of the isolates used in this study

Leaf rust isolate	Avirulent for host *Lr* genes	Virulent for host *Lr* genes
MRDSD[b] (PRTUS 60[a])	*2a*, *2c*, *24*, *11*, *30*, *21*, *28*, *42*, *52*, *19*	*1, 3a, 9, 16, 26, 3ka, 17, B, 10, 14a, 18, 39, 50, 23*
TKLSQ[b] (PRTUS 55[a])	*16*, *3ka*, *11*, *17*, *30*, *18*, *21*, *39*, *42*, *52*, *19*	*1, 2a, 2c, 3a, 24, 26, B, 10, 14a, 23*
MTPTB[b] (PRTUS 54[a])	*2a*, *2c*, *9*, *11*, *39*, *42*, *50*, *19*	*1, 3a, 16, 24, 26, 3ka, 17, 30, B, 10, 14a, 28, 50,*
MKPSG[b] (PRTUS 50[a])	*2c*, *9*, *11*, *18*, *21*, *19*	*1, 2a, 3a, 16, 24, 26, 3ka, 17, 30, B, 10, 14a, 28, 23,*
TTRSD[b] (PRTUS 45[a])	*17*, *18*, *21*, *19*	*1, 2a, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 30, B, 10, 14a, 28, 39, 50, 23*
TNRSD[b] (PRTUS 35[a])	*16*, *26*, *17*, *B*, *18*, *21*, *28*, *42*, *19*	*2a, 2c, 3a, 9, 24, 3ka, 11, 30, 10, 14a, 39, 50, 23*
MFBJG[b] (PRTUS 25[a])	*2a*, *2c*, *9*, *16*, *26*, *3ka*, *11*, *17*, *30*, *B*, *18, 21, 39, 42, 50, 19,*	*1, 3a, 24, 10, 14a, 28, 23,*
MGNTQ[b] (PT1)	*2a, 2c, 9, 16, 24, 3ka, 11, 30, B, 10, 14a, 21, 28, 41, 42*	*1, 3a, 26, 17, 18*
PPSMTJ[b] (PT2)	*2a, 16, 26, 11, 17, 14a*	*1, 2c, 3a, 9, 24, 3ka, 30, B, 10, 18*
TFGSB[b] (PT3)	*9, 16, 3ka, 17, 30, 10, 14a, 18*	*1, 2a, 2c, 3a, 24, 26, 11, B*
TDRSH[b] (PT4)	*9, 16, 26, 17, 18, 21, 39*, *19*	*1, 2a, 2c, 3a, 24, 3ka, 11, 30, B, 10, 28, 42, 50, 23,*
TTBSG[b] (PT5)	*9, 24, 3ka, 11, 17, 30, 10, 14a, 18*	*1, 2a, 2c, 3a, 16, 26, B*
LBGTG[b] (PT6)	*2a, 2c, 3, 9, 16, 24, 26, 3ka, 11, 17, 30, 14a, 18*	*1, B, 10*
MBRTG[b] (PT7)	*2a, 2c, 9, 16, 24, 26, 17, 21, 39/41, 52, 19*	*1, 3a, 3ka, 30, B, 10, 18, 28, 42, 23*
BBBPB[b] (PT8)	*1, 2a, 2c, 3a, 9, 16, 24, 26, 3ka, 11, 17, 30*	*14a*

[a] P = Puccinia; R = recondita; T = tritici; US = United States
[b] Nomenclature based on Long and Kolmer (1989)

Table 2.5 Seedling ITs[†] of differential lines inoculated with 16 virulence phenotypes of the leaf rust fungus *Puccina triticina*

Differential line-*Lr* gene	MRDSD	TKLSQ	MTPTB[†]	MKPSG[†]	TTRSD	TNRSD	MFBJG	MGNTQ[†]	PSMTJ	TFGSB	TDRSH	TTBSG	LBGTG[†]	MBRTG	BBBPB
RL6003-*Lr1*	4	3^{+}	3^{+}	3	3^{+}	3^{+}	3^{+}	3^{+}	3^{+}	3^{+}	3^{+}	3	3	3	;
RL6016-*Lr2a*	$0;^{\S}$	3	$0;^{\S}$	$0;^{\S}$	3^{+}	4	$0;^{\S}$	$0;^{\S}$	$;1^{+\S}$	3^{+}	3	3	0	0	$0;^{\S}$
RL6047-*Lr2c*	$0;^{\S}$	$33^{+\S}$	$0;^{\S}$	$0;^{\S}$	3^{+}	3^{+}	$0;^{\S}$	$;1^{\S}$	3	3^{+}	3	3	0	$0;^{\S}$	;
RL6002-*Lr3a*	4	3^{-}	3	3	3^{+}	4	3	3^{+}	3^{+}	3^{+}	3	3	$;1^{\S}$	3	;
RL6010-*Lr9*	4	;	3^{-}	;	3^{+}	4	0	;	3^{+}	0	0	3	0	0	0
RL6005-*Lr16*	3	$33^{+\S}$	3	3	3^{+}	2^{+}C	1N	3^{+}	3^{-}	2	2^{+}N	3^{+}	$;1^{\S}$	2N	$;2N^{\S}$
RL6064-*Lr24*	$;1^{\S}$	$33^{+\S}$	3	3	3^{+}	3	3	$;1^{\S}$	3	3^{+}	3^{+}	3	$;2^{\S}$	0	$;1^{-\S}$
RL6078-*Lr26*	4	3	3	3	3^{+}	2^{+}N	3^{-}	$;2^{+\S}$	$;1^{\S}$	3^{+}	;1	3	2^{+}N	$;2^{\S}$	$;1^{\S}$
RL6007-*Lr3ka*	$23C^{\S}$	3	3	3	3^{+}	4	$12^{\S}$	3	3	$12^{\S}$	3^{+}	2^{+}	$;1^{\S}$	3	$;1^{\S}$
RL6053-*Lr11*	$23C^{\S}$	$22^{+\S}$	;2C	1N	$2^{-}3^{+}$	4	2	2^{+}	2^{+}C	3^{-}	3^{+}	$;1^{+\S}$	3	3	2^{+}
RL6008-*Lr17*	3^{+}	$;,3^{\P}$	3	3	$2^{+}3$	;	;	3	$0;^{\S}$	$0;^{\S}$	$;1^{\S}$	$;1^{\S}$	0	$;1^{\S}$	;
RL6049-*Lr30*	2C	2^{+}	3	3	3	4	1^{+}	2^{+}N	3^{+}	$;1^{\S}$	3^{+}	2^{+}	$;1^{\S}$	3	2^{+}C
RL6051-*LrB*	3^{+}	3^{-}	3	3	33^{+}	3^{-}	2^{+}	3^{+}	3^{+}	3^{+}	3^{+}	3^{+}	3	3	3
RL6004-*Lr10*	3^{+}	3^{+}	3	3	3^{+}	3^{+}	3	4	3	3^{+}	3	3^{+}	3	3	2^{+}
RL6013-*Lr14a*	3^{+}	$33^{+\S}$	3	3	3^{+}	4	3	4	3	3^{+}	3^{+}	3^{+}	3	3	3
RL6009-*Lr18*	2^{+}	1^{+}	3	1^{+}	2N	2^{+}	2^{+}	3	3	3	$;1^{\S}$	1^{+}C	3	3	3
KS89WGRC07-*Lr21*	2^{+}C	3^{+}	2^{+}	$;2^{-\S}$	1^{+}N	$;1^{-\S}$	$;1^{\S}$	3	$12^{-\S}$	$;1^{\S}$	$;2^{\S}$	$;1^{\S}$	$;1^{\S}$	2^{-}	$;1C^{\S}$
RL6079-*Lr28*	$0;^{\S}$	3	2^{+}	3	$2^{+}3^{-\S}$	$;1^{\S}$	3	3	3	;	3^{+}	3^{+}	3	3	;
Overly-*Lr39*	3^{+}	0	0	0	3	3	0	0	3	0	0	0	0	0	0
KS91WGRC11-*Lr42*	$0;^{\S}$	$12C^{\S}$	2	2^{+}	$2^{+}3^{\S}$	2^{+}	2	;	$;1C^{\S}$	2	3^{+}	$0;^{\S}$	0	0	;
KS96WGRC36-*Lr50*	3^{+}	3^{-}	3	$;1^{\S}$	3	3	2^{+}C	3	3	2C	3	2^{+}N	$0;^{\S}$	3	$2^{+}3$
PI245487-*Lr52*	$0;^{\S}$	2	-	-	-	-	$;1^{\S}$	-	-	$;1^{\S}$	-	-	-	$0;^{\S}$	$0;^{\S}$
Morocco-*Lr19*	$0;^{\S}$	$0;^{\S}$	;	0	$0;^{\S}$	$0;^{\S}$	0;	$;1^{\S}$	$0;^{\S}$	$0;^{\S}$	0	$0;^{\S}$	$0;^{\S}$	0	$0;^{\S}$
RL6012-*Lr23*	3^{+}	3	2^{+}	23^{-}	3^{+}	3^{+}	3^{+}	$2^{+}3^{\S}$	3^{+}	3^{+}	3^{+}	3^{-}	2^{+}N	3	$2^{+}3C^{\S}$
Thatcher	4	4	3^{+}	3	4	4	4	3^{+}	3^{+}	4	4	3^{+}	3^{+}	3	4
Avocet S	3^{+}	3^{+}	3^{+}	3	3^{+}	3	3^{+}	3	3^{+}	3^{+}	3^{+}	3^{+}	3	3	$0;^{\S}$
Amadina	3^{+}	3^{+}	2N	$;1^{\S}$	1^{+}	$;1^{\S}$	1^{+}N	$;1^{\S}$	$0;^{\S}$	3^{+}	$0;^{\S}$	$;1^{\S}$	$;1^{\S}$	2^{+}	$0;^{\S}$

[‡]Infection types: 0 = no flecks or uredinia, 0; = faint hypersensitive flecks, ; = hypersensitive flecks, 1 = small uredinia, 2 = small uredinia with chlorosis, 3 = moderate size uredinia, 4 = large uredinia, + indicates slightly larger uredinia, - indicates slightly smaller uredinia; C = more than usual degree of chlorosis; N = more than usual degree of necrosis; [§]ITs with more than one symbols denote a range in IT; [¶]plants with low and high ITs that were classified as segregating. [†]'Amadina', 'Avocet S', 'Avocet S' x 'Amadina' derived RILs, 'Thatcher', and the Thatcher differential RL6012-Lr23 were tested with wsolates MTPTB, MKPSG, MGNTQ, and LBGTB at 25° C for postulation of the temperature-sensitive resistance gene *Lr23*.

Table 2.6 Segregation of leaf rust reaction in seedling plants of 'Avocet S' x 'Amadina' RILs

Isolate	Lines[†]			χ^2-value[‡]			
	Res	Susc	Seg	1 gene H_o: 1:1	2 genes H_o: 1:3	3 genes H_o: 1:7	4 genes H_o: 1:15
MRDSD	0	149	0	-	-		
TKLSQ	0	149	0	-	-		
MTPTB	40	101	7	26.39****	1.56^{NS}	23.31****	114.06****
MKPSG	81	61	6	2.82^{NS}	6.23****	97.83****	320.78****
TTRSD	52	89	7	9.71**	0.80^{NS}	56.66****	219.45****
TNRSD	62	77	9	1.62^{NS}	8.32*	103.02****	333.72****
MFBJG	39	104	5	29.55****	2.54^{NS}	20.99****	90.42****
MGNTQ	67	77	4	0.69^{NS}	11.28**	117.97****	349.15****
PSMTJ	57	87	4	6.25*	3.16^{NS}	72.25****	74.49****
TDRSH	55	89	4	8.03*	1.53^{NS}	64.45****	217.61****
TTBSG	51	92	5	11.76**	0.28^{NS}	55.86****	180.74****
LBGTG	59	81	8	3.46^{NS}	4.61^{NS}	88.73****	296.86****

[†]Res = resistant (IT 0 to 2^+); [†]Susc = susceptible (IT 3 to 4); [†]Seg = segregating (plants with low and high ITs); [‡]In RIL, 1:1, segregation of one gene; 1:3, segregation of two epistatic genes; 1:7, segregation of three epistatic genes; 1:15, segregation of four epistatic genes. NS – not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$

Table 2.7 Pearson correlation coefficients (r_p) between IT data for the 'Avocet S' x 'Amadina' population in response to 10 *P. triticina isolates*

	MTPTB	MKPSG	TTRSD	TNRSD	MFBJG	MGNTQ	PSMTJ	TDRSH	TTBSG	LBGTG
MTPTB	1.00	0.33****	0.26**	0.15^{NS}	0.17*	0.62****	0.20*	0.21NS	0.39****	0.28***
MKPSG		1.00	0.38****	0.31***	0.31****	0.27***	0.34****	0.30***	0.36****	0.34****
TTRSD			1.00	0.76****	0.69****	0.40****	0.94****	0.93****	0.73****	0.77****
TNRSD				1.00	0.58****	0.35****	0.75****	0.74****	0.48****	0.59****
MFBJG					1.00	0.34****	0.73****	0.70****	0.57****	0.56****
MGNTQ						1.00	0.42****	0.38****	0.46****	0.41****
PSMTJ							1.00	0.93****	0.71****	0.77****
TDRSH								1.00	0.63****	0.77****
TTBSG									1.00	0.61****
LBGTG										1.00

NS – not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$

Table 2.8 PCR-based markers linked to *Lr* genes and primer sequences

Gene	Marker set	Chromosome location	Sequence of primers 5'-3'	Gene source	Reference
Lr1	pTAG621-5 pTAG621-3	5DL	GGG TCA CGT ACT ACT ATA TA CCT TGC CAG CCC AAA AGA AG	*Triticum aestivum*	Feuillet et al. 1995
Lr3	Mwg798-F Mwg798-R	6BL	GGC TGT CTA CAT CTT CTG CA CAA GTG TTG AGA AGG AGA GT	*Triticum. aestivum*	Sacco et al. 1998
Lr9	J13/1 J13/2		TCC TTT TAT TCC GCA CGC CGG CCA CAC TAC CCC AAA GAG ACG	*Aegilops umbellulata*	Schachermayr et al. 1994
Lr10	F1.2245 Lr10-6/r2	1AS	GTG TAA TGC ATG CAG GTT CC AGG TGT GAG TGA GTT ATG TT	*Triticum aestivum*	Feuillet (personal communication)
Lr13	Barc183-F Barc183-R	2BS	CCC GGG ACC ACC AGT AAG T GGA TGG GGA ATT GGA GAT ACA GAG	*Triticum. aestivum*	Cakir et al. (pers. comm.)
Lr24	Lr24/Sr24#50-F Lr24/Sr24#50-R	3DL	CCC AGC ATC GGT GAA AGA A ATG CGG AGC CTT CAC ATT TT	*Thinopyrum ponticum*	Mago et al. 2005
Lr26	SCM9	T.1RS.1BL	TGA CAA CCC CCT TTC CCT CGT TCA TCG ACG CTA AGG AGG ACC C	*Secale cereale*	Saal and Wricke 1999
Lr47	PS10R PS10L	7AS	GCT GAT GAC CCT GAC CGG T TCT TCA TGC CCG GTC GGG T	*Aegilops speltoides*	Helguera et al. 2000

Table 2.9 Thermocycle temperature profile for the specific *Lr* PCR primers used in the present study

Gene	Cycle conditions	Amplified marker fragment size	'Amadina'	'Avocet S'
Lr1	94° C – 5 min; 39 cycles (94°C – 1 min; 55° C – 1 min; 72° C – 2 min); 72° C – 10 min	560 bp	+	+
Lr3	94° C – 5 min; 39 cycles (94° C – 1 min; 60° C – 1 min; 72° C – 2 min); 72° C – 10 min	365 bp	+	+
Lr9	94° C – 6 min; 45 cycles (94° C – 1 min; 62° C – 1 min; 72° C – 2 min); 72° C – 10 min	1100 bp	-	-
Lr10	94° C – 3 min; 39 cycles (94° C – 45 sec; 57° C – 45 sec; 72° C – 30 sec); 72° C – 3 min	310 bp	-	+
Lr13	95° C – 3 min; 39 cycles (94° C – 40 sec; 58° C – 40 sec; 72° C – 1 min); 72° C – 10 min	190 bp	+	-
Lr24	20° C – 1 min; 94° C – 3 min; 39 cycles (94° C – 30 sec, 57° C – 30 sec, 72° C – 40 sec); 72°C – 10 min	200 bp	-	-
Lr26	94° C – 3 min; 30 cycles (95° C – 1 min, 60° C – 1 min, 72° C – 1 min), 72° C – 5 min	206 bp	+	-
Lr47	94° C – 3 min; 7 cycles of touchdown (94° C – 30 sec, 70° C → 64° C – 30 sec, 72° C – 30 sec); 35 cycles (94° C – 30 sec, 63° C – 30 sec, 72° C – 30 sec); 72° C – 7 min	282 bp	-	-

Table 2.10 PCR-based DArT markers and primer sequences

Marker set	Sequence of primers 5'-3'	PCR amplification conditions	Amplified marker fragment size	Chrom location
wPt6442-F	GCT TCC GTG AGT GAC GAT TT	94°C 5 min; 40 cycles (94°C – 1 min; 52°C – 1	203	1BL
wPt6442-R	TGA CGG CTT AGC CAC AAG TA	min; 72°C – 2 min); 72°C – 10 min		
wpt8986-F	TGT GTG GGA GAT GTC TTG AGT C	94°C 5 min; 40 cycles (94°C – 1 min; 60°C – 1	356	1BL
wPt8986-R	CAA CAG TGC ATT GTC CGT CT	min; 72°C – 2 min); 72°C – 10 min		
wPt1560-F	CAG GTC CAA TTC CAA TCT CC	94°C 5 min; 40 cycles (94°C – 1 min; 60°C – 1	370	1BL
wPt1560-R	AAC CCG TAA GCT GGT TCT GA	min; 72°C – 2 min); 72°C – 10 min		
wPt6343-F	AGC AGG CAC CGT CTG ATT T	94°C 5 min; 40 cycles (94°C – 1 min; 60°C – 1	234	2DS
wPt6343-R	ACA TGG TTG GGA AGG AAG G	min; 72°C – 2 min); 72°C – 10 min		

Table 2.11 Estimated additive (A) effects of leaf rust seedling genes detected by the mixed linear-model approach for leaf rust resistance in 'Amadina'

Isolate	Chromosomal location	Interval[‡]	A[§]	SE (A)[§]	H^2(A)[¶]
MTPTB	2BS	*XwPt615*[†]*–Xbarc183*	-0.2466****	±0.030	0.2061
	2DS	*XwPt6419*[†]*–XwPt6200*	-0.1860****	±0.024	0.2155
MKPSG	1BL	*XwPt3753*[†]*–XwPt4434*	-0.2030****	±0.029	0.2562
	2BS	*XwPt6932*[†]*–XwPt8492*	-0.1584****	±0.031	0.0831
TNRSD	1BL	*XwPt1781*[†]*–XwPt8267*	-0.5105****	±0.043	0.4827
	2DS	*XwPt9780*[†]*–XwPt6419*	+0.1007***	±0.030	0.0998
MFBJG	1BL	*XwPt3451*[†]*–XwPt7094*	-0.4164****	±0.027	0.5884
MGNTQ	1BL	*XwPt8949*[†]*–XwPt3852*	-0.3311****	±0.026	0.3221
	2BS	*XwPt6477*[†]*–Xbarc7*	-0.1595****	±0.031	0.1002
	2DS	*XwPt4144*[†]*–XwPt9780*	-0.0974****	±0.026	0.0808
LBGTG	1BL	*XwPt1684*[†]*–XwPt1781*	-0.4032****	±0.032	0.4996

[‡]Gene-i and Gene-j are the genes of testing points i and j respectively; [‡]Interval is the interval of testing point; [§]A is the additive effect in the testing point; [¶]H^2(A) represents the phenotypic variation explained by A; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; NS – not significant

Table 2.12 Epistatic genomic regions detected by QTLNetwork 2.0 for seedling resistance in 'Amadina'

Isolate	Chromosomal location	Interval-i[‡]	Chromosomal location	Interval-j[‡]	AA[§]	SE(AA)[§]	H^2(AA)[¶]
MTPTB	2BS	*XwPt615–Xbarc183*	2DS	*XwPt6419–XwPt6200*	-0.2382****	±0.0300	0.1663
MKPSG	1BL	*XwPt3753–XwPt4434*	2BS	*XwPt6932–XwPt8492*	-0.1023**	±0.0310	0.0378
TNRSD	1BL	*XwPt1781–XwPt8267*	2DS	*XwPt9780–XwPt6419*	-0.0959*	±0.0440	0.0146
MGNTQ	1BL	*XwPt8949–XwPt3852*	2BS	*XwPt6477–Xbarc7*	+0.0842**	±0.0320	0.0312
	2BS	*XwPt6477–Xbarc7*	2DS	*XwPt4144–XwPt9780*	-0.1500****	±0.0316	0.0566
	1BL	*XwPt359–XwPt5067*	2DS	*XwPt4144–XwPt9780*	+0.0259****	±0.0259	0.0490

[‡]Gene-i and Gene-j are the genes of testing points i and j respectively; [‡]Interval-i and Interval-j are the intervals of testing points i and j; [§]AA is the additive-by-additive interactions between testing points i and j, respectively; [¶]H^2(AA) represents the phenotypic variation explained by AA; **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001; NS – not significant

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CHAPTER 3 - Genetic mapping of slow-rusting resistance against leaf rust and stripe rust in the spring wheat 'Amadina'

Abstract

Leaf and stripe rust diseases are important constraints to wheat production worldwide, and can be controlled through the use of genetic resistance. Due to pathogen adaptation, race-specific resistance in wheat cultivars is short-lived. Slow-rusting resistance is a desirable type of resistance because it does not lose its effectiveness when new pathogen races occur. The CIMMYT wheat breeding line 'Amadina' carries slow-rusting resistance to both leaf rust and stripe rust that has remained effective for many years. In order to identify the quantitative trait loci (QTL) for slow-rusting resistance against leaf rust and stripe rust, a population of 148 F_5 lines derived from a cross between 'Amadina' and the leaf rust- and stripe rust-susceptible cultivar 'Avocet S' was evaluated for adult-plant leaf rust and stripe rust resistance at two field sites in Mexico during different seasons. Segregation in the F_5 generation indicated that there were at least four additive genes involved in resistance for leaf rust and at least three for stripe rust. The parents and RIL population were genotyped with a total of 436 polymorphic SSR (SSR) and DArT markers. Using partial linkage mapping, we identified ten independent loci that contributed to adult plant resistance (APR) to the two rust diseases in the 'Avocet S' x 'Amadina' population. The loci identified on chromosomes 1BL and 5BS influenced resistance to both leaf and stripe rust. The loci on chromosomes 3AL, 4AL, and 7BL had effects only on leaf rust. The loci on chromosomes 4AL, 5AL, 2BL, 4BL, 2DL, and 4D were associated with APR for stripe rust, with two additional loci having an effect only through epistatic interaction. The percentage of phenotypic variance explained by each QTL varied from 0.68 to 19.48% for leaf rust, and 1.78 to 23.22% for stripe rust.

Introduction

Developing resistant varieties is the most efficient and environmentally sustainable means of reducing losses due to leaf and stripe rust diseases caused by *Puccinia triticina* f. sp. *tritici* and *P. striiformis* f. sp. *tritici*. The majority of the known resistance genes present in hexaploid wheat (*Triticum aestivum* L.) (McIntosh et al 1998) are

characterized by a hypersensitive response in the host plant upon infection by a pathogen race that possesses the corresponding avirulence allele. However, high genetic variation in the pathogen and the ability of the pathogen to evolve into new races with added virulence have been the major factors limiting successful long-term management of leaf rust and stripe rust by race-specific resistance genes. In recent years, geneticists and plant breeders have emphasized the importance of developing and deploying cultivars that carry durable, or slow-rusting, resistance based on quantitatively inherited multiple genes (Caldwell 1968; Parlevliet 1975; Johnson 1988). Slow-rusting resistance is characterized by the combined effect of an increased latent period and reduced uredinial size, infection frequency and spore production (Caldwell 1968; Parlevliet 1975; Ohm and Shaner 1976).

To date, only two independent loci, *Lr34/Yr18* on chromosome 7DS (Dyck 1987) and *Lr46/Yr29* on 1BL (Singh et al. 1998) have been shown to confer slow-rusting resistance to both leaf and stripe rusts. *Lr34*, which is the same as stripe rust adult-plant resistance gene *Yr18*, powdery mildew resistance gene *Pm38*, and leaf tip necrosis gene *Ltn1*, was recently cloned (Krattinger et al. 2009). This gene codes for a putative ATP-binding cassette (ABC)-type membrane protein (ABC transporter) which functions as ATP-driven efflux pumps that transport chemical energy within cells for metabolism (Davies et al. 2000). In plant pathogenic fungi, members of this transporter group play a role in providing resistance to phytoalexins, and to antifungal compounds or act as novel pathogenicity factors (Campbell et al. 2003). *Lr34/Yr18* and *Lr46/Yr29* have remained effective in commercial production for more than 50 and 30 years, respectively. Identification of other slow-rusting genes has not been easy due to their small effects, but classical genetic studies and allelism tests have revealed that at least twelve of these genes are present in CIMMYT germplasm (Singh and Rajaram 1994), where such resistance has been a major target for selection for over 30 years. In addition, using molecular tools, researchers have identified at least 21 loci with slow-rusting effects against leaf rust on all wheat chromosomes except 1A, 3D, 6B, 6D and 7A (William et al. 1997; Faris et al. 1999; Messmer et al. 2000; Suenaga et al. 2003; Schnurbusch et al. 2004a; Navabi et al. 2005; Singh et al. 2005; Xu et al. 2005a, b; Rosewarne et al. 2008). Similarly, at least 21 loci for stripe rust resistance have been reported (Börner et al. 2000; Bariana et al. 2001; Boukhtem et al. 2002; Suenaga et al. 2003; William et al. 2003;

Ramburan et al. 2004; Mallard et al. 2005; Navabi et al. 2005; Singh et al. 2000, 2005; Rosewarne et al. 2008), indicating significant diversity for slow-rusting genes in wheat germplasm.

Characterization and introduction of additional sources of slow-rusting resistance, as found in the CIMMYT germplasm, may complement and enhance resistance against leaf rust and stripe rust in other wheat growing regions of the world (Kolmer et al. 2007). Furthermore, information on the identity and effectiveness of the slow-rusting resistance genes in the CIMMYT germplasm can be used to help diversify leaf rust and stripe rust resistance in breeding programs. To more effectively develop and deploy resistance based on diverse slow-rusting genes, it is important to determine their chromosomal locations and develop diagnostic markers for marker-assisted selection (MAS).

The spring wheat 'Amadina' is an important parent in the leaf rust- and stripe rust-resistance breeding programs of CIMMYT and Kansas State University. 'Amadina' displays a high level of resistance to leaf rust, and an intermediate level of resistance to stripe rust across Mexico and the United States over a period of time. The adult plant resistance in 'Amadina' is not associated with seedling resistance or hypersensitive response. 'Amadina' may contain *Lr46/Yr29* as it has 'Pavon 76' in its pedigree and also carries the best available marker for the gene. Based on the absence of the leaf tip necrosis phenotype and the diagnostic marker, *csLV34* (Lagudah et al. 2006), 'Amadina' does not carry *Lr34/Yr18*. From previous genetic studies, Singh et al. (2004) hypothesized that 'Amadina' carries a minimum of four additive genes for resistance to leaf rust and a minimum of three additive genes for resistance to stripe rust. A population of 148 recombinant inbred lines (RILs) was used in this study. The advantages of using RILs for detecting QTL have been shown by Austin and Lee (1996). RILs undergo multiple cycles of meiosis before reaching homozygosity. Consequently, linked genes have a greater probability of recombining and their pleiotropic effects can be more easily detected (Burr and Burr 1991). This increases the efficacy of testing differences between genotypic classes.

The objective of our study was to identify genomic regions of the other minor, slow-rusting resistance genes associated with leaf rust and stripe rust reactions using a

mapping population developed from the slow-rusting parent ‘Amadina’ and susceptible parent ‘Avocet S’.

Materials and methods

Plant material

The plant material was comprised of ‘Avocet S’, ‘Amadina’, and a population of 148 recombinant inbred lines (RILs) derived from the cross of ‘Avocet S’ (WW-119/WW-15//Egret) with ‘Amadina’ (Bobwhite/Crow//Buckbuck/Pavon 76/3/Veery#10). The RIL population was developed at the International Center for Maize and Wheat Improvement (CIMMYT). A total of 148 individual F_4 derived F_5 lines, each tracing back to an individual F_2 plant, were obtained by harvesting a random spike from each F_3 and a random plant from each F_4 line.

Field studies

Three-way crossed non-replicated field trials for disease screening of the F_5 lines were conducted in Mexico for stripe rust during 2001, 2002, and 2003 crop seasons at CIMMYT’s research station at Toluca, and two-way crossed non-replicated field trials for leaf rust during the 2001-2002 and 2002-2003 crop seasons at CIMMYT’s research station in Ciudad Obregon, northwestern Mexico. Approximately 60 seeds of each line were sown in 75 cm wide paired-row plots, 1 m in length, with 20 cm row spacing and a 50 cm pathway between plots. Stripe rust and leaf rust epidemics were initiated approximately four and six weeks after planting, respectively. Spreader rows of the highly susceptible cv. Morocco, planted in hills on one side of the plots in the pathway, were sprayed with a suspension of urediniospores in the lightweight mineral oil Soltrol once a day for three consecutive days. Both field locations have favorable environments for development of the respective diseases. The *P. striiformis* culture used in the study, Mex96.11, has the avirulence/virulence formula *Yr1, 4, 5, 8, 10, 15, 17, 24, Sp/2, 3, 6, 7, 9, 27, A*. The Mexican *P. triticina* race MCJ/SP (nomenclature based on Singh (1991)), used in the study has the avirulence/virulence formula *Lr2a, 2b, 2c , 3ka, 9, 16, 19, 21, 24, 25, 28, 29, 30, 32, 33/1, (3), 3bg, 10, 11, 12, 13, 14a, 15, 17, 18, 20, 22b, 23, 26,*

27+31. Both 'Amadina' and 'Avocet S' display susceptible seedling reactions to these cultures.

Visual estimation of the host response to infection (infection types) to stripe rust followed a 0–to–9 scale as described by McNeal et al. (1971). The first rust severities and reactions were recorded on flag leaves at flowering for stripe rust and at about the milk stage of grain development for leaf rust, when the susceptible parent displayed severity between 80 and 100%. The severity range (i.e. the most resistant and most susceptible plants) and average rust severity (estimated visually) were recorded for each F_5 line. The lines were evaluated a second time approximately 12 to 15 days after the first evaluation, when rust had killed leaves of the susceptible parent. Previous CIMMYT studies (Singh et al., 1998; Singh et al., 2000) have shown that the timing of the first rating is critical to identify plants or lines that are similar in susceptibility level to the susceptible parent, where as the second rating is critical to identify plants or lines similar in resistance levels to the resistant parent. On the basis of the two data sets obtained during each of the two and three years of testing, the F_5 lines were grouped into three categories: parental-type resistant (PTR) with mean rust severity less than, equal to, or up to 5% higher than that of the resistant parent 'Amadina'; parental-type susceptible (PTS) with mean rust severity either the same or higher than that of the susceptible parent 'Avocet S'; and intermediate.

DNA extraction

Genomic DNA was extracted using a modified CTAB (hexadecyltrimethylammonium bromide) method (Murray and Thompson 1980). For each 150-200 mg powdered sample in a 2 ml centrifuge tube, 1000 µl 2 X CTAB extraction buffer containing 2% 2-Mercaptoethanol (Sigma, St. Louis, MO) and 100 µg/mL proteinase K (Roche Diagnostics, USA) was added. Tubes were incubated for 60 minutes at 60 °C in a water bath, followed by 10 minutes cooling at room temperature. Chloroform:isoamyl alcohol (24:1) extraction was performed twice. DNA in aqueous phase was precipitated by adding approximately two-thirds volume of isopropanol and placing the tubes at -20 °C overnight. DNA was re-dissolved in 500 µL 1 X TE pH 8.0, and RNA was removed by RNAse A (Roche Diagnostics, USA) treatment at 37 °C for 30 minutes. To extract the

DNA, chloroform:isoamyl alcohol (24:1) extraction was performed twice. Intact high quality genomic DNA was precipitated using half volume of 7.5M ammonium acetate and 2 volumes of 100% ethanol.

SSR assay

The two parents were screened for polymorphism with a total of 1600 SSRs (SSRs). These markers consisted primarily of Gatersleben Wheat SSRs (GWM; Röder et al. 1995) and markers from the Wheat SSR Consortium (WMC; Gupta et al. 2002), INRA Clermont-Ferrand (CFA and CFD; Guyomarc'h et al. 2002), Beltsville Agriculture Research Center (BARC; Song et al. 2005), and Kansas State University (KSUM; Yu et al. 2004). The entire population of 148 RILs and two parents were characterized with 103 polymorphic markers. For each polymerase chain reaction (PCR) reaction, 100 ng genomic DNA was used in a 25 µL solution containing 250 µM of each dNTP, 1 X PCR buffer, 0.4 pmol of each primer with 2.5 mM $MgCl_2$ and approximately 0.1 U *Taq* polymerase. An MJ Research (Watertown, MA) PTC-200 thermal cycler was programmed as follows for Xbarc: 'hot start' 95 ^oC for 1 min, followed by 40 cycles of 40 sec at 94 ^oC, 40 sec of annealing, and 1 min of extension at 72 ^oC, with a final extension at 72 ^oC for 10 min. For the remaining markers the program was: 'hot start' at 94 ^oC for 5 min, followed by 45 cycles of 1 min at 94 ^oC, 1 min of annealing, and 2 min of extension at 72 ^oC, with a final extension at 72 ^oC for 10 min. PCR products were size-separated on 6% polyacrylamide gels and silver stained. Visual allele identification followed a conservative approach, i.e. only clearly different bands were accepted as to be different. In case of doubt, e.g. null alleles, experiments were repeated.

Capillary fragment analysis

For genotyping the entire RIL population, PCR products were analyzed by capillary electrophoresis on an ABI3730 (Applied Biosystems, Foster City, CA). Each forward primer was 5'-tailed with the M13 forward consensus sequence. The M13-tailed forward primers were then used in combination with a standard M13 primer dye-labeled (VIG, NED, FAM, PET) at its 5'-end (Boutin-Ganache et al. 2001). Samples were prepared by pooling 3 µL of PCR product from separate primer sets, each with a different fluorescent dye. The DNA pool was mixed and centrifuged. One microliter of the pooled DNA was

added to a mixture of 6 μL of Hi-Di formamide (Applied Biosystems, Foster City, CA), 0.25 μL of Genescan 500-LIZ size standard (Applied Biosystems, Foster City, CA), and 3 μL of water. The samples were again mixed well and centrifuged. The 96-well plate was placed on an MJ Research PTC-200 thermal cycler for 5 min at 95°C and then on an ice slurry for 5 min. Raw data files from the ABI3730 were imported into GeneMarker v1.1 (SoftGenetics, State College, PA) for fragment analysis.

DArT assay

The development of the DArT marker set is described by Akbari et al. (2006), and was used in this study to provide additional genome coverage. DArT genotyping of wheat is offered as a commercial service by Triticarte Pty. Ltd. Yarralumla, ACT, Australia, (www.triticarte.com.au) who conducted the analyses for this study. Briefly, a genomic representation of a mixture of the entire population was produced with *Pst*I-*Taq*I digestion, spotted on microarray slides, and the individual genotypes were screened for polymorphism based on fluorescence signals. DNA from the parents ('Amadina' and 'Avocet S') was first screened for polymorphism and then the individual RILs were genotyped. A total of 501 loci were scored as present (1) or absent (0). Names of loci that were previously mapped by Triticarte Pty. Ltd include the prefix "wPt" (followed by numbers corresponding to a particular clone); loci that were mapped for the first time on the current map are presented by clone ID number. DArT technology is protected by patent No. WO 01/73119.

The Primer3 Software ver. 0.40 (Rozen and Skaletsky 2000) was used for designing PCR-based DArT primers (Table 3.13) from DNA sequences of DArT probes available online at http://www.diversityarrays.com/sequences.html with amplicon sizes ranging from 200 to 400 bp. For DArT probes whose sequence information is not yet available, primers were designed from probes flanking them.

Cytogenetic stocks

A total of 54 cytogenetic stocks of 'Chinese Spring' wheat (Sears 1954; Endo and Gill 1996) were obtained from Dr. B. S. Gill, Department of Plant Pathology, Kansas State University. Twenty compensating nullisomic tetrasomic (NT) lines were used for

assigning markers to individual chromosomes, and 34 ditelosomic (DT) lines were used for assigning markers to short arm or long arm of individual chromosomes.

Statistical and genetic analyses

The data were analyzed statistically after first checking residuals for normality across environments using the SAS (SAS Institute, Cary, NC, USA) procedure UNIVARIATE. Residual plots for leaf rust and stripe rust revealed a random distribution for the percentage scores, therefore data were left untransformed. Leaf rust and stripe rust scores were taken from similar locations in different years and observed differences could be interpreted as genotype x year interactions. However, in keeping with standard statistical nomenclature, we described this study as a multi-environment analysis, with environments representing results obtained in a similar location but from different years. Combined analyses of variance over environments were then performed for both rusts using the SAS mixed linear models procedure MIXED (Littell et al. 1996). Heritabilities (h^2) were calculated from the variance estimates using the formula: $h^2 = \sigma^2_G/\sigma^2_{GxE}$, where σ^2_G is the variance of the genotypic effect and σ^2_{GxE} is the variance of the genotype x environment interaction (i.e. the phenotypic effect). Estimate of the entry (RILs) covariance from the MIXED procedure represented σ^2_G, and the residual covariance represented σ^2_{GxE}. Frequency distribution and Chi-squared analysis of the population was performed on both the leaf and stripe rust data. A Chi-squared analysis of each segregating marker was also performed to test for deviation from the 1:1 expected segregation ratio. The r_p value of the Pearson Correlation Coefficient was analyzed for leaf and stripe rust data between years by using the SAS computer program.

The scores of all polymorphic DArT and SSR markers were converted into genotype codes (‘A’, ‘B’) according to the scores of the parents; heterozygotes were recorded as missing data. Genetic linkage maps were constructed using the computer program MAPMAKER v3.0 (Lander et al. 1987). Centimorgan (cM) values were calculated according to Haldane mapping function. Linkage groups were identified using a minimum logarithm of the odds (LOD) threshold value of 3.0 after preliminary analysis using LOD scores ranging from 3.0 to 20. Pair-wise, three-point and multi-point analyses

were used in order to determine the best order of marker loci within the linkage groups. Any locus whose location was ambiguous was placed in the interval in which they were best fitted using the "try" command. Markers from multi-locus primers or those that were different from the reported locus were distinguished with a suffix a, b, or c, with the suffix "a" given to the first mapped locus. The linkage groups were assigned to chromosomes by comparing the marker positions to previously published hexaploid wheat maps (Somers et al. 2004; Sourdille et al. 2004; Song et al. 2005; Akbari et al. 2006; Semagn et al. 2006; Crossa et al. 2007; Francki et al. 2009). Non-informative loci which did not meet the threshold value for entry with respect to jumps in goodness of fit, and/or they changed the order of anchoring markers were excluded from mapping. Final mapping was done by combining 2 or more linkage groups that belong to the same chromosome.

Composite interval mapping (CIM; Zeng 1994) was used to search for QTL using the disease severity scores for the 'Avocet S' x 'Amadina' RILs. QTL analyses for each environment separately and then across environments were undertaken using the CIM from QTL Cartographer ver. 2.5, and mixed linear CIM in QTLNetwork 2.0 (Yang et al. 2005). For the QTL Cartographer the parameter set-up of "model 6 standard analysis" was used; for both programs, walk speed 1-cM step, "forward and backward" regression for the selection of the markers to control the genetic background (control markers or cofactors) with a probability into and out of the model of 0.05 and a blocked window size of 10 cM to exclude closely linked control markers at the testing site were used. Significant thresholds for QTL detection were calculated for each dataset using 1,000 permutations (Churchill and Doerge 1994) and a genome-wide LOD threshold (experiment-wise P value $\leq$ 0.05). The final genetic model incorporated significant additive effects and epistatic effects as well as their environment interaction. Negative values indicate a contribution towards slow-rusting resistance while positive values represent that for susceptibility. The mean of disease severity for haplotypes carrying a marker was compared with that of haplotypes not carrying the marker. When slight shifts of the QTL peaks during different years were observed during the CIM analysis, the peak identified with the analysis across environments using the mixed linear CIM program from QTLNetwork ver. 2.0 was considered to be the common peak associated with that

QTL. Linear regression analysis using the SAS procedure PROC CORR PEARSON was used for estimating the phenotypic variance explained by the closest marker of a detected QTL in each environment.

Results

Phenotypic distribution

The disease scores for the parents, population means, population maxima and minima for all environments are listed in Table 3.1. The resistant parent 'Amadina', displayed a maximum leaf rust severity of 0–1% with no or only few pustules with uredinia spores, and 25–30% for stripe rust, whereas the susceptible parent 'Avocet S' showed maximum leaf rust severity of 80-100%, and stripe rust severity of 90% in all three trials. The population showed a normal distribution in the field for leaf rust with the frequency distribution being skewed from the mid-parental values toward the resistant parent 'Amadina' (Figure 3.1), indicating that some of the genes could have a larger effect. The average stripe rust severity showed a continuous distribution with the population exhibiting transgressive segregation (Figure 3.2). Few RILs had the leaf or stripe rust disease levels similar to either the resistant or susceptible parents. This supports a quantitative mode of inheritance of adult plant resistance (APR) to leaf rust and stripe rust in the evaluated genotypes. The mean leaf rust and stripe rust severity values (Table 3.1) were similar to the mid-parent values, suggesting that additive effects are the predominant mode of inheritance for these two traits. Furthermore, as field inoculated races were virulent to all major seedling resistances, we concluded that the genes involved in resistance were of "adult-plant" nature.

Number of genes segregating in the RIL population for leaf rust and stripe rust

Based on the leaf rust and stripe rust data, 7 RILs were considered resistant for leaf rust, 136 intermediate for leaf rust, 7 susceptible for leaf rust, 11 resistant for stripe rust, 124 intermediate for stripe rust, and 13 susceptible for stripe rust (Table 3.3). Classification of the RILs into three response categories based on severity, viz. parental-type resistant (PTR), parental-type susceptible (PTS), and intermediate revealed that a minimum of

four, and three additive genes conferred adult plant resistance to leaf rust, and stripe rust, respectively. The observed frequencies of the three response categories significantly differed from expected frequencies for two and three genes for leaf rust, and two genes for stripe rust. Singh et al. (1998) previously reported the presence of the slow-rusting additive *Lr46/Yr29* locus, derived from 'Pavon 76', in 'Amadina'.

Phenotypic correlation between data

Genotype x environment interaction variance was significantly different from zero for both the leaf rust and stripe rust scores (Table 3.4). Genotype x environment interactions were approximately 30% and 25% as large as the genotypic variances for leaf rust and stripe rust, indicating strong correlation (r_p = 0.85 for leaf rust, and r_p = 0.73–0.82 for stripe rust from Table 3.4) of genotype performance across environments. The high Pearson correlation coefficient (r_p) of 0.85 between the leaf rust severity data of the lines collected over 2 years is a strong indication of the reliability and consistency of the field data in our non-replicated trials. Similarly, stripe rust severity of the RILs recorded over 3 years also showed high and significant Pearson correlation coefficient (r_p) values ranging between 0.73 and 0.82. Highly significant r_p values (0.35–0.53) between leaf rust severity data recorded over 2 years and stripe rust severity data recorded over 3 years indicated that at least some genes were common to the two traits. The calculated narrow sense estimate of heritability from the SAS procedure MIXED was 0.87 for slow leaf-rusting, and 0.93 for slow stripe-rusting.

Molecular mapping of 'Avocet S' x 'Amadina' RIL population

Among the 537 loci (434 DArTs, and 103 SSRs) used for preliminary mapping in the 'Avocet S' x 'Amadina' population, 436 (367 DArTs, and 69 SSRs) mapped to 35 linkage groups, each with 2-42 loci. A total of 101 non-informative loci (19%) were excluded from mapping because they did not meet the threshold value for entry with respect to jumps in goodness of fit, and/or they changed the order of anchoring markers. This map spanned 1877.7 cM, representing an average of 53.6% of the entire wheat genome (Röder et al. 1998), and had a mean genetic distance of 4.3 cM per locus.

Cytogenetic analysis of markers flanking putative QTL associated with resistance of leaf rust

An initial single environment QTL analysis using leaf rust scores from environment 1 (2000) and environment 2 (2002) identified only two QTL at intervals *XwPt1313–XwPt4721* and *XwPt7108–Xwmc276*. Using compensating nullisomic-tetrasomics and ditelosomics, DArT markers *XwPt1313* and *XwPt7108* (Table 5) were assigned to chromosomes 1BL (Figure 3.7) and 7BL (Figure 3.11). Sequence information of *XwPt4721* was not available. In addition to the loci on 1BL and 7BL, multi-environment analysis using the QTLNetwork 2.0 (Yang et al. 2005) identified three other loci at the intervals *XwPt5133–XwPt9238*, *XwPt6603–XwPt4064*, and *XwPt4577–Xwmc99*. Sequence information for DArT markers *XwPt5133*, *XwPt9238*, *XwPt4064* and *XwPt4577* was not available. The closest marker, *XwPt5133*, to the likelihood peak at the interval *XwPt5133–XwPt9238* was previously mapped to chromosome 3AL (http://maswheat.ucdavis.edu; Crossa et al. 2007). PCR-based *XwPt6603* and *XwPt4064* markers were not polymorphic on 3.0% agarose gel. We converted sequence information of DArT markers *XwPt9675*, which was 1.7 cM from the interval *XwPt6603–XwPt4064*, and *XwPt3030*, which was 6.3 cM from the interval *XwPt4577–Xwmc99* to PCR-based monoplex assays (Table 3.5) that were polymorphic on 3.0% agarose gel, and assigned the markers to chromosomes 4AL and 5BL.

QTL analysis of leaf rust reaction

Close linkage of the *Lr46/Yr29* STS marker *Xth4301* [designed at Kansas State University (unpublished report)], to the interval *XwPt1313–XwPt4721* flanking the QTL on 1BL confirmed the gene was *Lr46/Yr29*, which was derived from 'Pavon 76'. Simple linear regression analysis using the closest markers to the likelihood peak plot indicated that *Lr46/Yr29* accounted for 26% and 37% of the phenotypic variation in environments 1 and 2, and the second locus, *QSLr.ksu-7BL.16*, on chromosome 7BL explained 14% of the phenotypic variance in environment 1 (Table 3.6).

The resistant parent 'Amadina' contributed alleles for the QTL on 3AL (*QSLr.ksu-3AL.3*), 1BL (*Lr46/Yr29*), and 5BL (*QSLr.ksu-5BL.19*). Resistance alleles from 'Amadina' with additive effect (A) -36.99, accounted for 29.83% of the observed

phenotypic variation. The *QSLr.ksu-4AL.1* and *QSLr.ksu-7BL.16* QTL on chromosomes 4AL and 7BL were derived from the susceptible parent 'Avocet S'. Resistance alleles from 'Avocet S' with A -14.83, accounted for 5.78% of the phenotypic variation observed. All 5 detected QTL showed additive main effects (A), but did not show significant interaction with environment (Table 3.7).

Epistatic effects between QTL for leaf rust reaction

We found a significant digenic epistatic effect between *QSLr.ksu-1BL.2* (9.6-19.1 cM) and *QSLr.ksu-7BL.16* (34.0-47.1 cM) (Table 3.8). The *QSLr.ksu-1BL.2* x *QSLr.ksu-7BL.16* interaction with AA -6.87, accounted for 2.2% of the phenotypic variance for leaf rust reaction. The additive x additive interaction had a very small but significant interaction with environment 1 (AAE_1). There was a poor correlation for presence of alleles between flanking markers for loci involved in epistatic interactions (Table 3.9).

Cytogenetic analysis of markers flanking putative QTL associated with resistance of stripe rust

In addition to the *Lr46/Yr29* locus, single environment QTL analysis (Table 3.11) using stripe rust scores from environments 1, 2, and 3 also identified four additional QTL at intervals *XwPt6149–XwPt7062*, *XwPt1301–XwPt3281*, and *XwPt2379–Xgwm149b* associated with variation in stripe rust score (Figure 3.3c, d, e, f and g). PCR-based monoplex assay (Table 3.10) of *XwPt6149* was not polymorphic on 3.0% agarose gel, and sequence information for *XwPt1301*, *XwPt3281*, and *XwPt2379* was not available. Using the cytogenetic stocks, *XwPt7062* was assigned to chromosome 4BS. Marker *XwPt6209*, which was 1.1 cM from *XwPt7062*, could not be assigned to a chromosome because the amplified fragment was missing in Chinese Spring. However the 260 bp fragment amplified by *XwPt6209* was present in the resistant parent 'Amadina', and absent in the susceptible parent 'Avocet S'. Markers *XwPt1301* and *XwPt6343* mapped in the same region. PCR-based assay of *XwPt6343* which was polymorphic on 3.0% agarose gel was assigned to chromosome 2DS. Marker *XwPt2379* was assigned to chromosome 4D based on previous report (http://wheat.pw.usda.gov/GG2/index.shtml). The multi-environment analysis (Table 3.12) identified all the loci identified in single environments plus three additional loci at intervals *XwPt7919–XwPt8657*, *XwPt4210–Xgwm526*, and

XwPt4577–Xwmc99. PCR-based monoplex assay (Table 3.10) of *XwPt7919* was polymorphic on 3.0% agarose gel, but it could not be assigned to a specific chromosome because the fragment was absent in Chinese Spring. However, we were able to assign polymorphic PCR-based assay of *XwPt150* (Table 3.10), which was 0.2 cM from *XwPt7919*, to chromosome 4BS. Marker *XwPt4210* sequence information was not available, but it was assigned to chromosome 2BS based on previous reports (http://wheat.pw.usda.gov/ GG2/index.shtml; Crossa et al. 2007). The QTL at the interval *XwPt4577-Xwmc99* (Table 3.12) was the same *QSLr.ksu-5BS.19* QTL (Table 3.7) associated with resistance to leaf rust.

QTL analysis of stripe rust reaction

In the single environment analysis (Table 3.11) of stripe rust reaction in each tested environment, *Lr46/Yr29* accounted for 25%, 23% and 24% of the phenotypic variation observed in environments 1 (2000), 2 (2002), and 3 (2003). Simple linear regression analysis using the closest marker to the likelihood peak plot, *XwPt1313*, indicated that the proportion of total phenotypic variation explained by this locus ranged between 32% and 34% in the three environments. The *QSYr.ksu-4BS.3* QTL located on chromosome 4BS, with A -8.88 and -7.35, accounted for 12% and 11% of the phenotypic variance observed in environments 1 and 2, but was not present in environment 3. Conversely, *QSYr.ksu-2DS.14* and *QSYr.ksu-4D.2* QTL on chromosomes 2DS and 4D with A -22.12 and -11.06 each accounting for 7% of the phenotypic variation were observed in environment 3. A fifth QTL on 2BS was detected by the CIM from QTL Cartographer analysis only in environment 2.

In addition to the *Lr46/Yr29 locus*, 'Amadina' contributed alleles for the QTL, *QSYr.ksu-4AS.9*, *QSYr.ksu-5BS.19*, *QSYr.ksu-2DS.14*, and *QSYr.ksu-4D.2*, on chromosomes 4AS, 5BS, 2DS, and 4D, whereas alleles for *QSYr.ksu-2BS.16*, and *QSYr.ksu-4BS.3* on chromosomes 2BS and 4BS were contributed by 'Avocet S'. Resistance alleles from 'Amadina' with A -35.2, explained 39.11% of the observed phenotypic variance, whereas resistance alleles from 'Avocet S' with A -9.16, accounted for 15.74% of the phenotypic variance observed. All detected loci generally showed no interaction with environment (Table 3.12).

Epistatic effects between QTL for stripe rust reaction

Two digenic epistatic interactions involving loci associated with stripe rust were detected (Table 3.13). The first interaction involved the *QSYr.ksu-2BS.16* (130.8–146.2 cM) and *QSYr.ksu-4D.2* QTL (1.2–46.6 cM) with AA +2.09, and it accounted for 1% of the phenotypic variation. The second interaction was between two non-additive (DD) QTL, [*QSYr.ksu-5AL.3* (9.5-10.4 cM) and *QSYr.ksu-5AL.5* (47.3–56.0 cM) on chromosome 5AL with DD +4.11%, and it accounted for 3% of the observed phenotypic variation (Table 3.13). Correlation analysis showed poor estimates between flanking markers of putative QTL involved in epistatic interactions (Table 3.14).

Discussion

Genetic basis of durable leaf rust and stripe rust resistance

The objective of our study was to elucidate the genetic basis of the slow rusting resistance of the spring wheat cultivar ‘Amadina’. Genetic analysis of the ‘Avocet S’ x ‘Amadina’ RIL population indicated that there were at least four additive genes involved in slow-rusting resistance for leaf rust and at least three for stripe rust. CIM analysis across environments identified the *Lr46/Yr29* locus on 1BL, and 8 other linkage groups with minor but significant effects to either or both diseases. Negative additive main effect and additive x additive effect indicate increased slow-rusting resistance, whereas positive additive main effect and additive x additive effect indicate increased susceptibility. In the investigated population, both parental cultivars contributed alleles to an improved slow-rusting resistance. This is confirmed by pronounced transgressive segregation. Thus, transgression breeding is a possible strategy to improve slow-rusting resistance.

Leaf rust reaction

Three genomic regions, *QSLr.ksu-3AL.3*, *QSLr.ksu-1BL.2* (i.e. the *Lr46/Yr29* locus), and *QSLr.ksu-5BS.19* on chromosomes 3AL, 1BL, and 5BL from the resistant parent ‘Amadina’ were associated with leaf rust. ‘Amadina’ contributed allele for *QSLr.ksu-3AL.3*. Chu et al. (2009) identified a putative QTL (*QLr.fcu-3AL*) on chromosome 3AL that conditioned both seedling and adult plant resistance to more than one race. Messmer

et al. (2000) also mapped a QTL on 3AL conferring leaf rust resistance in a RIL population developed from the Swiss winter wheat variety 'Forno' and the Swiss winter spelt (*Triticum spelta* L.) 'Oberkulmer' RIL population.

Allele for the l *QSLr.ksu-5BL.19* QTL, detected on the short arm of chromosome 5B by the sensitive multi-environment analysis, had additive effect -20.73, and accounted for 8% of the observed phenotypic variation. Resistance allele for *QSLr.ksu-5BL.19*, located at the interval *wPt4577–Xwmc99*, was contributed by the resistant parent 'Amadina'. Based on the ITMI and Consensus maps and the location of the *Xwmc99* locus, *QSLr.ksu-5BL.19* should be located between 5BL1–0.55–0.75 and 5BL9–0.76–0.79 bins of the linkage group 5B. Homoeologous group-5 chromosomes of wheat contain at least seven catalogued genes for rust resistance. Except for, *Yr19*, whose arm location is unknown on chromosome 5B, most of the resistance genes were mapped on the long arms of homoeologous group-5 chromosomes (*Lr18* and *Yr3* on 5BL, *Lr1* and *Sr30* on 5DL and *Yr34* on 5AL) (http://www.ars.usda.gov/Main/docs.htm/docid=10342). However, based on our previous gene postulation study, both 'Amadina' and 'Avocet S' do not carry the *T. timopheevi*-derived *Lr18* gene (Unpublished). Chu et al. (2009) identified QTL, *QLr.fcu-5BL*, significantly associated with adult-plant resistance in a doubled-haploid population derived from the cross between the synthetic hexaploid wheat line (x *Aegilotriticum* spp.) TA4152-60 and the North Dakota hard red spring wheat breeding line ND495.

The effect of the QTL on 1BL corresponding to *Lr46/Yr29* on both leaf rust and stripe rust was highly significant (Table 3.5 and 3.9). This is also the case with the *Lr34/Yr18* adult plant resistance QTL located on chromosome 7DS (Suenaga et al. 2003). Both of these genomic regions on chromosomes 1BL and 7DS are also reported to be gene rich regions (Erayman et al. 2004). Close associations of the *Lr46/Yr29* with powdery mildew have been reported by Liu et al. (2001). The region associated with *Lr34/Yr18* (Suenaga et al. 2003) also seems to have an effect on barley yellow dwarf tolerance (Singh 1993), spot blotch resistance (Joshi et al. 2004) and powdery mildew resistance (Spielmeyer et al. 2005). Mateos-Hernandez et al. (2005) narrowed the physical location of the *Lr46/Yr29* locus to a sub-microscopic region between the breakpoints of deletion lines 1BL-13 [fraction length (FL) = 0.89–1] and 1BL–10

[fraction length (FL) = 0.89–3]. Additive x additive interaction between the *Lr46/Yr29* locus and the QTL, *QSLr.ksu-7BL.16*, on chromosome 7BL was detected. The *QSLr.ksu-7BL.16* QTL was flanked by *XwPt7108* and *Xwmc276*. If we refer to the ITMI and Consensus map and the location of the *Xwmc276* locus (Somers et al. 2004; Song et al. 2005), *QSLr.ksu-7BL.16* should map to the 7BL10–0.79–1.00 bin of linkage group 7B. Rosewarne et al. (2008) identified an epistatic interaction for stripe rust between the region around the *Lr46/Yr29* locus and another unmapped region. The additive x additive interaction for leaf rust identified in this study was positive, indicating its role in increasing susceptibility. It is plausible that *Lr46/Yr29* may not always act syngerstically with some APR genes, but also antagonistically with others, with the former set being particularly important for slow-rusting resistance. Although the *QSLr.ksu-7BL.16* QTL located on chromosome 7BL had additive main effect on leaf rust severity similar to that of *Lr46/Yr29* in the multi-environment analysis, it accounted for only 5% of the observed phenotypic variation. A number of QTL studies have identified loci on 7B as conferring adult plant resistance to leaf rust, indicating the importance of this region. Crossa et al. (2007) reported a significant association of the DArT marker *XwPt7108*, which was the closest marker to the likelihood peak plot on 7BL, in this study with resistance to leaf rust in five historical wheat multi-environment international trials from the International Maize and Wheat Improvement Center (CIMMYT). Up to now, three important leaf rust resistance genes have been mapped in the distal regions of chromosome group 7: *Lr19*, introgressed from the short terminal 7EL segment of *Lophopyrum ponticum* (Sharma and Knot 1966), and the two closely linked genes *Lr14a* and *Lr14b* (distal portion of chromosome 7BL; Dyck and Samborski 1970). APR QTL on 7BL were reported in the CIMMYT spring wheat cultivars ‘Parula’ (William et al. 1997) and ‘Opata 85’ (Faris et al. 1999; Nelson et al. 1997), and a Swiss winter wheat cultivar ‘Forno’ (Messmer et al. 2000; Schnurbush et al. 2004b). However, some of these QTL may correspond to different genes. Two QTL, one with a larger effect than the other, were identified on 7BL in ‘Forno’. The APR QTL on 7BL in ‘Opata 85’, which was effective in Ithaca, NY, and not effective in Ciudad Obregon, was in a cluster of defense response genes (Faris et al. 1999). Xu et al. (2005a, b) reported a QTL on the long arm of chromosome 7B in CI 13227 with effects on AUDPC, final severity, infection rate, and latent period. Recently

APR genes located on the long arm of chromosome 7B were reported in the CIMMYT spring wheat cultivars 'Weebill' (Zhang et al. 2008) and 'Attila' (Rosewarne et al. 2008). *Lr46/Yr29* was involved in epistatic interaction with the QTL, *QSLr.ksu-7BL.16* in this study.

In addition to the *QSLr.ksu-7BL.19* QTL on the long arm of chromosome 7B, the susceptible parent 'Avocet S' contributed resistance allele at the *QSLr.ksu-4AL.1* QTL on the long arm of chromosome 4A with a very minor but significant effect. The *QSLr.ksu-4AL.1* QTL was located at the interval *XwPt6603–XwPt4064*, and the locus *Xbarc78* was 17.7 cM from the *XwPt4064* locus. Based on the ITMI and Consensus maps and the location of the *Xbarc78* locus, *QSLr.ksu-4AL.1* should map to the deletion bin 4AL4–0.80–1.00 of the linkage group 4A. The *Ae. speltoides*-derived *Lr28* gene is located on chromosome 4AL (McIntosh et al. 1995). However, based on our previous gene postulation study, both 'Amadina' and 'Avocet S' do not carry *Lr28* (Unpublished). Except for *Lr25*, whose arm location is unknown, (Crossa et al. 2007), no other leaf rust resistance genes have been mapped to chromosome 4AL. Contribution of the resistance alleles by 'Avocet S' confirms earlier report by William et al. (2006) that 'Avocet S' seems to posses some genetic factors that contribute to slow rusting resistance, which results in significant delays in becoming completely susceptible. However, the presence of *QSLr.ksu-4AL.1* and *QSLr.ksu-7BL.19* was not sufficient enough to prevent 'Avocet S' from becoming completely susceptible. This clearly shows that effectiveness of slow-rusting resistance is dependant on a combination of a number of slow-rusting genes as well as the size of the effect of each individual gene involved.

Stripe rust reaction

In addition to the *Lr46/Yr29* locus, multi-environment CIM analysis identified 6 other QTL, *QSYr.ksu-4AL.9*, *QSYr.ksu-2BS.16*, *QSYr.ksu-4BL.3*, *QSYr.ksu-5BL.19*, *QSYr.ksu-2DL.14*, and *QSYr.ksu-4D.2* on chromosomes 4AL, 2BL, 4BL, 5BL, 2DL, and 4D with minor effects on stripe rust severity. The resistant parent 'Amadina' contributed alleles for the *QSYr.ksu-4AL.9*, *QSYr.ksu-5BL.19*, *QSYr.ksu-2DL.14*, and *QSYr.ksu-4D.2* QTL. Previously, DArT markers *XwPt2084*, *XwPt3795*, and *XwPt7807* on chromosome 4AL showed significant association with stripe rust, grain yield, and stem rust (Crossa et al.

2007). That region is 24.6 cM distal to the DArT marker, *XwPt7919*, which was the marker closest to the likelihood peak plot on 4AL in this study.

The *QSYr.ksu-5BL.19* QTL detected on the long arm of chromosome 5B by the multi-environment analysis was the same *QSLr.ksu-5BL.19* QTL on 5BL that was associated with leaf rust resistance in this study, indicating evidence of cross-cutting resistance between leaf rust and stripe rust. The *QSYr.ksu-5BL.19* QTL accounted for 8% of the observed phenotypic variation. A number of QTL studies have identified loci on 5BL as conditioning APR to stripe rust, indicating the importance of this region in stripe rust resistance. Mallard et al. (2005) identified two QTL, *QYr.inra-5BL.1* and *QYr.inra-5BL.2* on 5BL, accounting for 25% and 35% of the phenotypic observed variation. The *QYr.inra-5BL.2* QTL was located on the translocated region of chromosome 5BS in the long arm of chromosome 5B. Law and Worland (1997) suggested that the genes involved in resistance to yellow rust are most probably located on the short arm of chromosome 5B and may be closely linked to the break point of the translocation. Dilbirligi et al. (2004) also physically mapped a putative nucleotide binding site-leucine-rich repeat (NBS–LRR) gene (sequence BM136556a) in the 5BS–5 bin where the *Xgwm639* marker is located (Sourdille et al. 2004).

QSYr.ksu-2DL.14 QTL located on the short arm of chromosome 2D was detected only in environment 3. Resistance allele for *QSYr.ksu-2DL.14* with A -22.12 accounted for 6.51% of the observed phenotypic variance. However, in the multi-environment analysis allele, *QSYr.ksu-2DL.14* with A -10.30 accounted for only 2% of the phenotypic variance. *QSYr.ksu-2DL.14* was located at the interval *XwPt1301–XwPt6343*, and the locus *Xgwm301* was 10.2 cM from the *XwPt6343* locus. If we refer to the ITMI and Consensus wheat maps and the location of the *Xgwm301* locus, *QSYr.ksu-2DL.14* should be located at the deletion bin 2DL9–0.76–1.00 bin of the linkage group 2D. Thus, *QSYr.ksu-2DL.14* could not correspond to the APR gene *Yr16* which is located in the centromeric region of chromosome 2D (Worland and Law 1986). Melichar et al. (2008) mapped a minor QTL, *QPst.jic-2D*, on chromosome 2D of the UK wheat cultivar, Guardian that expresses a partial, growth-stage specific resistance to stripe rust. The QTL, *QPst.jic-2D*, seemed to be expressed late in the season or detectable only under high disease pressure.

QSYr.ksu-4BL.3 was flanked by the DArT markers *XwPt6149* and *XwPt7062*. The marker, *XwPt6149*, was the closest marker to the likelihood peak plot of *QSYr.ksu-4BL.3* on 4BL in this study. *QSYr.ksu-4BL.3* was consistently detected in environments 1 and 2, respectively although its position shifted slightly in the two environments. *QSYr.ksu-4BL.3* with A -8.88 and -7.45 accounted for 12.04% and 11.45% of the observed phenotypic variations in environments 1 and 2, respectively. Multi-environment analysis also detected *QSYr.ksu-4BL.3*. Significant association of the DArT marker, *wPt6149,* with reaction to stripe rust on 4BL had also been reported by Crossa et al. (2007). Thus we assumed *QSYr.ksu-4BL.3* was the same locus reported by Crossa et al. (2007). Based on the ITMI and Consensus maps and the location of the locus *Xgwm149* which was 3.6 cM from *XwPt7062*, *QSYr.ksu-4BL.3* should map to the deletion bin 4BL1-0.86-1.00. In a study on a segregating population of 113 recombinant inbred F_6 lines from the cross between 'Avocet-*YrA*' and 'Saar', Lillemo et al. (2008) identified a major QTL in 'Avocet-*YrA*' associated with powdery mildew resistance on chromosome 4BL (close to *XwPt6209*). The QTL identified by Lillemo et al. (2008) was located on chromosome 4BL at the same position as similar QTLs for powdery mildew resistance detected in the 'Forno' x 'Oberkulmer' (Keller et al. 1999), 'Synthetic' x 'Opata' (Börner et al. 2002) and 'Fukuho-komugi' x 'Oligoculm' (Liang et al. 2006) populations. The same QTL from Avocet showed significant effects on leaf rust and stripe rust in the 'Avocet' x 'Pavon' population (William et al. 2006). In the present study, the locus *XwPt6209* was 11.5 cM from the locus *XwPt6149* and 0.9 cM from the locus *XwPt7062*. Thus, QSYr.ksu-4VL.3 could correspond to the QTL for powdery mildew resistance reported by Lillemo et al. (2008).

The QTL *QSYr.ksu-4D.2* detected in this study could not be physically mapped due to insufficient marker information and unavailability of sequence information. *QSYr.ksu-4D.2* with A -11.06 was detected only in environment 3, and it accounted for 7.87% of the observed phenotypic variation. In the multi-environment analysis, allele for *QSYr.ksu-4D.2* with A -3.32 explained 4.18% of the observed phenotypic variation. Association of regions on both the short arm and long arm of chromosome 4D with stripe rust had been reported (Singh et al. 2000; Suenaga et al. 2003).

The susceptible parent 'Avocet S' contributed alleles for the loci *QSYr.ksu-2BL.16*, and *QSYr.ksu-4BL.3* on chromosomes 2BL and 4BL. The QTL *QSYr.ksu-2BL.16* was located at the interval *XwPt4210-Xgwm526* on the linkage group 2B, and it explained 4.61% of the observed phenotypic variation. If we refer to the ITMI and Consensus genetic maps and the location of the locus *Xgwm526*, *QSYr.ksu-2BL.16* should be located in C–2BL2–0.36 bin, the centromeric part of the long arm of chromosome 2B. Mallard et al. 2005 identified a major QTL, *QYr.inra-2BL*, on the centromeric region of chromosome 2BL, using F_7 RILs derived from the cross between the stripe rust-durable resistant 'Camp Rémy' and the susceptible Récital. The QTL *QYr.inra-2BL* explained 42-61% of the observed phenotypic variation. The peak of *QYr.inra-2BL* corresponded to a seedling-stage resistance gene and co-located with the seedling gene *Rsp*, which accounted for 30% of the phenotypic variation observed. According to Mallard et al. (2005) The *QYr.inra-2BL* QTL corresponds to a QTL, *QYR1*, reported by Boukhatem et al. (2002). A group of three race-specific resistance genes (the possibly allelic *Yr5* and *Yr7* for stripe rust, and *Sr9* for stem rust) has been mapped on 2BL, about 15 cM from the centromere (Hart et al. 1993; Bariana et al. 2001; Mallard et al. 2005). However, *QSYr.ksu-2BL.16* does not correspond to either *Yr5* or *Yr7* because they are not present in 'Avocet S' (Rosewarne et al. 2008). The genes *Yr27* and *Yr31* are linked closely with *Lr13* and *Lr23* respectively, which are in the centromeric region (http://wheat.pw.usda.gov). The *QSYr.ksu-2BL.16* detected by the more sensitive multi-environment analysis in this study could not be attributed to *Yr27* as the Mexican *P. striiformis* culture, Mex96.11, had virulence on this gene. Furthermore, *QSYr.ksu-2BL.16* is 89.2 cM from the *Lr13*, which lies in the interval between *XwPt615–XwPt2314* in our previous study (Unpublished). Minor QTL, with significant effects on stripe rust, have been previsously identified on 6A (William et al. 2006; Singh et al. 2005) and the distal end of 2BL (Rosewarne et al. 2008) in 'Avocet S'. The QTL *QSYr.ksu-2BL.16* identified in this study is different from the QTL reported by Rosewarne et al. (2008) due to their different locations on 2BL. According to William et al. (2006), the 'Avocet'-derived QTL on 6AL is likely due to a translocation from *Agropyron elongatum* in 'Avocet S'that is known to carry the stem rust resistance gene *Sr26*. As this present study and that of William et al. (2006) and Rosewarne et al. (2008) utilized partial linkage mapping, the

unidentified loci in each of the corresponding studies could be a reflection of limitations of this approach in identifying all minor QTL. Positive additive x additive interaction for stripe rust between *QSYr.ksu-2BL.*16 and *QSYr.ksu-4D.2* was identified in this study. Furthermore, two loci with non-additive main effects showed no phenotypic effects when alone, but, nevertheless, contributed significantly to the genetics of slow stripe-rusting trait, when analyzed for gene-gene interaction. Both loci interactions reduced levels of slow-rusting resistance against stripe rust.

Possible influences on adult plant resistance

The detected QTL in this study represent a minimum number considering that our genetic map did not cover the whole genome of wheat. The number of QTL for slow leaf-rusting and stripe-rusting in our study is the same as the number of genes estimated by the segregation analysis (Geiger and Heun 1989; Shaner et al. 1997; VanderGaag and Jacobs 1997) under the assumption of equal gene effects. According to our data, the assumption of equal gene effects is not met because the additive effects of individual QTL for leaf rust (ranging from -11.36 to -20.73) and stripe rust (ranging from -10.65 to -10.93) differed considerably. In previous studies where one of the parents contained either *Lr34/Yr18* or *Lr46/Yr29*, as well as other race-specific resistance genes, not more than two genomic regions conferring slow-rusting resistance were identified (Nelson et al. 1997; Rosewarne et al. 2008; Zhang et al. 2008). In contrast, several minor QTL for resistance in the ‘Avocet S’ x ‘Amadina’ RIL population were identified in our study, and the possible explanations here might be differences in genetic background, the population size, the number of environments and/or marker coverage. However, all QTL in this study except *Lr46/Yr29* could not be detected in all environments. Since environments differed in the time of plant development when leaf rust and stripe rust infection started, the infection pressure and, most likely, in the pathogen population, different genes might be relevant for resistance in different environments. Replicating the disease tests at the studied locations might have permitted a comparison of within-site with between-year variation. However, we propose that qualitative variations in resistance-gene expression were due to macro-environmental (across year) and not to micro-environmental variation. This proposition is based on the uniformity of disease

pressure with the spreader method used. Epidemics were almost certainly monotypic since typical natural epidemics do not occur in the experimental field locations. From these data and from the high correlation between environments we can conclude that QTL from the multi-environment analysis are certainly more reliable and thus, relevant for practical breeding.

Conclusion

We have identified and mapped 5 genomic regions for resistance to leaf rust, and 9 genomic regions for resistance to stripe rust. All loci, except the *Lr46/Yr29* locus, had relatively minor effects on response to leaf and stripe rust diseases, highlighting the value of phenotypic selection under epidemic field conditions and making marker development difficult. Two additional loci, *QSYr.ksu-5AL.3* and *QSYr.ksu-5AL.5*, located 35.5 cM apart on chromosome 5AL were involved in non-additive x non-additive interaction for stripe rust. The susceptible parent 'Avocet S' contributed allele for *QSYr.ksu-5AL.3*, and the resistant parent 'Amadina' contributed allele for *QSYr.ksu-5AL.5*. Both loci had no phenotypic effect alone, i.e. when they were not interacting with each other. Although it is difficult to have a clear explanation to this finding due to lack of sufficient evidence, we do think that either the interaction is an example of epistasis between two susceptibility alleles leading to increase in disease severity than would be predicted by simply adding together their individual phenotypes, or it is a type of interaction involving a susceptibility allele and an epistatic modifier leading to significant suppression of the susceptibility allele phenotype. The QTL analyses presented here clearly show the very complicated nature of slow-rusting resistance. Our results indicated that the major portion of genetic variability for slow-rusting resistance against leaf and stripe rust in the 'Avocet S' x 'Amadina' population was additive gene action while, to a certain degree, epistatic effects were associated with slow-rusting. This is in agreement with Das et al. (1992), who found predominantly additive genetic variance for partial leaf rust resistance in advanced spring wheat populations and, to some extent, additive x additive genetic variance. In the statistical analysis, it is possible to confuse loosely linked markers as having epistatic interactions. Correlation analysis revealed poor estimates between markers flanking putative loci involved in interactions for leaf rust and stripe rust. This

indicates that although genetic control is predominantly through simple additive genes, the effects of one allele can also be dependent on the presence of an appropriate allele at a second locus, which in itself does not have an effect on the phenotype.

Whether the mapping locations of any of the previously reported resistance QTL on 3AL, 4AL, 2BL, 4BL, 5BL, 7BL, 2DL, and 4D QTL are coincident with that of *QSLr.ksu-3AL.3*, *QSLr.ksu-4AL.1*, *QSYr.ksu-4AL.9*, *QSLr.ksu-2BL.16*, *QSYr.ksu-4BL.3*, *QSYr.ksu-5BL.19*, *QSLr.ksu-7BL.16*, *QSYr.ksu-2DL.14*, and *QSYr.ksu-4D.2* revealed in this study is not known and needs to be determined. The locus on 5BS in this study conferred resistance to both leaf rust and stripe rust. Since the regions of chromosomes 7DS (*Lr34/Yr18/Pm38*) and 1BL (*Lr46/Yr29/Pm39*) are associated with partial resistance to leaf rust, stripe rust, and powdery mildew, *QSYr.ksu-5BL.19* and it is possible some of the QTL in this study may also confer resistance to other wheat pathogens. The availability of genetic stocks for the above mentioned genes/QTL in a homogeneous background could facilitate testing for allelism and gene postulation studies. The exploitation of the rice-wheat synteny and wheat genomic sequence information will help in fine mapping of adult plant resistance detected in this study, and in the identification of additional markers suitable for accumulating partial leaf rust and stripe rust resistance to develop wheat cultivars with potentially long-lasting resistance. Recent success in cloning of the closely linked/pleiotropic slow-rusting gene, *Lr34/Yr18,* (Krattinger et al. 2009) has enhanced our understanding of the functional aspects of durable disease resistance. *Lr34* has been predicted to encode a pleiotropic drug resistance (PDR)-like ATP binding cassette (ABC) transporter. The same gene controlled resistance based on *Lr34*, *Yr18*, *Pm38* as well as *Ltn1* (a controlling premature senescense of the leaf tips about 1-2 weeks after flowering, which is widely used as a phenotypic marker for rust resistance). The nucleotide sequence of *Lr34* spans 11,805 bp and consists of 24 exons. Lillemo et al. (2008) identified a powdery mildew resistance gene, *Pm39*, in a region corresponding to the location of *Lr46/Yr29*. Another common feature of *Lr46/Yr29* is the expression of *Ltn2*, (Rosewarne et al. 2006; Lillemo et al. 2008). *Lr46* is most likely the same gene as the stripe rust adult-plant resistance gene, *Yr29*, powdery mildew resistance gene, *Pm39*, and leaf tip necrosis gene, *Ltn2*.

This research has highlighted the importance of chromosome 5BL for both leaf and stripe rust resistance, and the centromeric region of chromosome 2B for stripe rust resistance. This region has been implicated in other resistance systems. Our results are relevant to hexaploid wheat breeding because of the recent interests in 'Amadina' and its derivatives as a preferred source of slow-rusting resistance against leaf rust and stripe and other valuable traits in many wheat breeding programs at CIMMYT and academic institutions in the United States. Additionally, the characterization of genetic variation controlling slow-rusting resistance components (e.g. additive effects, non-additive effects, additive x additive epistatic interaction, non-additive x non-additive epistatic interaction) offers breeders and researchers alike insight into the significance of QTL with additive main effects and interacting QTL in conferring acceptable levels of slow-rusting resistance in wheat. The ultimate goal of linkage analysis is the identification of genes and genetic pathways that mediate resistance to rust diseases. Although a complete understanding of slow-rusting resistance must await cloning and comparison of all genes associated with the trait, it is reasonable to conclude that additive gene action and, at least to some extent, epistasis reflect important features of the genetics of slow-rusting resistance.

Figures and Tables

Figure 3.1 Histogram of leaf rust severity for the 'Avocet S' x 'Amadina' population. Two data sets are presented for leaf rust.

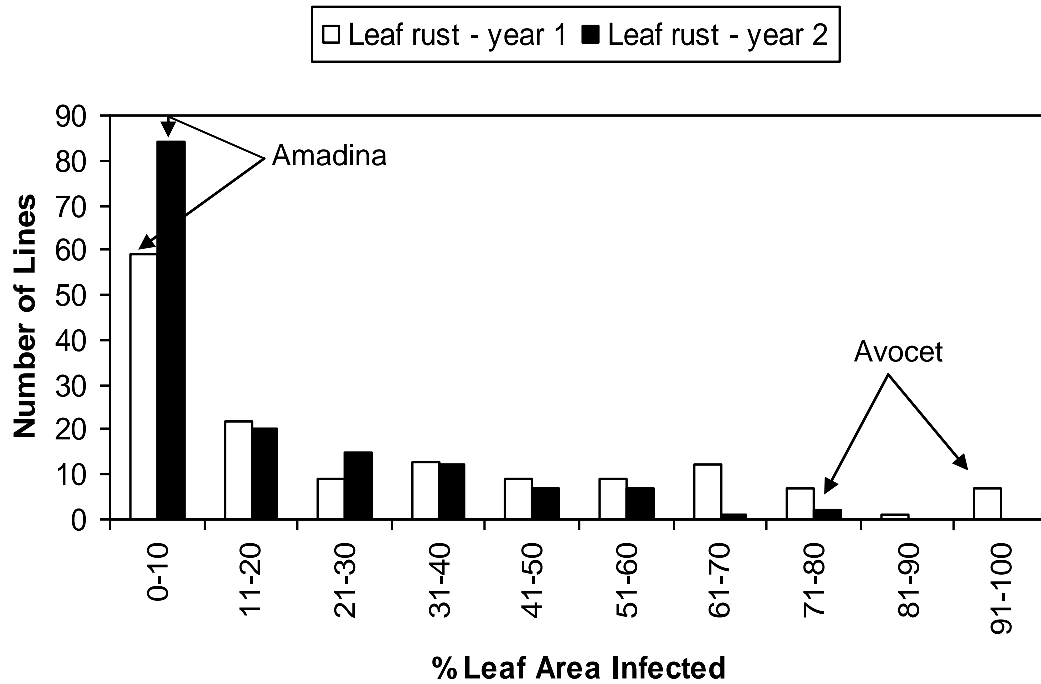


Figure 3.2 Histogram of stripe rust severity for the 'Avocet S' x 'Amadina' population. Three data sets for stripe rust.

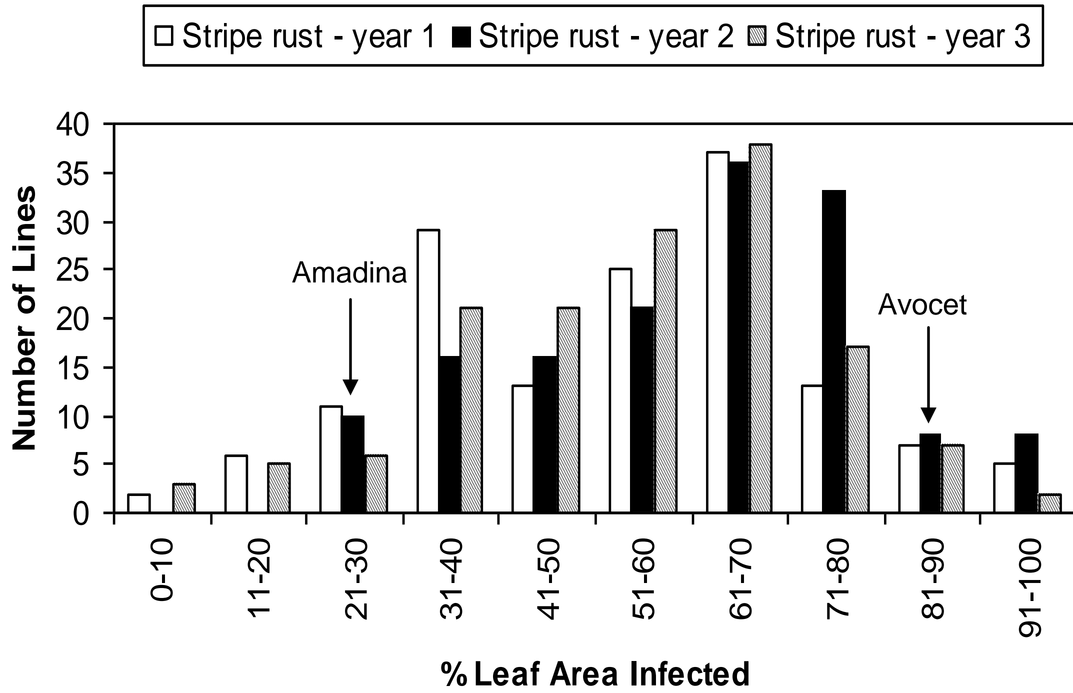


Figure 3.3 Composite interval mapping (CIM) in the 'Avocet S' x 'Amadina' population in response to leaf rust (a and b) and stripe rust (c, d, e, f)
[†](____2000; …….2002; _ _ _2003)

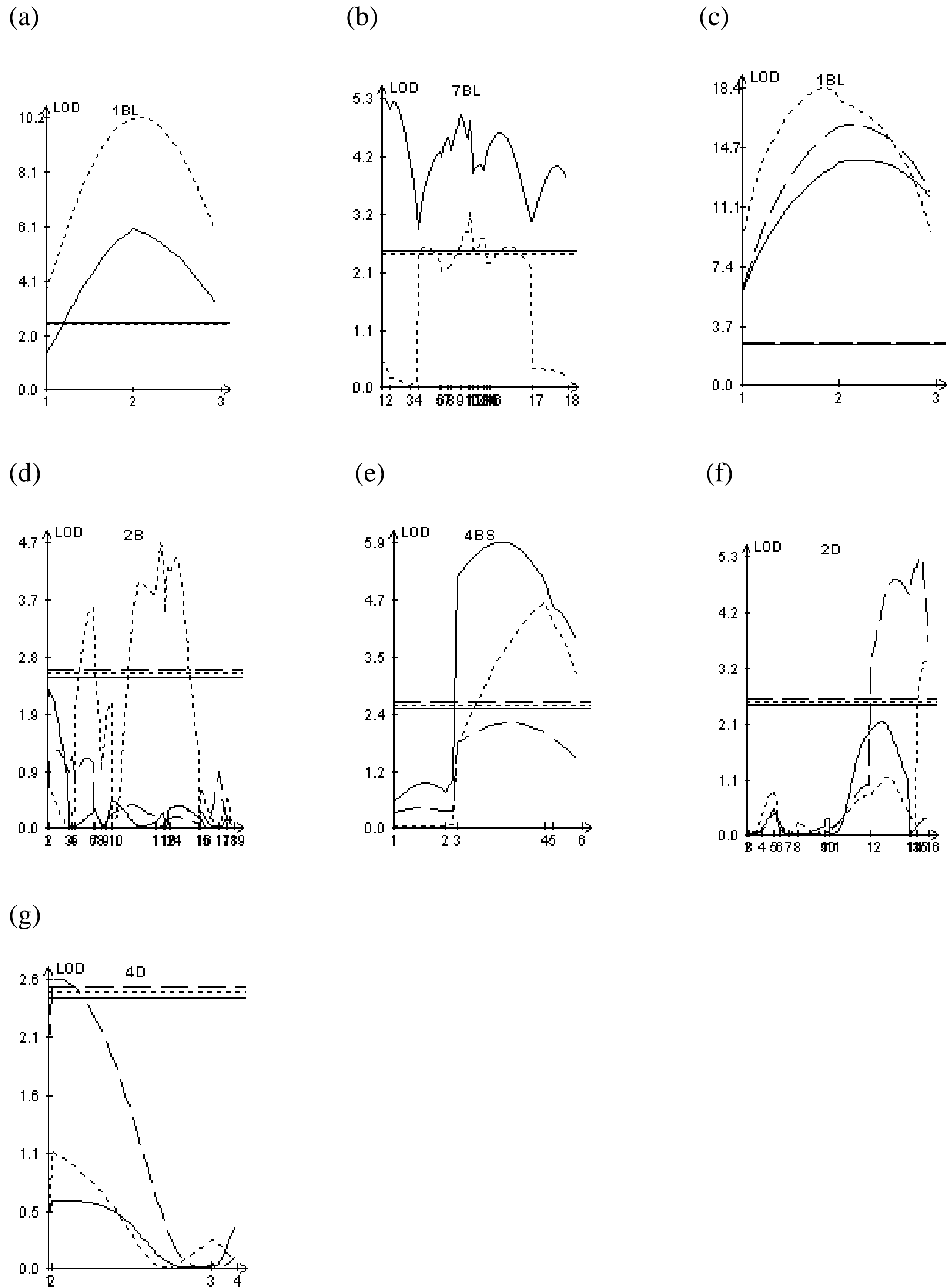
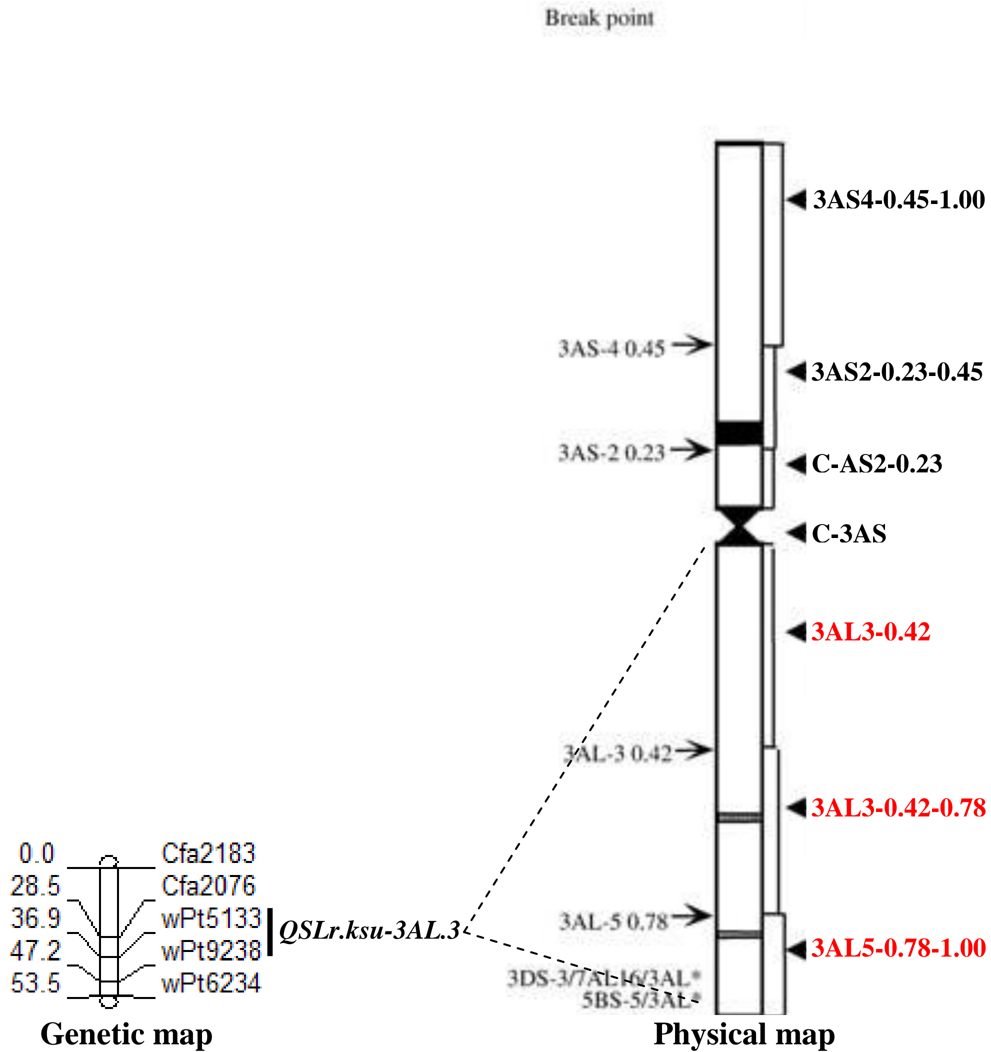


Figure 3.4 Partial Linkage map showing likely genomic location of slow leaf-rusting (*QSLr.ksu-*) and slow stripe-rusting (*QSYr.ksu-*) resistance in chromosome 3A.

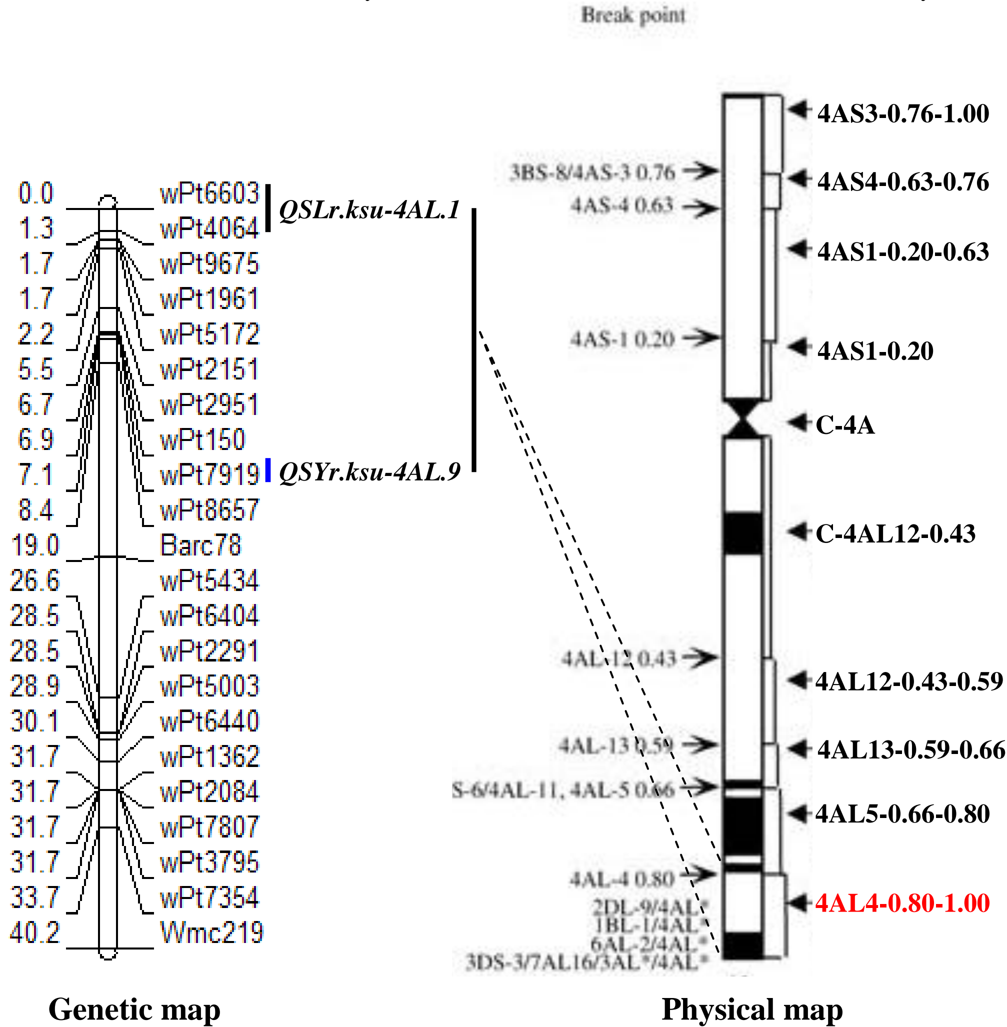


[†]Black bar – multi-environment analysis for leaf rust.

[‡]The locus *QSLr.ksu-3AL.3*. was only detected by the multi-environment analysis of QTLNetwork v2.0. Resistant allele for the QTL was derived from the resistant parent ‘Amadina’.

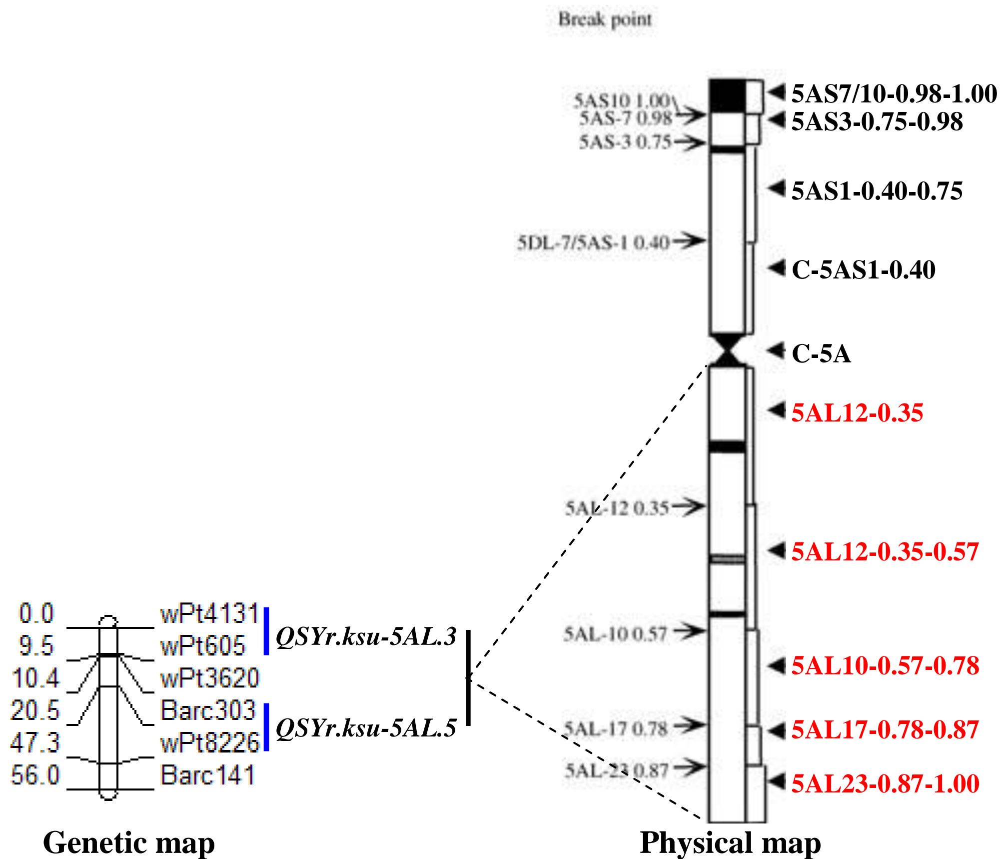
Figure 3.5 Partial Linkage map showing likely genomic locations of slow leaf-rusting (*QSLr.ksu-*) and slow stripe-rusting (*QSYr.ksu-*) resistance in chromosome 4A.

Black bar – multi-environment analysis for leaf rust; Blue bar – multi-environment analysis for stripe rust.



‡The loci, *QSLr.ksu-4AL.1* and *QSYr.ksu-4AL.9*, were both detected on the long arm of chromosome 4A by the more sensitive multi-environment analysis only of QTLNetwork v2.0. Resistance allele for *QSLr.ksu-4AL.1* was derived from the susceptible parent 'Avocet S', and allele for *QSYr.ksu-4AL.9* was derived from the resistant parent 'Amadina'.

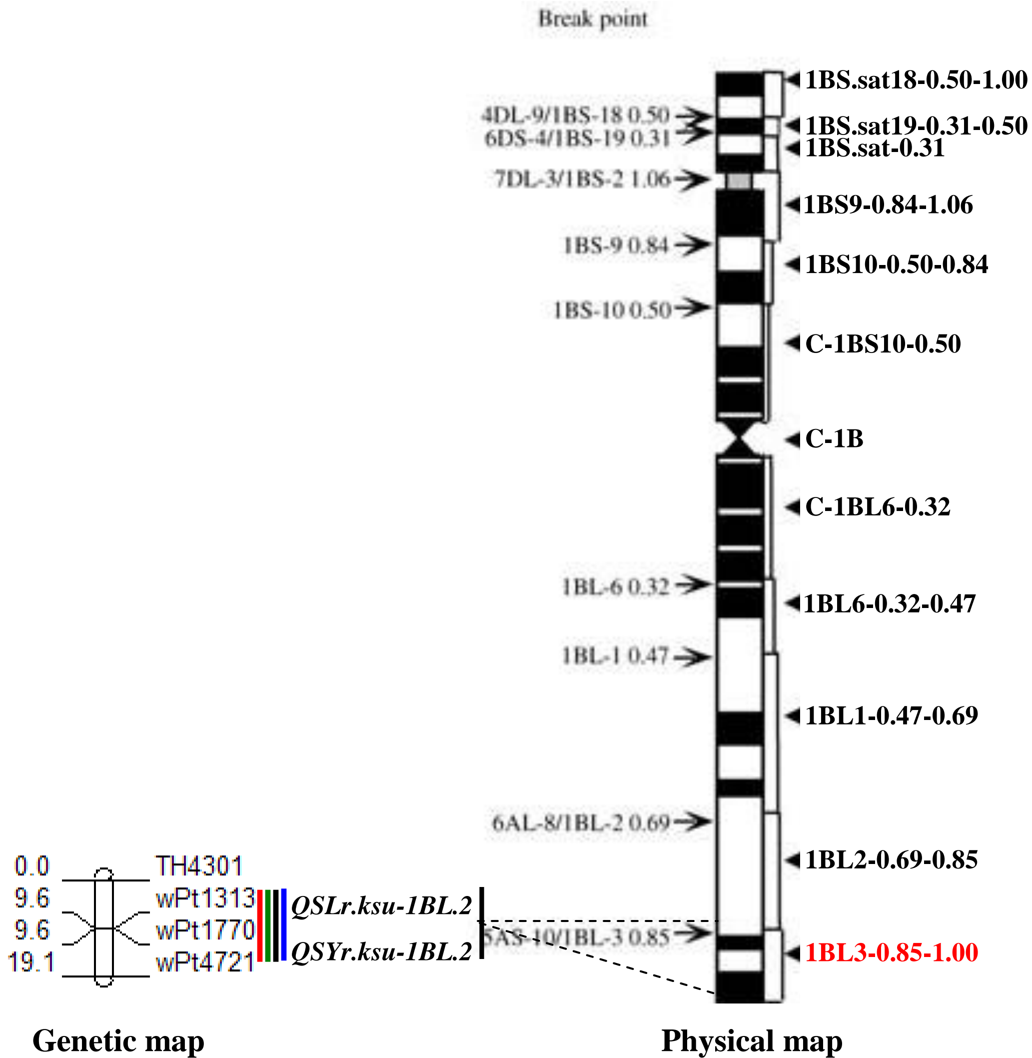
Figure 3.6 Partial Linkage map showing likely genomic locations of slow stripe-rusting (*QSYr.ksu-*) resistance in chromosome 5A.



†Blue bar – multi-environment analysis for stripe rust.

‡Both loci, *QSYr.ksu-5AL.3* and *QSYr.ksu-5AL*, on the long arm of chromosome 5A were detected only through epistatic interaction by the multi-environment analysis of QTLNetwork v2.0. Allele for *QSYr.ksu-5AL.3* was derived from the susceptible parent 'Avocet S', and allele for *QSYr.ksu-5AL.5* was derived from the resistant parent 'Amadina'.

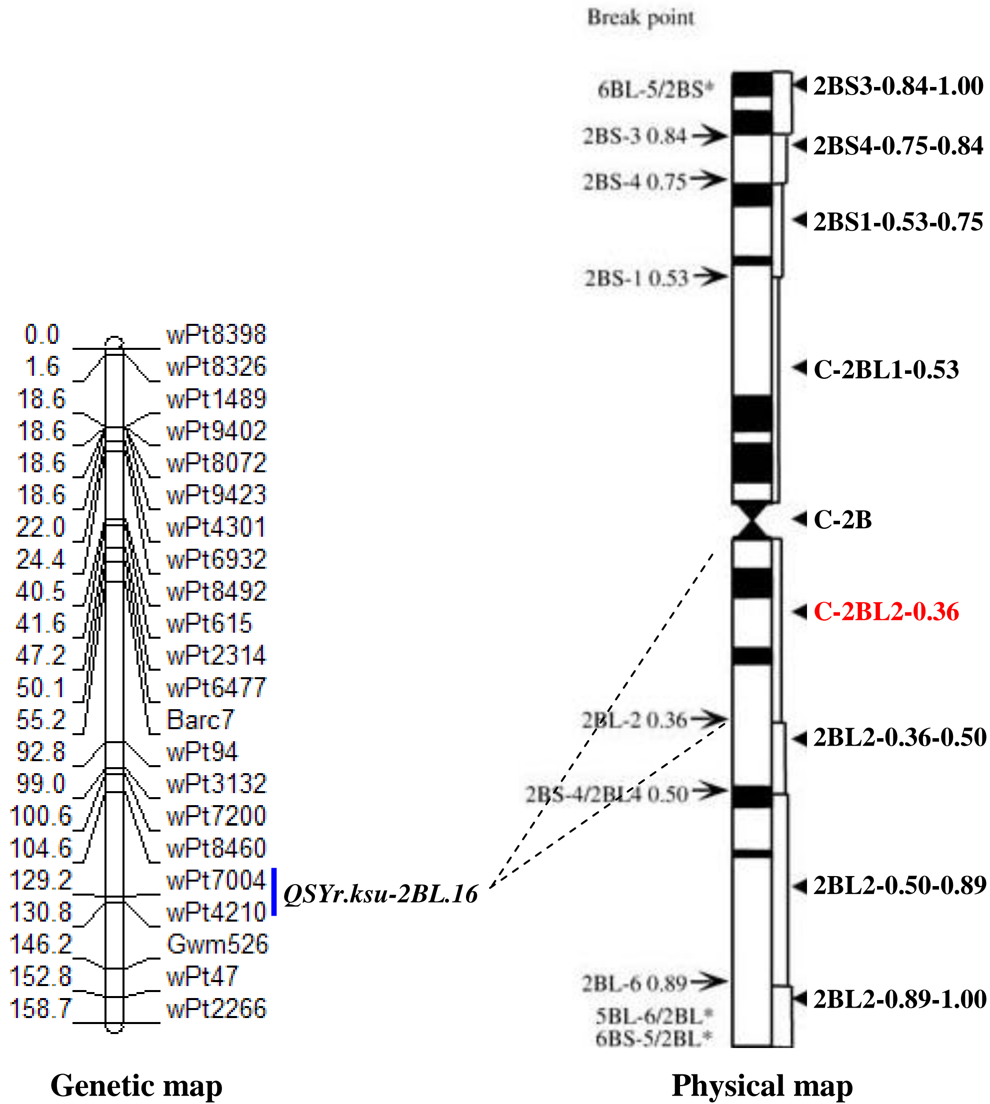
Figure 3.7 Partial Linkage map showing likely genomic location of slow leaf- (*QSLr.ksu-)* and stripe-rusting (*QSYr.ksu*-) resistance in chromosome 1B.



†Black bar – multi-environment analysis for leaf rust; Red bar – single-environment analysis for leaf rust; Blue bar – multi-environment analysis for stripe rust; Green bar – single-environment analysis for stripe rust.

‡Both loci, *QSLr.ksu.1BL.2* and *QSYr.ksu-1BL.2*, were detected by both single and multi-environment analyses of QTLNetwork v2.0. Resistance allele for the QTL was derived from the resistant parent 'Amadina'.

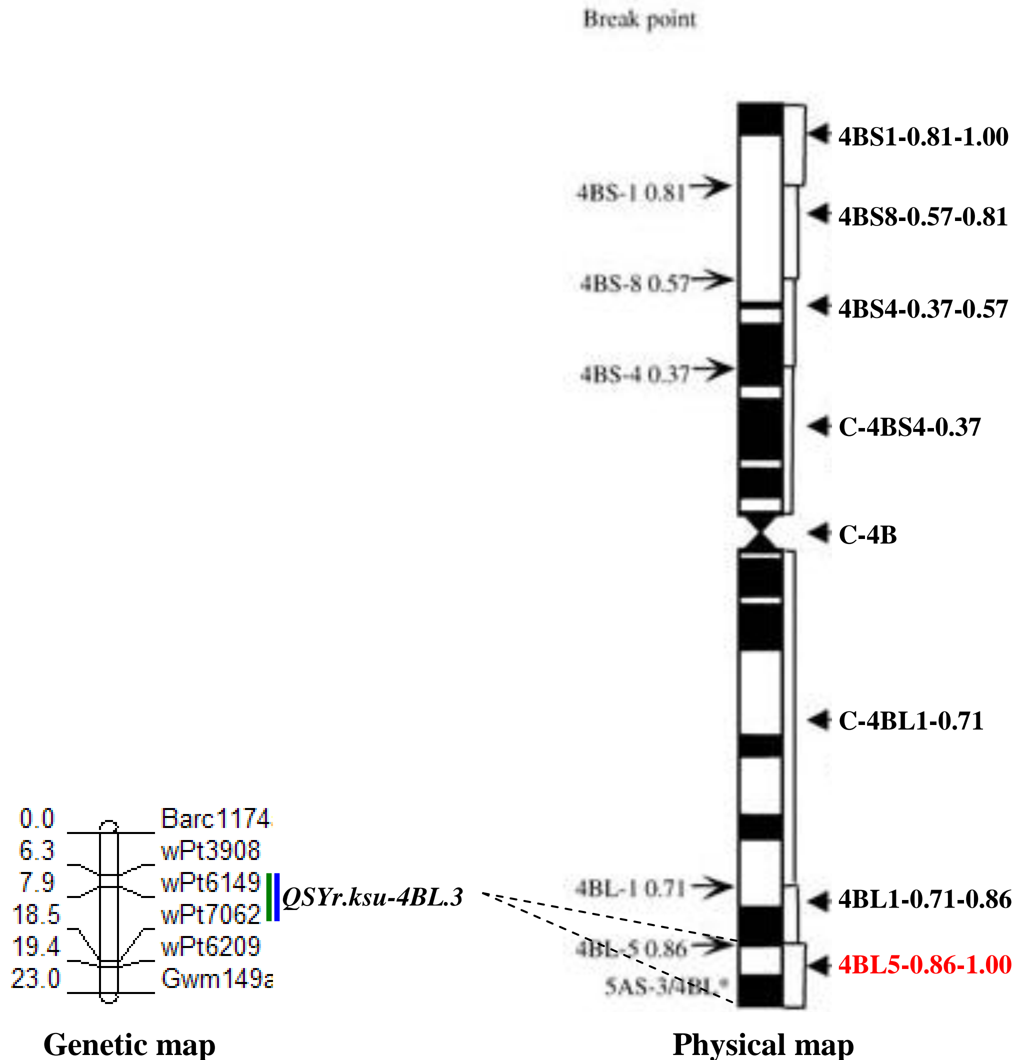
Figure 3.8 Partial Linkage map showing likely genomic location of slow stripe-rusting (*QSYr.ksu-*) resistance in chromosome 2B.



†Blue bar – multi-environment analysis for stripe rust.

‡The locus *QSYr.ksu-2BL.16* was only detected by multi-environment analysis of QTLNetwork v2.0. Resistant allele for the QTL was derived from the susceptible parent 'Avocet S'.

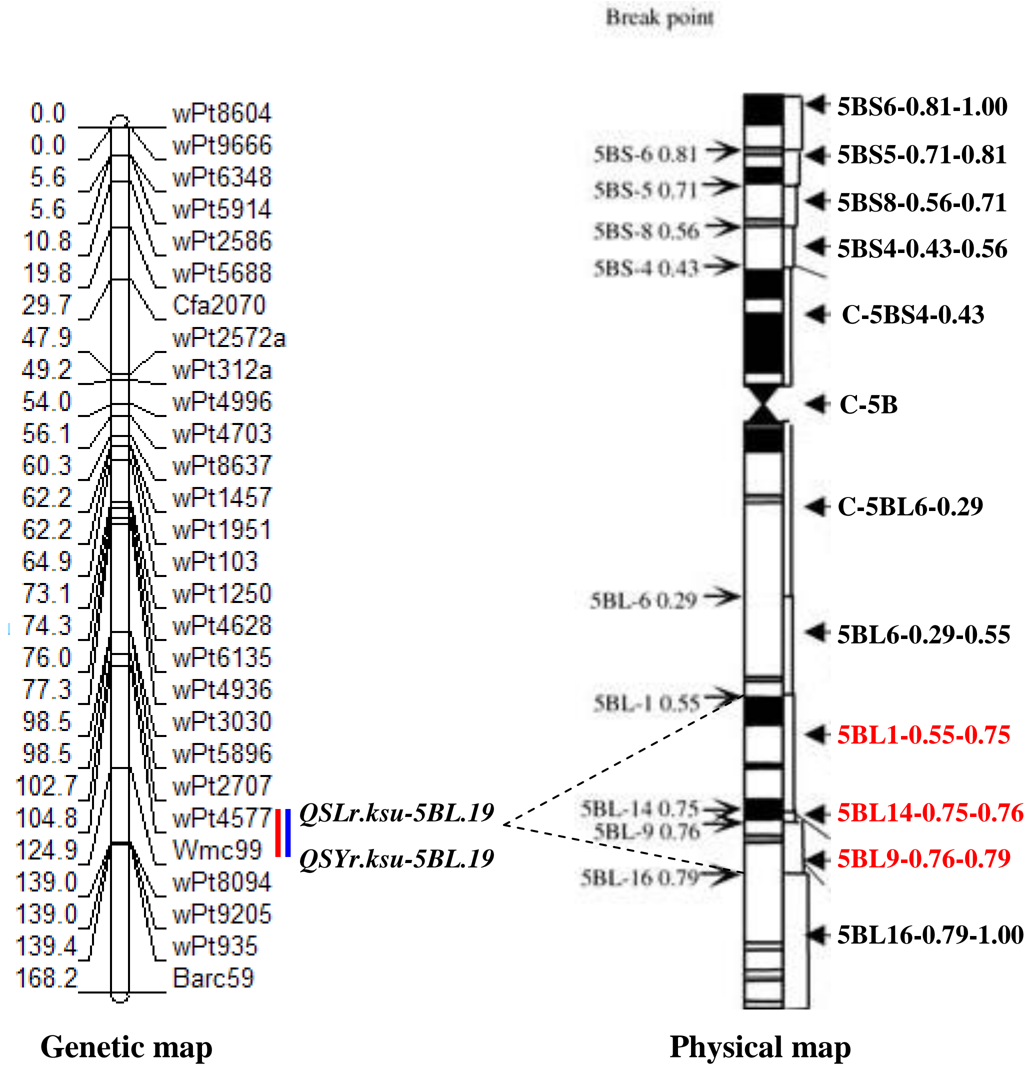
Figure 3.9 Partial Linkage map showing likely genomic location of slow stripe-rusting (*QSYr.ksu*-) resistance in chromosome 4B.



[†]Blue bar – multi-environment analysis for stripe rust; Green bar – single-environment analysis for stripe rust.

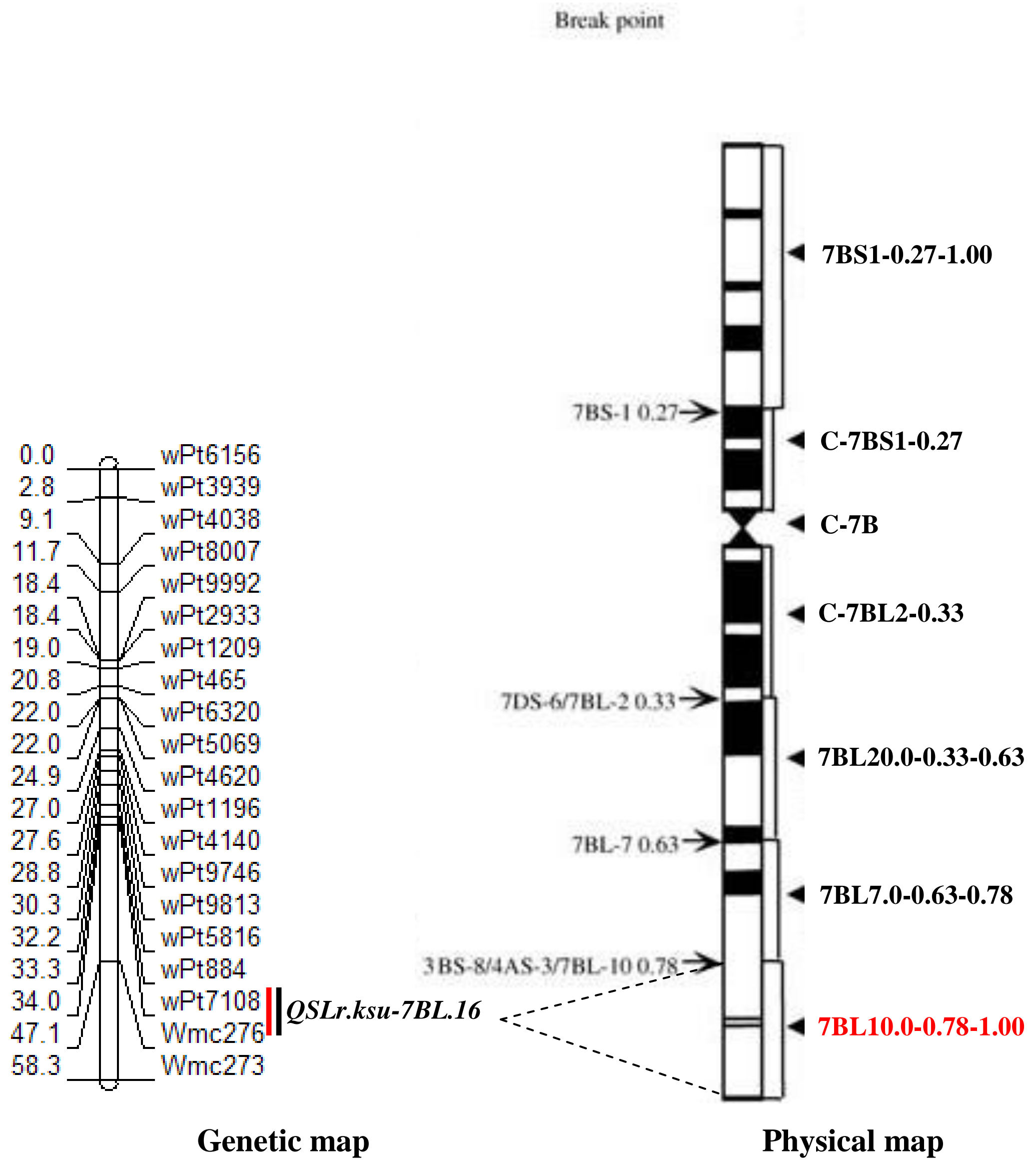
[‡]The locus *QSYr.ksu-4BL.3* was detected by both single and multi-environment analyses of QTLNetwork v2.0. Resistant allele for the QTL was derived from the susceptible parent 'Avocet S'.

Figure 3.10 Partial Linkage map showing likely genomic location of slow leaf- (*QSLr.ksu-*) stripe-rusting (*QSYr.ksu-*) resistance in chromosome 5B.



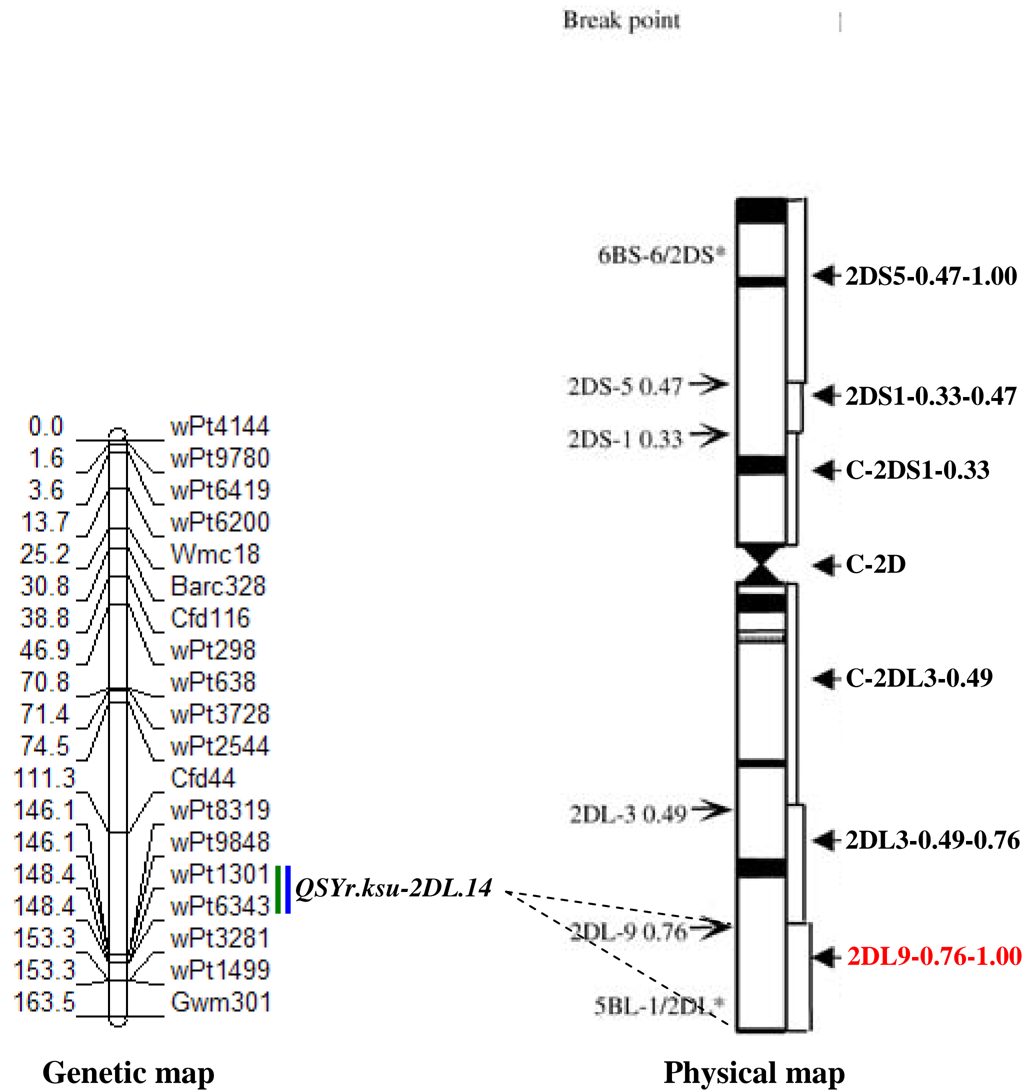
‡The loci, *QSLr.ksu-5BL.19* and *QSYr.ksu-5BL.19*, were only detected by multi-environment analysis of QTLNetwork v2.0. Resistance allele for *QSYr.ksu-5BL.19* was derived from the resistant parent 'Amadina'.

Figure 3.11 Partial Linkage map showing likely genomic location of slow leaf-rusting (*QSLr.ksu-*) resistance in chromosome 7B.



‡The locus *QSLr.ksu-7BL.16* was detected by both single and multi-environment analyses of QTLNetwork v2.0. Resistant allele for the QTL was derived from 'Avocet S'.

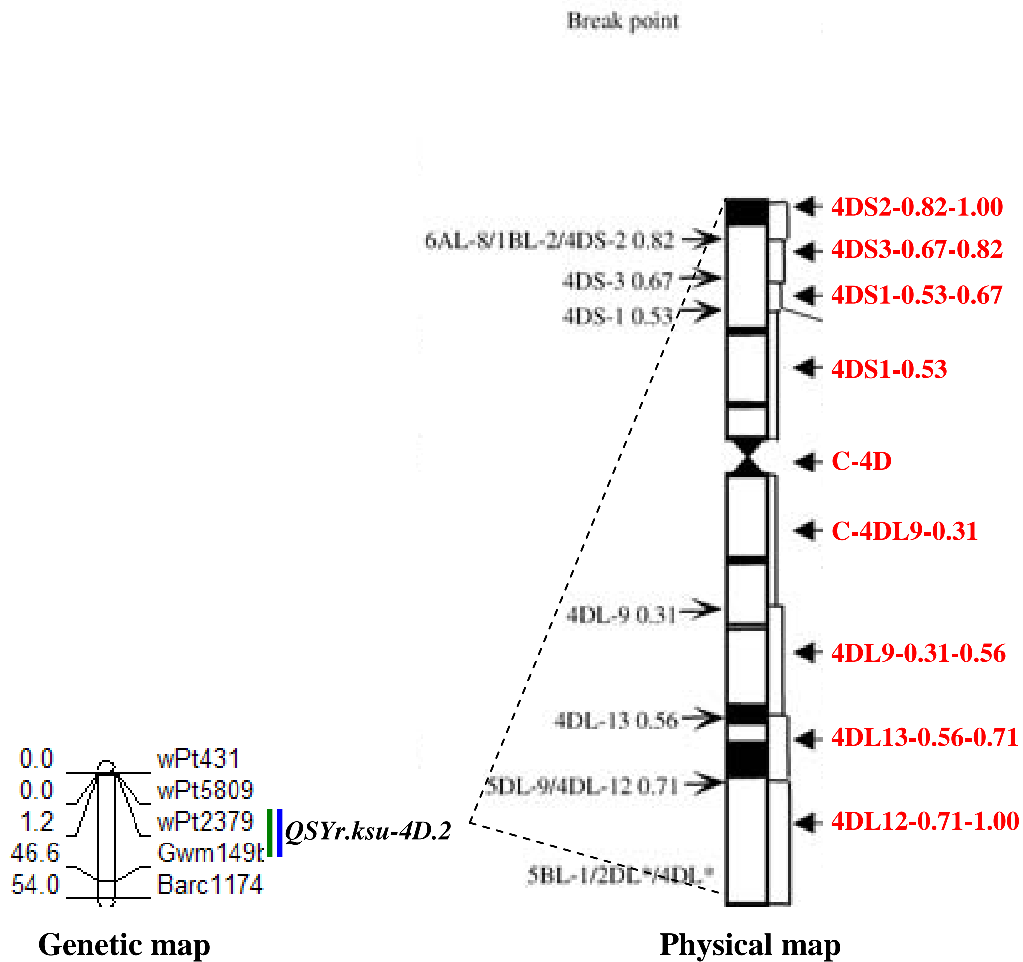
Figure 3.12 Partial Linkage map showing likely genomic location of slow leaf-rusting (*QSLr.ksu-*) resistance in chromosome 2D.



[†]Blue bar – multi-environment analysis for stripe rust; Green bar – single environment analysis for stripe rust.

[‡]The locus *QSYr.ksu-2DL.14* was detected by both single and multi-environment analyses of QTLNetwork v2.0. Resistant allele for the QTL was derived from the resistant parent ‘Amadina’.

Figure 3.13 Partial Linkage map showing likely genomic location of slow stripe-rusting (*QSYr.ksu-*) resistance in chromosome 4D.



†Blue bar – multi-environment analysis for stripe rust; Green bar – single environment analysis for stripe.

‡The locus *QSYr.ksu-4D.2* was detected by both single and multi-environment analyses of QTLNetwork v2.0. The locus could not be assigned to a chromosome arm due to unavailability of sequence information of the DArT markers, *XwPt431*, *XwPt5809*, and *XwPt2379*. Resistant allele for the QTL was derived from the resistant parent 'Amadina'.

Table 3.1 Summary of leaf rust and stripe rust reactions for the 'Avocet S' x 'Amadina' RIL population (% average leaf area covered by rust)

	Leaf rust		Stripe rust		
	2000	2002	2000	2002	2003
'Avocet S'	100	80	90	90	90
'Amadina'	1	0	25	30	30
Population mean	32	18	57	65	59
Range low	1	0	10	30	5
Range high	100	80	100	100	100

‡Scores are given for the parents, population means, and highest and lowest scoring lines in each environment.

Table 3.2 Estimate of genetic (σ^2_G) and genetic x environment ($\sigma^2_{G \times E}$) variances from a random effect model of leaf rust and stripe rust for the 'Avocet S' x 'Amadina' RIL population

Covariance parameters		
	Leaf rust	Stripe rust
σ^2_G	468.59	308.72
[Entry (RILs) Covariance Estimate]		
$\sigma^2_{G x E}$	134.83	71.1967
[Residual Covariance Estimate]		
h^2 [Heritability estimate]	0.87	0.93

Table 3.3 Frequency distribution of 'Avocet S' x 'Amadina' RIL population for leaf rust response categories based on 2 years of field data, and stripe rust response categories based on 3 years of field data.

Disease	Number of RILs with response			X^2 value[a]		
	PTR[b]	PTS[c]	Other[d]	2 genes	3 genes	4 genes
Leaf rust	7	7	134	99.23*	19.64*	1.38[NS]
Stripe rust	11	13	124	59.72*	3.63[NS]	4.52[NS]

* Indicates significant X^2 value at $P = 0.01$; [a] Expected ratios for X^2 tests: 2 genes; [b]Parental-type resistant; [c]Parental-type susceptible; [d]Intermediate; NS = not significant

Table 3.4 Pearson correlation coefficients (*r*) between leaf rust and stripe rust severity data for 2 and 3 years

	Leaf rust		Stripe rust		
Trait/year	2000	2002	2000	2002	2003
Leaf rust 2000	1.00	0.85****	0.49****	0.53****	0.44****
Leaf rust 2002		1.00	0.50****	0.54****	0.47****
Stripe rust 2000			1.00	0.82****	0.81****
Stripe rust 2002				1.00	0.79****
Stripe rust 2003					1.00

NS = Not significant; **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001.

Table 3.5 PCR-based DArT markers and primer sequences for putative QTL associated with resistance to leaf rust

Marker set	Sequence of primers 5'-3'	PCR amplification conditions	Amplified marker fragment size	Chrom location
wPt9675-F wPt9675-R	TAC TCC CTC CGT TTC ACG AT GAT GAG CGC TGT GGT TAT CA	94°C 5 min; 40 cycles (94°C – 1 min; 60°C – 1 min; 72°C – 2 min); 72°C – 10 min	277 bp	---
wPt1313-F wPt1313-R	GTA CTC AGC GGG CTT CAG TG GGC TAG TTT ATG AGG CGG TTT C	94°C 5 min; 40 cycles (94°C – 1 min; 61°C – 1 min; 72°C – 2 min); 72°C – 10 min	206 bp	1BL
wPt3030-F wPt3030-R	CTC CAG CCT GAA CCC ATA AA AGA AGG CTA CCG CAC AAT CA	94°C 5 min; 40 cycles (94°C – 1 min; 61°C – 1 min; 72°C – 2 min); 72°C – 10 min	311 bp	5BS
wPt7108-F wPt7108-R	GTC GTT GGT CAG CAA GTG AA GCA ACA CAC CAT GTT TGG TC	94°C 5 min; 40 cycles (94°C – 1 min; 58°C – 1 min; 72°C – 2 min); 72°C – 10 min	251 bp	7BL

Table 3.6 Single environment QTL analysis for leaf rust reaction in the 'Avocet S' x 'Amadina' RIL population

Flanking interval[‡]	Loc	Leaf rust										
		Year 2000							Year 2002			
		R^2 (%)	A[§]	SE (A)[¶]	H^2(A)[¶]	AA[§]	SE (AA)	H^2(AA)[¶]	R^2 (%)	A[§]	SE (A)[¶]	H^2(A)[¶]
XwPt1313[†]*-XwPt4721*	1BL	26.1****	-12.77****	±2.07	0.2033	7.27*	±3.13	0.0252	36.7****	- 10.3****	-1.39	0.2707
XwPt7108[†]*-Xwmc276*	7BL	14***	-12.52****	±3.08	0.0715				-	-		-

[‡]Flanking interval is the interval of testing point; [†]Marker nearest to the peak of likelihood ratio plot; [§]A is the additive effect in the testing point; [¶]H^2(A) represents the phenotypic variation explained by A; [§]AA is the additive x additive epistatic interaction; [¶]H^2(AA) represents the phenotypic variation explained by AA; - not detected; NS = not significant; *P<0.05; **P<0.01; ***P<0.001, ****P<0.0001. The resistance alleles on 1BL (*Lr46* locus) was derived from the resistant parent 'Amadina', and resistance allele on 7BL was derived from the susceptible parent 'Avocet S'. Digenic epistatic interaction (AA) was detected between loci on 1BL (*Lr46* locus) and 7BL in environment 1 (2000).

Table 3.7 Estimated additive (A) and additive x environment interactions (AE) of QTL detected by the mixed linear-model approach for slow leaf-rusting resistance in the population derived from 'Avocet S' x 'Amadina'

QTL effect						QTL heritability
QTL name	Flanking interval[‡]	A[§]	SE (A[§])	AE_1[§]	AE_2[§]	H^2(A)[¶]
QSLr.ksu-3AL.3	*XwPt5133-XwPt9238*	-5.13****	±1.31	NS	NS	0.0235
QSLr.ksu-4AL.1	*XwPt6603-XwPt4064*	-3.47**	±1.15	NS	NS	0.0068
QSLr.ksu-1BL.2	*XwPt1313-XwPt4721*	-11.13****	±1.29	NS	NS	0.1948
QSLr.ksu-5BL.19	*XwPt4577-Xwmc99*	-20.73****	±4.44	NS	NS	0.0800
QSLr.ksu-7BL.16	*XwPt7108-Xwmc276*	-11.36****	±1.69	NS	NS	0.0510

[‡]Flanking interval is the interval of testing point; [§]A is the additive effect in the testing point; [§]AE_1 and [§]AE_2 represent QTL x environment interaction effects for environments 1 (year 2000) and 2 (2002) respectively; [¶]H^2(A) represents the phenotypic variation explained by A; NS - not significant, *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. Resistance alleles on 3AL, 1BL (*Lr46* locus), and 5BL were derived from the resistant parent 'Amadina', and resistance allele on 4AL and 7BL were derived from the susceptible parent 'Avocet S'.

Table 3.8 Estimated additive x additive epistatic (AA) and additive x additive epistasis x environment interaction (AAE) effects of QTL detected for slow leaf-rusting resistance using data of two environments

Epistasis effect							Epistasis heritability	
QTL-i[††]	Flanking interval-i[‡]	QTL-j[††]	Flanking interval-j[‡]	AA[§] score	AAE_1[§]	AAE_2[§]	H^2(AA)[¶]	H^2(AAE)[¶]
QSLr.ksu-1BL.2	*XwPt1313-XwPt4721*	*QSLr.ksu-7BL.16*	*XwPt7108-Xwmc276*	6.87***	0.0001*	NS	0.0221	0.0019

[††]QTL-i and QTL-j are the QTL of testing points i and j respectively; [‡]Flanking interval-i and Flanking interval-j are the intervals of testing points i and j; [§]AA is the additive-by-additive interactions between testing points i and j, respectively; AAE_1 and AAE_2 is the additive x additive epistasis x environments 1 (year 2000) and 2 (year 2002), respectively; [¶]H^2(AA) represents the phenotypic variation explained by AA; [¶]H^2AAE represents the phenotypic variation explained by AAE; NS = not significant; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. Resistance allele for *QSLr.ksu-1BL.2* was derived from the resistant parent 'Amadina', and resistance allele for *QSLr.ksu-7BL.16* was derived from the susceptible parent 'Avocet S'.

Table 3.9 Pearson correlation coefficients for presence of alleles between flanking markers for two putatively epistatic loci affecting leaf rust reaction in the 'Avocet S' x 'Amadina' RIL population

	XwPt1313-1BL.2	*XwPt4721-1BL.3*	*XwPt7108-7BL.16*	*Xwmc276-7BL.17*
XwPt1313-1BL.2	1.00	-7.00****	-0.01^{NS}	-0.04^{NS}
XwPt4721-1BL.3		1.00	-0.17^{NS}	0.18*
XwPt7108-7BL.16			1.00	-0.63****
Xwmc276-7BL17				1.00

NS = not significant; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. Resistance allele for QTL on 1BL was contributed by the resistant parent 'Amadina'. Resistance allele for QTL on 7BL was contributed by susceptible parent 'Avocet S'.

Table 3.10 PCR-based DArT markers and primer sequences for putative QTL associated with resistance to stripe rust

Marker set	Sequence of primers 5'-3'	PCR amplification conditions	Amplified marker fragment size	Chrom location
wPt150-F wPt150-R	TTA CAA CTG AAG GAC GCC AGT C TGT TCA CCT GGA GGA AGT TG	94°C 5 min; 40 cycles (94°C – 1 min; 60°C – 1 min; 72°C – 2 min); 72°C – 10 min	326 bp	4AS
wPt4131-F wPt4131-R	GTG CAA ACG AGA TGC CAG TA AGG ACC GTG CAA AAG AGC TA	94°C 5 min; 40 cycles (94°C – 1 min; 60°C – 1 min; 72°C – 2 min); 72°C – 10 min	240 bp	5AL
wPt1313-F wPt1313-R	GTA CTC AGC GGG CTT CAG TG GGC TAG TTT ATG AGG CGG TTT C	94°C 5 min; 40 cycles (94°C – 1 min; 61°C – 1 min; 72°C – 2 min); 72°C – 10 min	206 bp	1BL
wPt7062-F wPt7062-R	AGC CAC ATT GAG AAG GAG GA AAT ACA CCG GAG AAG CCA TC	94°C 5 min; 40 cycles (94°C – 1 min; 57°C – 1 min; 72°C – 2 min); 72°C – 10 min	281 bp	4BS
wPt6209-F wPt6209-R	GCT TGC ACT CGC AGC TAA T CTA CTC CCT TCG TCC CAC AA	94°C 5 min; 40 cycles (94°C – 1 min; 60°C – 1 min; 72°C – 2 min); 72°C – 10 min	260 bp	---
wPt3030-F wPt3030-R	CTC CAG CCT GAA CCC ATA AA AGA AGG CTA CCG CAC AAT CA	94°C 5 min; 40 cycles (94°C – 1 min; 61°C – 1 min; 72°C – 2 min); 72°C – 10 min	311 bp	5BS
wPt6343-F wPt6343-R	AGC AGG CAC CGT CTG ATT T ACA TGG TTG GGA AGG AAG G	94°C 5 min; 40 cycles (94°C – 1 min; 60°C – 1 min; 72°C – 2 min); 72°C – 10 min	234	2DS

Table 3.11 Single environment QTL analysis for stripe rust reaction in the 'Avocet S' x 'Amadina' RIL population

Flanking interval[‡]	Loc	Stripe rust										
		Year 2000			Year 2002			Year 2003				
		R^2 (%)	A[§]	H^2(A)[¶]	R^2 (%)	A[§]	H^2(A)[¶]	R^2 (%)	A[§]	H^2(A)[¶]	AA[§]	H^2(AA)[¶]
XwPt1313[†]-*XwPt4721*	1BL	34.5****	-12.58****	0.2542	33.8****	-10.27****	0.2345	32.1****	-11.22****	0.2422	-12.75*	0.0175
XwPt6149[†]-*XwPt7062*	4BL	8.0*	-8.88****	0.1204	-	-		-	-	-		
XwPt7062[†]-*XwPt6209*	4BL	9.1*	-	-	-	-7.45****	0.1145	-	-	-		
XwPt1301[†]-*XwPt3281*	2DL	3.4[NS]	-	-	-	-		-	-22.12****	0.0651		
XwPt2379[†]-*Xgwm149b*	4D	6.6*	-	-	-	-		-	11.06****	0.0787		

[‡]Flanking interval is the interval of testing point; [†]Marker nearest to the peak of likelihood ratio plot; [§]A is the additive effect in the testing point; [¶]H^2(A) represents the phenotypic variation explained by A; [§]AA is the additive x additive epistatic interaction; H^2(AA)[¶] represents the phenotypic variation explained by AA; - not detected, NS = not significant; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; [†]likely same allele. The resistance alleles on 1BL (*Yr29* locus), 2DL, and 4D were derived from the resistant parent 'Amadina', and resistance allele on 4BL was derived from the susceptible parent 'Avocet. Digenic epistatic interaction (AA) was detected between loci on 1BL (*Yr29* locus) and 2DL in environment 3 (2003).

Table 3.12 Estimated additive (A) and additive x environment interactions (AE) of QTL detected by the mixed linear-model approach for slow stripe-rusting resistance in the population derived from 'Avocet S' x 'Amadina'

QTL effect						QTL heritability
QTL name	Flanking interval[‡]	A[§]	AE_1[§]	AE_2[§]	AE_3[§]	H^2(A)[¶]
QSYr.ksu-4AS.9	*XwPt7919-XwPt8657*	-	-	-	-	0.0178
QSYr.ksu-1BL.2	*XwPt1313-XwPt4721*	-10.65****	NS	NS	NS	0.2322
QSYr.ksu-2BL.16	*XwPt4210-Xgwm526*	-3.81****	NS	NS	NS	0.0461
QSYr.ksu-4BL.3	*XwPt6149-XwPt7062*	-5.35****	NS	NS	NS	0.1113
QSYr.ksu-5BL.19	*XwPt4577-Xwmc99*	-10.93****	NS	NS	NS	0.0793
QSYr.ksu-2DL.14	*XwPt1301-XwPt3281*	-10.30****	NS	NS	NS	0.0200
QSYr.ksu-4D.2	*XwPt2379-Xgwm149b*	-3.32****	NS	NS	NS	0.0418

[‡]Flanking interval is the interval of testing point; [§]A is the additive effect in the testing point; [§]AE_1, [§]AE_2, and AE_3 represent QTL x environment interaction effects for environments 1 (year 2000), 2 (2002), and 3 (2003); [¶]H^2(A) represents the phenotypic variation explained by A; NS - not significant; - not detected; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. Alleles on 4AL, 1BL (*Yr29* locus), 5BL, 2DL and 4D were derived from the resistant parent 'Amadina.' and alleles on 2BL and 4BL were derived from the resistant susceptible parent 'Amadina'.

Table 3.13 Estimated additive x additive epistatic (AA) and additive x additive epistasis x environment interaction (AAE) effects of QTL detected for slow stripe-rusting resistance using data of three environments

Epistasis effect								Epistasis heritability	
QTL-i[††]	Flanking interval-i[‡]	QTL-j[††]	Flanking interval-j[‡]	AA[§]	AAE_1[§]	AAE_2[§]	AAE_3[§]	H^2(AA)[¶]	H^2(AAE)[¶]
QSYr.ksu-2BL.16	*XwPt4210-Xgwm526*	*QSYr.ksu-4D.2*	*XwPt2379-Xgwm149b*	2.09**	NS	NS	NS	0.0108	0.0011
QSYr.ksu-5AL.3	*XwPt3620-Xbarc303*	*QSYr.ksu-5AL.5*	*XwPt8226-Xbarc141*	4.11***	NS	NS	NS	0.0289	0.0008

[††]QTL-i and QTL-j are the QTL of testing points i and j respectively; [‡]Flanking interval-i and Flanking interval-j are the intervals of testing points i and j; [§]AA is the additive-by-additive interactions between testing points i and j, respectively; [§]AAE_1, [§]AAE_2 and [§]AAE_3 are the additive x additive epistasis x environments 1 (year 2000), 2 (year 2002), and 3 (2003; [¶]H^2(AA) represents the phenotypic variation explained by AA; [¶]H^2AAE represents the phenotypic variation explained by AAE; NS = not significant; *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$. Alleles for *QSYr.ksu-2BL.16* and *QSYr.ksu5AL.3* were derived from the susceptible parent 'Avocet S', and alleles for *QSYr.ksu-4D.2* and *QSYr.ksu-5AL.5* were derived from the resistant parent 'Amadina'.

Table 3.14 Pearson correlation coefficients for presence of alleles between flanking markers for two putatively epistatic loci affecting stripe rust reaction in the 'Avocet S' x 'Amadina' RIL population

	XwPt4210-2BS	*Xgwm526-2BS*	*XwPt2379-4D.3*	*Xgwm149b-4D.4*	*XwPt3620-5AL.3*	*Xbarc303-5AL.4*	*XwPt8226-5AL.5*	*Xbarc141-5AL.6*
XwPt4210-2BL.19	1.00	0.56****	-0.10^{NS}	0.19*	-0.02^{NS}	0.05^{NS}	0.15^{NS}	0.27**
Xgwm526-2BL.20		1.00	0.02^{NS}	0.06^{NS}	0.05^{NS}	0.02^{NS}	0.20^{NS}	0.14^{NS}
XwPt2379-4D.3			1.00	-0.30***	0.08^{NS}	-0.17^{NS}	-0.05^{NS}	-0.03^{NS}
Xgwm149b-4D.4				1.00	-0.03^{NS}	-0.01^{NS}	0.04^{NS}	0.01^{NS}
XwPt3620-5AL.3					1.00	-0.72****	-0.39****	-0.40****
Xbarc303-5AL.4						1.00	0.44****	0.44****
XwPt8226-5AL.5							1.00	0.73****
Xbarc141-5AL.6								1.00

NS = not significant; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. Alleles for QTL on 2BL and 5AL.3 were derived from susceptible parent 'Avocet', and alleles for QTL on 4D and 4AL.5 were derived from the resistant parent 'Amadina'.

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CHAPTER 4 - Genetic characterization of slow leaf-rusting and its components in the CIMMYT wheat 'Amadina'

Abstract

Slow leaf-rusting and its components, latent period and infection frequency, were studied in a population of 148 recombinant inbred lines (RILs) derived from the cross between the leaf rust susceptible cultivar 'Avocet S' and the slow leaf-rusting resistant cultivar 'Amadina'. The RILs and the two parents were inoculated at the flowering stage with the TPBS isolate of the wheat leaf rust pathogen. Transgressive segregations for latent period (LP), infection frequency (IF), and final disease severity (FS) were observed in the population, indicating predominant additive genetic control of slow leaf-rusting resistance. Heritabilities (h^2) were approximately 95.3, 91.8 and 98.7% for FS, LP and IF. It was estimated that at least five genes conditioning FS and LP, and four genes conditioning IF, segregated in the cross 'Avocet S' x 'Amadina'. Correlations between LP and FS and LP and IF were moderately negative, and that between IF and FS was moderately positive. Two QTL on chromosomes 1BL and 6BL, denoted *QLp.ksu-1BL.2* and *QLp.ksu-6BL.7*, were associated with LP, three QTL on chromosomes 1BL, 6BL and 2DS, denoted *QIf.ksu-1BL.2*, *QIf.ksu-6BL.15*, and *QIf.ksu-2DS.3*, were associated with IF, and two QTL on chromosomes 1BL and 6BL, denoted QFs.ksu-1BL.1 and QFs.ksu-6BL.10, were associated with FS. *QLp.ksu-1BL.2*, *QIf.ksu-1BL.2*, and *QFs.ksu-1BL.1* correspond to the *Lr46/Yr29* locus. Digenic epistatic interactions with dominance x dominance effects were also detected for the IF trait.

Introduction

Monogenic hypersensitive resistance has been a major weapon used by wheat breeders to control the leaf rust (*Puccinia triticina*) pathogen. However, the generally race-specific and hence non-durable nature of monogenic hypersensitive resistance shifted focus of wheat breeders to the race-non-specific slow-rusting type of resistance (Singh and Rajaram 1992; Singh et al. 2000a) in which the infection is not completely stopped but the spread of the disease is delayed. In general, slow leaf-rusting resistance is characterized by the combined effect of a prolonged latent period (time from inoculation

to sporulation of the resulting pustule), reduced infection frequency (number of pustules per unit area), and reduced pustule size (reduced infection size) (Ohm and Shaner 1976; Kolmer 1996). Latent period in wheat is negatively associated with the area under disease progress curve (AUDPC), final rust severity, uredinium size, and receptivity (Das et al. 1993; Herrera-Foessel et al. 2007a), whereas uredinium size is positively correlated with AUDPC and final disease severity in durum wheats (Herrera-Foessel et al. 2007a).

Several genetic studies have been performed in order to determine the inheritance of slow leaf-rusting in wheat (Das et al. 1992; Singh et al. 2004; Zhang et al. 2008). In most of these studies transgressive segregation for slow leaf-rusting resistance was found as well as predominantly additive genetic action and, to some extent, additive x additive genetic variance. One to three genes with predominantly additive gene action and with or without significant epistatic effects were implicated in the inheritance of slow leaf-rusting across several cultivars (Das et al. 1992; Singh et al. 2004; Zhang et al. 2008). Several authors have identified latent period as the most important component that explains most of the difference in slow rusting resistance (Parlevliet and Ommeren 1975; Ohm and Shaner 1976). Its heritability is moderately high and has varied from 0.46 to 0.90 (Lee and Shaner 1985a; Bjarko 1988; Jacobs and Broers 1989; Das et al. 1992). According to a number of studies, at least three to five genes with unequal and epistatic effects are involved in the control of latent period (Broers and Jacobs 1989; Shaner et al. 1997; VanderGaag and Jacobs 1997). Although slow leaf-rusting resistance can be measured in the field by recording disease severity at weekly intervals and then calculating AUDPC, accurate assessment of slow-rusting resistance in the field can be subject to large experimental error, because the phenotype is subtle and can be affected by environmental factors such as field heterogeneity, other pathogens or pests, as well as developmental stage of the crop. On the other hand, measurement of single components of slow-rusting resistance under a controlled environment involves much smaller experimental error values.

Characterization and dissection of slow leaf-rusting resistance into Mendelian factors of inheritance, and establishment of the genomic locations of such genetic loci through DNA markers are vital to slow leaf-rusting resistance breeding. Although several genomic regions have been related to slow-rusting resistance in various studies, our

understanding of the genetic basis of slow leaf-rusting resistance is still limited. The labor-intensive and complicated nature of phenotypic measurements of the components of slow leaf-rusting render QTL mapping approaches difficult if not impractical. To date latent period is the only component that has been dissected at the molecular level. Xu et al. (2005a) identified three QTL on chromosomes 2DS (*QLrlp.osu-2DS*), 2B (*QLrlp.osu-2B*) and 7BL (*QLrlp.osu-7BL*) using a recombinant inbred line (RIL) population developed from the cross CI 13227 (prolonged latent period) x Suwon 92 (short latent period). However, the marker-based analysis employed by Xu et al. (2005a) was based on the assumption of absence of epistasis among QTL which may result in biased estimates of the positions and effects of QTL and hence a lower precision and power for QTL detection (Wang et al. 1999). Epistasis has been revealed as one of the important genetic bases of complex quantitative traits such as slow leaf-rusting (Das et al. 1992; Rosewarne et al. 2008). Use of a methodology that directly maps QTL with additive and epistatic effects as well as additive x environment interaction is required for a better understanding of the genetic basis of the slow leaf-rusting trait, and its components. Therefore, this study was designed to increase our knowledge on slow leaf-rusting in bread wheat. Slow leaf-rusting resistance components – latent period, infection frequency and pustule size were investigated in a recombinant inbred line (RIL) population developed from a cross involving the slow leaf-rusting resistant CIMMYT spring wheat breeding line ‘Amadina’ and the leaf rust susceptible spring wheat ‘Avocet S’, and the relationships among the slow rusting components and leaf rust severity were studied. QTL conditioning the components of slow leaf-rusting resistance trait for main effects, epistatic effects, and QTL x environment interactions were characterized, and the relative contributions of these genetic components in controlling the expression of the slow leaf-rusting resistance components were compared. In addition, by estimating the heritability of these components, it is possible to account for genetic and non-genetic factors influencing slow leaf-rusting. The ultimate goal of the QTL analysis is to develop tools that are useful for marker-assisted selection (MAS) in practical breeding programs targeting enhanced slow leaf-rusting resistance.

Materials and methods

Plant material

The population was comprised of 148 recombinant inbred lines (RILs) derived from the cross of the leaf and stripe rust-susceptible spring wheat 'Avocet S' (WW-119/WW-15//EGRET) with the slow leaf-rusting resistant spring wheat 'Amadina '(BOW/CROW//BUC/PVN/3/VEE#10). The RIL population was developed at the International Center for Maize and Wheat Improvement (CIMMYT).

Greenhouse studies

Two of a greenhouse experiments were conducted at The Plant Sciences Center, Kansas State University, Manhattan, KS using a completely randomized design (CRD) with two replications. Each replication contained two pots each with three plants from each F_7 RIL. Experimental units were plants grown in two pots in pasteurized silt-loam/peat/coarse perlite soil mix (12:55:3, v/v/v) with 2% (w/w) gypsum. Pots were watered twice a day to maintain soil moisture near field capacity. Greenhouse temperatures were set at 19° C (night-time) and 23 ^{o}C (day-time), respectively. Day light was supplemented with light from High Pressure Sodium (HPS) 400 W bulbs (emitting 5000 lx) fitted with reflectors for 16 h. Relative humidity varied from approximately 70% during the day to 90% at night. Pots were fertilized at planting with 2 g/pot 14–14–14 Osmocote (The Scotts Company, Marysville, OH), 1 g/pot 0-46-0 phosphorus, and 2 g/pot 13–13–13 NPK, 2 g/pot gypsum, and micronutrients. For pest and disease control, granular Marathon 1% (OHP, Mainland, PA) was used for aphids, Malathion (Ortho, Marysville, OH) for thrips, and micro fine dusting sulfur (Georgia Gulf Sulfur, GA) for powdery mildew.

Plants with most secondary tillers at a similar stage of anthesis were uniformly and heavily inoculated with urediospores of the *P. triticina* isolate TKLSQsuspended in non-phytotoxic isoparafinic light oil (Soltrol 170) (Chevron Phillips Chemical Company, The Woodlands, TX). The TKLSQ isolate has the virulence/avirulence formula: *Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr26, Lr3ka, LrB, Lr10, Lr14a, Lr21, Lr28, Lr20, Lr23*/*Lr11, Lr17, Lr30, Lr18, Lr39, Lr42, Lr52, Lr19*. Inoculation was done at 6:00 P.M. in the evening and the inoculated plants were then incubated overnight in a mist chamber in a greenhouse at

20ºC ±3ºC with 100% relative humidity for 14 h. After the incubation, the plants were transferred back to the greenhouse.

Before first symptoms appeared on the inoculated leaves, a 1 cm^2 area in the middle of a single flag leaf of each four plants representing each F_7 RIL was marked. Clearly visible pustules were counted each day in the marked area until pustule increase stopped. From these data, latent period for each inoculated plant was calculated as LP_{50} (days to the appearance of 50% of the pustule) using the formula:

Latent Period (LP) = $[t_1 + (F/2 - nt_1)(t_2 - t_1)] / (nt_2 - nt_1)$

where F = final number of pustules, t_1 = day prior to 50% pustules observed, t_2 = day after 50% pustules observed, nt_1 = number of pustules observed at t_1, and nt_2 = number of pustules observed at t_2 (Das *et al*., 1993).

Infection frequency (IF) (number of pustules/cm^2) was calculated by dividing the number of pustules by area of the flag leaf where pustules were counted. LP and IF were estimated by averaging the values of the four flag leaves per pot. Final leaf rust severity (FS) in the form of percentage of infected leaf area, based on visual estimate scale was recorded on flag leaves 15 days after inoculation.

DNA extraction

Genomic DNA was extracted using a modified CTAB method (Murray and Thompson 1980). For each 150-200 mg sample in a 2 ml centrifuge tube, 1000 µl 2 X CTAB extraction buffer containing 2% 2-Mercaptoethanol (Sigma, St. Louis, MO) and 100 µg/ml proteinase K (Roche Diagnostics, USA) was added. Tubes were incubated for 60 min at 60 ^oC in a water bath, followed by 10 min cooling at room temperature. Chloroform:isoamyl alcohol (24:1) extraction was performed twice. DNA in aqueous phase was precipitated by adding approximately two-third volume of isopropanol and placing the tubes at -20 ^oC overnight. RNA was removed from DNA re-dissolved in 500 µl 1 X TE pH 8.0 by RNAse A (Roche Diagnostics, USA) treatment at 37 ^oC for 30 min. To extract the DNA, chloroform:isoamyl alcohol (24:1) extraction was performed twice. Intact high quality genomic DNA was precipitated using half volume of 7.5 M ammonium acetate (Sigma-Aldrich, Steinheim, Germany) and 2 volumes of 100% ethanol (Pharmco, CT).

SSR (microstaellite) assay

The two parents were screened with a total of 1600 SSRs (SSRs) for polymorphism. These markers consisted primarily of Gatersleben Wheat SSRs (GWM) and markers from Wheat SSR Consortium (WMC), INRA Clermont-Ferrand (CFA and CFD), Beltsville Agriculture Research Center (BARC), and Kansas State University (Ksum). For each polymerase chain reaction (PCR) reaction, 100 ng genomic DNA was used in a 25 µL solution containing 250 µM of each dNTP, 1 X PCR buffer, 0.4 pmol of each primer with 2.5 mM $MgCl_2$ and approximately 0.1 U *Taq* polymerase. An MJ Research PTC-200 thermal cycler (Watertown, MA) was programmed as follows for Xbarc: ‘hot start’ 95 ^{o}C for 1 min, followed by 40 cycles of 40 sec at 94 ^{o}C, 40 sec of annealing, and 1 min of extension at 72 ^{o}C, with a final extension at 72 ^{o}C for 10 min. For the remaining markers the program was: ‘hot start’ at 94 ^{o}C for 5 min, followed by 45 cycles of 1 min at 94 ^{o}C, 1 min of annealing, and 2 min of extension at 72 ^{o}C, with a final extension at 72 ^{o}C for 10 min. PCR products were size-separated on 6% polyacrylamide gels and silver stained. Visual allele identification followed a conservative approach, i.e. only clearly different bands were accepted as different. In case of doubt, e.g. null alleles, experiments were repeated.

Capillary fragments analysis

For genotyping the entire RIL population PCR products were analyzed by capillary electrophoresis on an ABI3730 (Applied Biosystems, Foster City, CA). Each forward primer was 5’-tailed with the M13 forward consensus sequence. The M13-tailed forward primers were then used in combination with a standard M13 primer dye-labeled (VIG, NED, FAM, PET) at its 5’-end (Boutin-Ganache et al. 2001). Samples were prepared by pooling 3 µL of PCR product from separate primer sets, each with a different fluorescent dye. The DNA pool was mixed and centrifuged. One microliter of the pooled DNA was added to a mixture of 6 µL of Hi-Di formamide (Applied Biosystems, Foster City, CA), 0.25 µL of Genescan 500-LIZ size standard (Applied Biosystems, Foster City, CA), and 3 µL of water. The samples were again mixed well and centrifuged. The 96-well plate was placed on an MJ Research PTC-200 thermal cycler for 5 min at 95° C and then on an

ice slurry for 5 min. Raw data files from the ABI3730 were imported into GeneMarker v1.1 (SoftGenetics, State College, PA) for fragment analysis.

DArT assay

DArT markers (Akbari et al. 2006) were used to provide additional genomic coverage. DArT genotyping of wheat is offered as a commercial service by Triticarte Pty. Ltd. Yarralumla, ACT, Australia (www.triticarte.com.au) who conducted the analyses for this study. Briefly, a genomic representation of a mixture of the entire population was produced with *Pst*I-*Taq*I digestion, spotted on microarray slides, and the individual genotypes were screened for polymorphism based on fluorescence signals. DNA samples from the parents were first screened for polymorphism and then the individual RILs were genotyped. A total of 501 polymorphic loci were scored as present (1) or absent (0). Names of loci that were previously mapped by Triticarte Pty. Ltd include the prefix "wPt" (followed by numbers corresponding to a particular clone); loci that were mapped for the first time on the current map were presented by clone ID number. DArT technology is protected by patent No. WO 01/73119.

Statistical and genetic analyses

LP, IF, and FS scores were taken from similar greenhouse in different years and observed differences could be interpreted as genotype x year interactions. However, in keeping with standard statistical nomenclature, environments represent results obtained in the same greenhouse but from different years. Genotype means, ranges, and standard errors of the traits were calculated across environments for the RIL lines and their parents from raw data. Parental midparent values were calculated, and deviations of the RIL population trait means from their midparent values were tested by paired t tests. Since the distribution of RIL trait means in the population were of interest, across environment means of nontransformed data were tested for normal distribution, skewness, and kurtosis by the UNIVARIATE procedure of SAS (SAS Institute, 2000).

Before analyses of variance, the Box et al. (1978) power transformation series was tested on all dependent variable data in an effort to achieve error variance homogeneity, stabilize variances among fixed effects, and to attain normality. On the basis of results from these tests, LP and FS data were not transformed, and IF data were

transformed by the square root of data. Analyses of variance of both the transformed and untransformed data were conducted by the SAS Mixed Procedure, in which lines, and environments were considered as fixed variables and replication within environments was considered as a random variable. Estimates of variance components were calculated by the restricted maximum likelihood method (REML). All variance component estimates were transformed to their respective coefficients of variation (CV%) to allow direct comparisons between traits. Narrow-sense heritabilities (h^2) were estimated by the ratio of the estimate of the genetic variance to the phenotypic variance of the RIL population mean. A model describing the data was:

$$Y_{ijk} = \mu + l_i + f_j + lf_{ij} + b_{k(i)} + e_{ijk}$$

where i = 1,2 (i.e. 2 environments), j = 150 (i.e. population size), k = 1,2 (i.e. 2 replications), $l_i \sim iid\ N(0, \sigma^2_{Environment})$, $f_j \sim iid\ N(0, \sigma^2_{Genotypic})$, $lf_{ij} \sim iid\ N(0, \sigma^2_{Environment\ x\ Genotype})$, $b_{k(i)} \sim iid\ N(0, \sigma^2_{Replication(Environment)})$, $e_{ijk} \sim iid\ N(0, \sigma^2_{Residual})$

The additive genetic variance component is $\sigma^2_{Genotypic}$, and the variance of the population mean was:

$$\sigma^2_{y.j.} = \sigma^2_{Run.}/2 + \sigma^2_{Genotypic} + \sigma^2_{Environment\ x\ Genotype}/2 + \sigma^2_{Replication(Environment)}/4 + \sigma^2_{Residual}/4.$$

For this design heritability was defined as $h^2 = \sigma^2_{Genotypic}/\ \sigma^2_{y.j.}$. The SAS mixed linear models procedure MIXED was used to fit the model to obtain REML estimates of variance components. Significant differences in means were determined by L.S.D. tests. Frequency distribution and Chi-squared analysis of 'Avocet S' x 'Amadina' population when tested against leaf rust was performed, and also a Chi-squared analysis of each segregating marker was performed to test for deviation from the 1:1 expected segregation ratio. The r_p value of the Pearson Correlation was calculated for LP, IF, and FS, and between the LP and IF, LP and FS, and IF and FS using the SAS procedure PROC CORR.

Genetic linkage maps were constructed using the computer program MAPMAKER v3.0 (Lander et al. 1987). Centimorgan (cM) values were calculated according to Haldane mapping function. Linkage groups were identified using a minimum logarithm of the odds (LOD) threshold value of 3.0 after preliminary analysis using LOD scores ranging from 3.0 to 20. Pair-wise, three-point and multi-point analyses

were used in order to determine the best order of marker loci within the linkage groups. Loci whose location were ambiguous were placed in the interval in which they best fit using the "try" command. Markers from multi-locus primers or those that were different from the reported locus were distinguished with a suffix a, b, or c, with the suffix "a" given to the first mapped locus.

Composite interval mapping was used to search for QTL using the LP, IF, and FS scores for the 'Avocet S' x 'Amadina' RILs. QTL analyses for each year (experiment) separately and then across runs were undertaken using the CIM function of QTL Cartographer v2.5 (Wang et al. 2005), and mixed-model based composite interval mapping in QTLNetwork v2.0. (Yang et al. 2005). For QTL Cartographer the parameter set-up of "model 6 standard analysis" was used; for both programs, walk speed 1 cM step, "forward and backward" regression for the selection of the markers to control the genetic background (control markers or cofactors) with a probability into and out of the model of 0.05, and a blocked window size of 10 cM to prevent tightly linked markers tagging single QTL from being included in the model. Significant thresholds for QTL detection were calculated for each dataset using 1,000 permutations and a genome-wide LOD threshold (experiment-wise P value ≤ 0.05). Mean of LP, IF, and FS of haplotypes carrying a marker linked to a trait was compared with that of haplotypes not carrying the marker. When slight shifts of QTL peaks during different years were observed during the CIM analysis, the peak identified with the analysis across runs using the mixed linear model (MLM) approach for CIM program from QTLNetwork v2.0. was considered to be the common peak associated with that QTL. In the mixed linear model CIM, QTL are fixed variables while molecular markers are random variables. Thus estimates of the QTL will not depend upon a particular fixed set of markers being in the model. The genetic model also incorporates significant additive effects and epistatic effects as well as their run interaction (Wang et al. 1999).

According to Wang et al. (1999), the MLM for simultaneous search of interacting QTL (Q_i between flanking markers M_{i-} and M_{i+}, and Q_j between flanking markers M_{j-} and M_{j+}), under the assumption of presence of QTL x environment interaction (QE) was:

$$y_{hk} = \mu + a_i x_{Aik} + a_j x_{Ajk} + aa_{ij} x_{AAijk} + u_{Ehk} e_{Eh} + u_{AiEhk} e_{AiEh} + u_{AjEhk} e_{AjEh} + u_{AAijEh} k e_{AAijEh} + \sum_{f(h)} u_{Mfk(h)} e_{Mf(h)} + \sum_{l(h)} u_{MMlk(h)} e_{MMl(h)} + \varepsilon_{hk}$$

where y_{hk} is the phenotypic value of the k–th RIL in environment h; μ is the population mean; a_i and a_j are the additive effects (fixed) of two putative QTL (Q_i and Q_j), respectively; aa_{ij} is the additive x additive epistatic effect (fixed) between Q_i and Q_j; x_{Aik}, x_{Ajk} and x_{AAijk} coefficients of QTL effects derived according to the observed genotypes of the markers (M_{i-}, M_{i+} and M_{j-}, M_{j+}) and the test positions ($r_{Mi\text{-}Qi}$ and $r_{Mj\text{-}Qj}$); e_{Eh} is the random effect of environment h with coefficient u_{Ehk}; e_{AiEh} (or e_{AjEh}) is the additive x environment interaction effect with coefficient u_{AiEhk} (or u_{AjEhk}) for Q_i (or Q_j); e_{AAijEh} is the epistasis x environment interaction effect with coefficient $u_{AAijEhk}$; $e_{Mf(h)}$ is the effect of marker f nested within the h-th environment with coefficient $u_{Mfk(h)}$; $e_{MMl(h)}$ is the effect of marker x marker interaction nested within the h–th environment with coefficient $u_{MMlk(h)}$; and ε_{hk} is the residual effect. Additive effects were negative if the allele of 'Amadina' or 'Avocet S' reduced IF, FS or increased LP score, and positive if the 'Amadina' or 'Avocet S' allele increased IF, FS or reduced LP. QTL names start with the prefix "*Q*", followed by either "*Lp*" for latent period, or "*If*" for infection frequency, or "*Fs*" for final leaf rust severity, "*ksu*" (Kansas State University), chromosome name, and number of the nearest marker to the QTL peak.

Cytogenetic stocks

Seeds of 54 cytogenetic stocks of 'Chinese Spring' wheat (Sears 1954; Endo and Gill 1996) were procured from Dr. B. S. Gill, Department of Plant Pathology, Kansas State University. Twenty compensating nullisomic tetrasomic (NT) lines were used for assigning markers to individual chromosomes, and 34 ditelosomic (DT) lines were used for assigning markers to chromosome arm of individual chromosomes.

Results

Segregation of Latent Period (LP), infection frequency (IF), and final leaf rust severity (FS) in RILs

The phenotypic behavior of LP, IF, and FS for the RIL population and its parents under the two environments along with heritabilities (h^2) are described in Table 4.1. In each environment the parental lines showed significant ($P < 0.05$) differences in LP, IF, and FS. In all replications and both years, 'Amadina' showed a higher level of resistance to

the *P. triticina* isolate TKLSQ than 'Avocet S', evidenced by prolonged LP, and reduced IF abd FS. LP score of replicated entries of 'Amadina' varied from 13–14 days, and the IF and FS scores were constantly 4 pustules/m^2 and 5%, i.e. 'Amadina showed in all 4 experiments prolonged LP, and few pustules with uredinia spores (i.e. reduced IF). On the other hand, replicated entries of 'Avocet S' varied from 12–13 pustules/cm^2 for IF, and were constantly 6 days for LP and 80% for and FS scores. Table 4.2 shows highly significant genotype x environment interactions for all traits, but the magnitude of the genotype x environment variance components was small [$\sigma^2_{GXE} = 0.4563$ ($P < 0.0001$) for LP, 0.1121 for IF ($P < 0.05$), and 9.5324 ($P < 0.05$) for FS compared to the highly significant ($P < 0.0001$) genotypic variance components for LP ($\sigma^2_G = 3.4705$), IF ($\sigma^2_G =$ 14.3747), and FS ($\sigma^2_G = 394.58$). This resulted in high heritability values of 0.918 for LP, 0.987 for IF, and 0.95 for FS.

Although the mean of the RIL population under the two environments was not significantly different, wide variation occurred among the RILs and trangressive segregants were observed across both environments with some lines having longer LP, and reduced IF and FS than that of the resistant parent 'Amadina', or shorter LP, and increased IF and FS than that of the susceptible parent 'Avocet S', confirming earlier reports on presence of resistance factor(s) in the susceptible parent 'Avocet S' (Singh et al. 2005; William et al. 2006). The RILs showed continuous distributions for non-transformed LP, and FS data, and transformed IF data, varying from 6 to 15 days for LP, 0 – 100% for FS, and 0 to 17 pustules/cm^2 for IF (Figures 4.1, 4.2, 4.3). Both skewness and kurtosis values for untransformed LP and IF, and transformed FS data were less than 1.0, suggesting that the data were suitable for QTL analysis.

Number of genes associated with latent period (LP), infection frequency (IF), and final leaf rust severity (FS)

The number of genes that condition the traits was estimated by comparing the observed frequencies of lines that resembled each of the parents, with frequencies that would be expected in a RIL population if 2, 3, 4, or 5 genes conditioned a trait. (Singh and Rajaram 1992). On the basis of the data sets, the RILs were grouped into three categories: parental-type resistant (PTR) lines when their LP, IF, and FS means were less, equal, or

up to 5% higher than that of the resistant parent 'Amadina'; parental-type susceptible (PTS) lines when their mean LP, IF, and FS were either the same or higher than that of the susceptible parent 'Avocet S'; and others (intermediate severity levels). The frequencies of observed lines in each category for LP, IF, and FS are summarized in Table 4.3. The χ^2 values indicate that there were at least four genes associated with LP and reduced IF, and at least five genes associated with FS.

Coefficients of correlation

A positive correlation was found between IF and FS (r_p = 0.58 to 0.64, $P < 0.0001$). LP was significantly ($P < 0.0001$) and negatively correlated with IF (r_p = -0.64 to -0.68) and FS (r_p = -0.59 to -0.60) (Table 4.4). These moderately significant correlations among the traits indicated that they are under the control of same gene(s).

Molecular mapping of 'Avocet S' x 'Amadina' RIL population

Among the 1600 SSR markers tested on 'Avocet S' and 'Amadina', 103 SSRs were polymorphic between the two parents. These SSRs were supplemented with 437 DArT markers. The loci of the linkage map constructed with the RIL population were grouped into 35 linkage groups. The total distance covered was 1878 cM. The average marker density was 5.26 cM with at least 2 marker loci per linkage group. Linkage groups were assigned to chromosomes by comparing the marker positions to previously published hexaploid wheat maps (Somers et al. 2004; Akbari et al. 2006; Semagn et al. 2006; Crossa et al. 2007; Huynh et al. 2008; Mantovani et al. 2008; Francki et al. 2009; Neumann et al. 2010). Final mapping was done by combining 2 or more linkage groups that belong to the same chromosome.

Cytogenetic analysis of markers flanking putative QTL associated with latent period (LP), infection frequency (IF), and final disease severity (FS)

Single environment analysis for all three traits identified a total of five intervals, *XwPt1313–XwPt4721* (Figure 4.8), *XwPt2689–XwPt6282*, *XwPt9532–XwPt3304*, *XwPt245–XwPt7207*, and *Xc117419–XwPt8721* (Figure 4.10) for putative QTL. "*c117419*" is a clone ID identifying DArT marker based on the clone it was derived from during development of the diversity array. PCR-based markers (Table 4.5) were assigned

to chromosome arms using the cytogenetic stocks. Markers *XwPt1313*, and *XwPt2689* and *XwPt3304* were assigned to chromosomes 1BL, and 6BL. Markers *XwPt6282*, *XwPt9532*, *XwPt245*, *XwPt7207*, *Xc117419*, and *XwPt8721* could not be assigned to specific chromosomes because their sequence information was not available. However, all these markers were in the same 6BL linkage group in this study. PCR-based monotypic assay of *XwPt5188* (0.4 cM from *wPt2689*), *XwPt7777* (0.5 cM from *XwPt6282*), and *XwPt3116* (0.7 cM from *wPt3304*, 3.7 cM from *wPt245*, and 32.3 cM from *c117419*) were assigned to 6BL linkage group. Multi-environment analysis identified intervals *XwPt2847–XwPt128*, *XwPt4301–XwPt6932*, and *XwPt6419–XwPt6200*. Based on previous reports, *wPt2847* and *wPt128* map to chromosome 1AL, and *XwPt4301* map to 2BS (Crossa et al. 2007; Huynh et al. 2008; Neumann et al. 2010). Marker *wPt4144*, which was assigned to chromosome 2DS (Crossa et al. 2007), was 1.6 cM from the *XwPt6419–XwPt6200* interval in this study.

Single environment QTL analysis and characterization for latent period (LP), infection frequency (IF), and final leaf rust severity (FS)

Initial single environment analyses for all three traits identified a total of four QTL, *QLp.ksu-1BL.2* (same as *QIf.ksu-1BL.2* and *QFs.ksu-1BL.1*) on chromosome 1BL, QIf.ksu-*6BL.7* (same as *QIf.ksu-6BL.7* and *QFs.ksu-6BL.10*), *QIf.ksu-6BL.15* and (same as *QIf.ksu-6BL.18*) on 6BL, and *QIf.ksu-2DS.3* on 2DS, (Table 4.6).

Latent period (LP)

We detected two putative QTL, both with additive main effects, *QLp.ksu-1BL.2* and *QLp.ksu-6BL.7*, at the intervals *XwPt1313–XwPt4721* and *XwPt2689-XwPt6282* on chromosomes 1BL and 6BL. The allele of *QLp.ksu-1BL.2* with significant additive effects (A) +0.77 and +0.76, accounted for 14.93 and 14.32% of the phenotypic variance for LP in environments 1 and 2 respectively. *QLp.ksu-6BS.7* with significant A +0.61, which was only detected in environment 2, accounted for 9.36% of the observed phenotypic variation for LP. Resistance allele for *QLp.ksu-1BL.2* was contributed by the resistant parent ‘Amadina’, whereas the resistance allele for *QLp.ksu-6BL.7* was contributed by the susceptible parent ‘Avocet S’ (Table 4.6).

Infection frequency (IF)

Two putative QTL, *QIf.ksu-1BL.2* and *QIf.ksu-6BL.15*, located at the intervals *XwPt1313-XwPt4721* and *XwPt245-XwPt7207* on chromosomes 1BL and 6BL were detected (Table 4.6). *QIf.ksu-1BL.2* with significant A -1.75 and -1.61, accounted for 20.88 and 19.27% of the observed phenotypic variation for IF in environments 1 and 2, respectively. *QIf.ksu-6BL.15* significant A -1.39 and -1.36 accounted for 13.69 and 14.02% of the observed phenotypic variation for IF in environments 1 and 2, respectively. Resistance allele for *QIf.ksu-1BL.2* was contributed by 'Amadina', whereas resistance allele for *QIf.ksu-6BL.15* was contributed by 'Avocet S'.

Final leaf rust severity (FS)

The QTL *QFs.ksu-1BL.1* and *QFs.ksu-1BL.2* located at the intervals *Xth4301-XwPt1313* and *XwPt1313-XwPt4721* on chromosome 1BL were each detected in single environment only (Table 4.6). *QFs.ksu-1BL.1* with significant A -10.81, accounted for 29.02% of the observed phenotypic variation for FS in environment 2. *QFs.ksu-1BL.2* also with significantl A -11.77, explained 30.51% of the phenotypic variance in environment 1. We believe both *QFs.ksu-1BL.1* and *QFs.ksu-1BL.2* are the same QTL, and the differences in the intervals were likely due to shifts in the QTL position as a result of environmental influence. Another QTL, *QFs.ksu-6BL.10* located at the interval *XwPt9532-XwPt3304* on chromosome 6BL, was detected in environment 2 only, and accounted for 15.20% of the observed phenotypic variation (Table 4.6). The resistance allele *QFs.ksu-6BL.10* was contributed by 'Avocet S'. Additive effects of *QFs.ksu-1BL.1* and *QFs.ksu-6BL.10*, indicated that they could significantly reduce the FS, and account for 44.22% of the phenotypic variance in environment 2.

Multi-environment QTL analysis and characterization for latent period (LP), infection frequency (IF), and disease severity (DS)

In order to determine QTL that are important for the components of slow leaf-rusting as well as severity under different environments, we performed QTL analysis across the two environments using QTL Network 2.0. (Table 4.7)

Latent period (LP)

We detected two putative QTL, *QLp.ksu-1BL.2*, and *QLp.ksu-6BL.7*, at the intervals *XwPt1313-XwPt4721*, and *XwPt2689-XwPt6282* on chromosomes 1BL and 6BL that significantly increased LP due to additive effects +0.75 and +0.56, and explained 14.41% and 8.64% of the phenotypic variance. Additive x environment interactions of the QTL were not significant (Fig. 4.13; Table 4.7). Resistance allele for *QLp.ksu-1BL.2* was contributed by 'Amadina', whereas resistance allele for *QLp.ksu-6BL.7* was contributed by 'Avocet S'.

Infection frequency (IF)

Three putative QTL, *QIf.ksu-1BL.2*, *QIf.ksu-6BL.15*, and *QIf.ksu-2DS.3*, at the intervals *XwPt1313-XwPt4721*, *XwPt245-XwPt7207*, and *XwPt6419-XwPt6200* on chromosomes 1BL, 6BL, and 2DS with significant A -1.77, -1.65, and -0.56, accounted for 20.07, 13.85, and 10.4% of the observed phenotypic variance for IF. Additive x environment interactions of the loci were not significant (Figure 4.14; Table 4.7). Resistance allele for *QIf.ksu-1BL.2* was contributed by 'Amadina', whereas resistance alleles for *QIf.ksu-6BL.15* and *QIf.ksu-2DS*.3 were contributed by 'Avocet S'.

Final leaf rust severity (FS)

We detected two putative QTL, *QFs.ksu-1BL.2* and QTL *QFs.ksu-6BL.10* with additive main effects, at the intervals *XwPt1313-XwPt4721* and *XwPt9532-XwPt3304* on chromosomes 1BL and 6BL (Figure 4.15; Table 4.7). *QFs.ksu-1BL.2* with significantl A -11.23, explained 29.49% of the phenotypic variance for FS. *QFs.ksu-6BL.10* with significant A -6.44, accounted for 11.78% of the observed phenotypic variance. Resistance allele for *QFs.ksu-1BL.2* was contributed by 'Amadina', whereas resistance allele for *QFs.ksu-6BL.10* was contributed by 'Avocet S'. The two resistance alleles together could significantly reduce FS due to additive effects, and account for 41.27% of the observed phenotypic variance. Additive interactions with environments (AE) were not significant.

Epistatic effects between QTL for latent period (LP), infection frequency (IF), and final disease severity (FS)

Genomic regions involved in epistatic interactions as well as epistasis x environment interactions were directly mapped by the mixed linear model based-QTL mapping approach (Wang et al. 1999) using the software QTLNetwork 2.0. Epistatic digenic effects were categorized as additive x additive effects (AA), additive x non-additive effects (AD), non-additive x additive effects (DA), and non-additive x non-additive effects (DD) (Table 4.8).

No epistatic interactions between QTL associated with prolonged LP and reduced FS were detected (Figures 4.13 and 4.15). However, epistatic interactions associated with reduced IF were detected (Figure 4.14; Table 4.8). Non-additive x non-additive epistatic effect (DD -0.92) was detected between a QTL, *QIf.ksu-1AL.2*, at the interval *XwPt2847-XwPt128* on chromosome 1AL with a QTL, *QIf.ksu-2BS.4*, at the interval *XwPt4301-XwPt6932* on 2BS, explaining 6.77% of the observed phenotypic variation for IF. A second epistatic interaction with additive x non-additive effect (AD -0.78) involving a QTL, *QIf.ksu-6BL.7*, at the interval *XwPt2689-XwPt6282* on 6BL and a QTL, *QIf.ksu-4D.3*, at the interval *Xgwm149b-Xbarc1174b* on 4D explained 5.4% of the observed phenotypic variation for IF. A third epistatic interaction with non-additive x non-additive effect (DD -0.97) between a QTL, *QIf.ksu-6BL.18*, on 6BL and a QTL, *QIf.ksu.4D.1*, on 4D accounted for 5.94% of the phenotypic variance for IF. Resistance alleles for the *QIf.ksu-1AL.2* x *QIf.ksu-2BS.4* and *QIf.ksu-6BL.18* x *QIf.ksu-4D.1* epistatic interactions were derived from 'Avocet S', whereas resistance alleles for the *QIf.ksu-6BL.7* x *QIf.ksu-4D.3* epistatic interaction were contributed by 'Amadina'. If we include the three digenic epistatic effects in the model for the simultaneous fit, the amount of the explained phenotypic variance for IF would increase from 34.96% to 53.07%. Poor correlation estimates between markers flanking putative QTL involved in epistatic interactions for IF were observed (Table 4.9).

Discussion

Genetic basis of slow leaf-rusting resistance and its components

The objective of our study was to elucidate the genetic basis of the slow leaf-rusting resistance of the CIMMYT wheat breeding line 'Amadina'. The leaf rust resistance of 'Amadina' has been shown to be durable across Mexico and the United States (Singh et al. 2004), and therefore, it is of great interest to transfer this resistance into other breeding lines. Singh et al. (2004) estimated a minimum number of four additive genes for leaf rust resistance in 'Amadina'. In this study, 'Amadina' clearly influenced expression of the components of resistance. The LP was prolonged, the pustules per square centimeter of flag leaf area were fewer, and FS was considerably reduced. High variability for the traits was observed among the RILs. Genotype x environment interaction variance was very small compared to the very large genotypic variance (Table 4.2), indicating stability of the expression of the traits. A test with the *Lr34/Yr18* closely linked marker *csLV34* (Lagudah et al. 2006) was negative.

Our studies determined that 'Amadina' has at least four genes prolonging LP, and at least four and five genes were associated with reductions in IF and FS, respectively (Table 4.3). This estimate of the genes associated with LP is consistent with estimates from earlier studies (Kuhn et al. 1980; Lee and Shaner 1985a, b; Shaner et al. 1997; VanderGaag and Jacobs 1997), and similar to estimates from studies on other cereals and rust species (Luke et al. 1975; Parlevliet 1976; Milus and Line 1986; Bjarko and Line 1988; Jacobs and Broers 1989). Furthermore, minimum number of genes associated with FS is consistent with earlier report of Singh et al. (2004) on inheritance of slow leaf-rusting resistance in 'Amadina'. The estimate of heritability (h^2 = 0.918) for mean latent period (MLP) in this study was similar to that of previous estimates which varies from 0.46 to 0.90 (Lee and Shaner 1985a; Jacobs and Broers 1989; Bjarko and Line 1988; Das et al. 1992; Xu et al. 2005a). Heritabilities for IF and FS were also high.

This study showed that both LP and IF are reliably measured components of slow rusting when plants are inoculated under controlled conditions in the greenhouse. LP was negatively correlated with IF (-0.64 to -0.68) and FS (-0.59 to -0.60). Das et al. (1993) also reported negative association of LP with AUDPC, uredinium size (pustule size), and receptivity (IF). On the other hand, IF was positively associated with FS (0.58 to 0.64). The significant but moderate correlation between the components of slow leaf-rusting

provides evidence for some level of interdependence for the components which could be under the control of identical genomic regions. The moderate levels of LP-FS and IF-FS correlations suggests that all components are important as selection criteria when breeding for genotypes with higher levels of slow leaf-rusting resistance. Further evidence in this regard was provided by Rossi et al (1999) who showed that improvements in single components of resistance reduced AUDPC for *Cercospora* leaf spot of sugar beet. When different components were modeled simultaneously to improve resistance, disease development was reduced more than additively (Rossi et al. 1999).

We have been able to characterize nine loci associated with components of slow leaf-rusting in RILs derived from the leaf rust susceptible 'Avocet S' and the leaf rust resistant 'Amadina'. In our study both parents contributed positive alleles for leaf rust resistance, thereby allowing for transgressive segregation. Based on the QTL results and the fact that the means for LP, IF, and FS of the RILs were not significantly different from the parental means, additive effects were the predominant mode of inheritance of the components of slow leaf-rusting. However, we found significant digenic non-additive x non-additive (DD), and additive x non-additive (AD) epistatic effects between QTL for IF. This finding is in agreement with Das et al. (1992) and Rosewarne et al. (2008), who found predominantly additive genetic variance for partial leaf rust resistance in advanced spring wheat populations, and, to some extent, epistatic genetic variance.

The mixed linear model CIM single environment analysis of the 'Avocet S' x 'Amadina' RIL population indentified a putative QTL on 1BL with additive effect as the main contributor for prolonged LP, and reduced IF and FS. The STS *Xth4301*, known to be closely associated with the slow-rusting locus *Lr46/Yr29* (Unpublished) on chromosome 1BL, mapped to the same linkage group as the major QTL accounting for 14.41, 20.07, and 29.49% of the phenotypic variance for LP, IF, and FS. Mateos-Hernandez et al. (2005) narrowed the physical location of the *Lr46/Yr29* locus to a sub-microscopic region between the breakpoints of deletion lines 1BL–3 [fraction length (FL) = 0.89–1] and 1BL–10 [FL = 0.89–3].

The multi-environment analysis identified a QTL, *QIf.ksu-2DL.3*, on chromosome 2DL associated with IF. The *QIf.ksu-2DL.3* QTL was located at the interval *XwPt6419* – *XwPt6200*, and the SSR *Xwmc18* was 11.5 cM from *XwPt6200*. If we refer to ITMI and

Consensus maps and the location of the *Xwmc18* locus, *QIf.ksu-2DL.3* should map in the centromeric region, C-2DL3-0.49 bin, of the long arm of linkage group 2D. QTL *QIf.ksu-2DL.3* with A -0.56, accounted for 1.04% of the observed phenotypic variation for IF. Resistance allele for the QTL was derived from 'Avocet S'. Crossa et al. (2007) reported significant association of *XwPt4144*, which was 3.6 cM from the QTL identified in this study on chromosome 2D, with grain yield. Since *wPt4144* was only 3.6 cM from the locus identified in this study, the 2D region reported by Crossa et al. (2007) may likely correspond to *QIf.ksu-2DL.3*. Schnurbusch et al. (2004) reported a minor QTL on 2DL, accounting for 11.4, 12.7, and 15.6% of observed phenotypic variation for infected leaf area, response to infection, and leaf tip necrosis. Leaf tip necrosis (LTN) is a morphological trait generally associated with gene *Lr34*/*Yr18* (Singh and Rajaram 1992) located on 7DS. The LTN trait has been shown to be quantitatively expressed, controlled by several QTLs and is variable in different backgrounds and environmental conditions (Messmer et al. 2000). The LTN trait described by Singh et al. (1992) to be associated with *Lr34/Yr18* locus was observed by investigating a number of crosses between *Lr34/Yr18/Ltn* positive lines and *Lr34/Yr18/Ltn* negative lines. LTN is also associated with the resistance *Bdv1*, a resistance gene to barley yellow dwarf virus (Singh 1993), and resistance to spot blotch disease of wheat caused by *Bipolaris sorokiniana* (Joshi et al. 2004). In the present study, 'Amadina' also showed some level of LTN. Rosewarne et al. (2006) reported association of LTN with the *Lr46/Yr29* locus in a population derived from the cross 'Avocet-*YrA*' x 'Atilla'. *Ltn-Lr46/Yr29* pleiotropism in other wheats has also been observed (Singh et al. 2001; Pathan and Park 2006). Scoring the 'Avocet S' x 'Amadina' population for LTN would have allowed us to know whether the *QIf.ksu-2DL.3* QTL derived from 'Avocet' is associated with LTN.

Two QTL, *QLp.ksu-6BL.7* (same as *QFs.ksu-6BL.10*), and *QIf.ksu-6BL.15* (same as *QIf.ksu-6BL.18*) on chromosome 6BL with additive main effects were also identified in this study. Due to lack of SSRs anchoring the DArT markers, we could not assign the QTL to a bin on 6B. Previous reports assigned most of DArT markers in the linkage group 6B in this study to the long arm of chromosome 6B (Akbar et al. 2006; Semagn et al. 2006; Crossa et al. 2007; Huynh et al. 2008; Mantovani et al. 2008; Francki et al. 2009; Neumann et al. 2010). *QLp.ksu-6BL.7* explained 8.64 and 11.78% of the observed

phenotypic variation for LP and FS, and *QIf.ksu-6BL.15* accounted for 13.85% of the phenotypic variance for IF. *Lr3a* is a known leaf rust resistance locus on the long arm of chromosome 6B with three reported alleles, *Lr3a*, *Lr3ka*, and *Lr3bg* (McIntosh et al. 1995, 1998; Herrera-Foessel et al. 2007b). We previously found the *P. triticina* isolate TKLSQ was virulent on *Lr3a*, but avirulent on *Lr3ka* (Unpublished). We could not test the reaction of Thatcher near-isogenic line (NIL) carrying *Lr3bg* to the TKLSQ isolate due to unavailability of a Thatcher NIL carrying the gene. Based on the previous gene postulation study, 'Amadina' does not carry *Lr9* which is also located on 6BL. Herrera-Foessel et al. (2007b) identified a leaf rust resistance gene linked to *Lr3a* in durum wheat. Additive x non-additive epistatic interaction for IF, involving *QIf.ksu-6BL.7* and a QTL, *QIf.ksu-4D.3*, on chromosome 4D was detected by the multi-environment analysis. The epistatic interaction significantly reduced IF, and accounted for 5.4% of the observed phenotypic variation. Resistance alleles for the interacting *QIf.ksu-6BL.7* and *QIf.ksu-4D.3* QTL were derived from 'Amadina'. Non-additive x non-additive interaction (DD -0.97) between *QIf.ksu-6BL.18* on 6BL and *QIf.ksu-4D.1* on 4D accounted for 5.94% of the phenotypic variance. Resistance alleles for the *QIf.ksu-6BL.18* and *QIf.ksu-4D.1* were derived from 'Avocet S'. In our previous field study, we identified a QTL, *QSYr.ksu-4D.1*, on chromosome 4D associated with adult-plant resistance to stripe rust (Unpublished). The nearest marker to *QIf.ksu-4D.2* was 1.2 cM from the nearest marker to *QSYr.ksu-4D.1*, and thus *QIf.ksu-4D.2* and *QSYr.ksu-4D.1* are highly likely the same QTL. William et al. (1997) identified a QTL on chromosome 4D associated with leaf rust resistance in bread wheat. Non-additive x non-additive (DD) interaction for IF involving *QIf.ksu-6BL.15* and *QIf.ksu-4D.1* on 4D was also detected. 'Avocet S' contributed alleles for *QIf.ksu-6BL.18* and *QIf.ksu-4D.1*, and the epistatic interaction accounted for 5.94% of the phenotypic variance for IF.

Non-additive x non-additive epistatic interaction for IF between a locus, *QIf.ksu-1AL.2*, on chromosome 1AL and a locus, *QIf.ksu-2BS.4*, on 2BS was also detected by the multi-environment analysis. 'Avocet S' contributed alleles for both interacting loci. Crossa et al. (2007) reported significant association of *XwPt128*, which was the nearest marker to *QIf.ksu-1AL.2*, with reaction to leaf rust as well as grain yield. 'Avocet S' carries the race-specific gene *Lr13* which is located on chromosome 2BS (Singh and

Rajaram 1991). In our previous gene postulation study, we found *XwPt615* linked to the race-specific gene, *Lr13*. However, *QIf.ksu-2BS.4* does not correspond to the *Lr13* locus because it is 19.6 cM from *XwPt615*. Referring to the Consensus and ITMI maps and the location of the *XwPt615* locus, *QIf.ksu-2BS.4* maps in the region between the 2BS1–0.53–0.75 and 2BS3–0.75–0.84 bins of the linkage group 2B. The race-specific gene, *Lr16*, is known to reside on chromosome 2BS. However, we previously postulated *Lr16* not to be present in either 'Amadina' or 'Avocet' (Unpublished). Rosewarne et al. (2008) identified a locus at the interval *Xgwm682-XP32/M62* on chromosome 2BS with additive main effect, reducing leaf rust severity by 4.4%. Xu et al. (2005b) reported a QTL, *QLr.osu-2B*, on chromosome 2BS explaining 18.8, 16.6, and 16.0% of the phenotypic variance for area under disease progress curve (AUDPC), final disease severity, and infection frequency.

It is likely that some defeated race-specific resistance genes such as *Lr1*, *Lr10*, *Lr13*, *Lr23*, and *Lr26*, which were identified previously in 'Amadina' and 'Avocet S' (Singh et al. 1991; Unpublished), had significant statistical effects on the levels of APR to the *P. triticina* isolate TKLSQ. Numerous studies have been conducted on the effects of gene pyramids on fungal diseases of cereals (Nass et al. 1981; Brodny et al. 1986; Pederson and Leath 1988; Roelfs 1988; Mundt 1990, 1991; Chantret et al. 1999). Using stem rust of wheat, Brodny et al. (1986) found that combination of defeated genes could significantly affect the number of spores produced per pustule. At least two underlying genetic mechanisms can lead to statistical relationships between the presence of a race-specific resistance and the level of non-race specific. Defeated race-specific resistance genes could have pleiotropic "residual" effects on non-race specific, or they could be linked to genes conferring non-race specific. Even though distinguishing between the two hypotheses is not straightforward, as it requires very precise control of the genetic background (Anderson 1982), the "residual effect" hypothesis was clearly favored in many studies (Nass et al. 1981; Pederson and Leath 1988; Chantret et al. 1999; Li et al. 2001). The linkage hypothesis follows that most race-specific resistance genes belong to tightly linked gene families in plant genomes (Hulbert et al. 2001) and that resistance gene analogs are frequently found in the vicinity of QTL for non-race specific (Geffroy et al. 2000; Zimnoch-Guzowska et al. 2000; Calenge et al. 2005; McIntyre et al. 2005;

Muylle et al. 2005). It would be interesting to study the interaction of different races with lines carrying different combinations of race-specific-non-race specific resistance-gene combinations may shed more light on the possible role of “defeated” race-specific resistance genes in slow-rusting resistance.

Conclusion

We used the mixed linear method for mapping QTL with additive and epistatic effects, as well as their QTL x environment interaction effects for slow leaf-rusting resistance and its components. Our results indicate that the major portion of genetic variability for slow leaf-rusting resistance and its components was additive gene action as well as digenic epistatic effects to some degree. Eight loci were associated with slow leaf-rusting, some of which were having additive effects whereas some were having non-additive effects, and some were derived from the susceptible parent ‘Avocet S’. In the statistical analysis, it is possible to confuse loosely linked markers as having epistatic interactions. Marwede et al. (2005) suggested three possible epistatic interactions: type I interactions between two QTL with additive effect, type II interactions between a QTL with additive effect and “background” locus without additive effect, and type III interactions between two loci showing epistatic effects only. In this study, all the epistatic interactions were type III (Figure 4.5; Table 4.6). The interacting loci seem not to have significant effects for IF alone but might affect its expression by epistatic effects with the other loci. The results also suggest that some of the additive QTL might be detected with effects confounded by epistasis, if epistatic effects were ignored in QTL mapping. Although the loci identified in this study explained a large portion of genetic variation, a significant proportion of the genetic variation for LP, IF and FS still remains unexplained. This is because our genetic map did not cover the whole genome of wheat. Thus, other minor QTL or modifiers may also be involved in this population. A larger population size would likely be needed to detect them. The mixed linear model CIM analysis presented here show the very complicated nature of slow leaf-rusting resistance trait and its components. Breeders must take into account such complexity and test for the effects of individual loci in targeted genetic backgrounds in order to obtain the expected phenotypes of the genes of interest. More work is required to verify whether the loci on chromosome 1AL, 2BS, 6BL, and

2DS have been previously implicated for resistance to leaf rust, or whether they represent new, unexploited QTL for slow leaf-rusting.

Figures and Tables

Figure 4.1 Histogram of latent period (LP) in the 'Avocet S' x 'Amadina' population

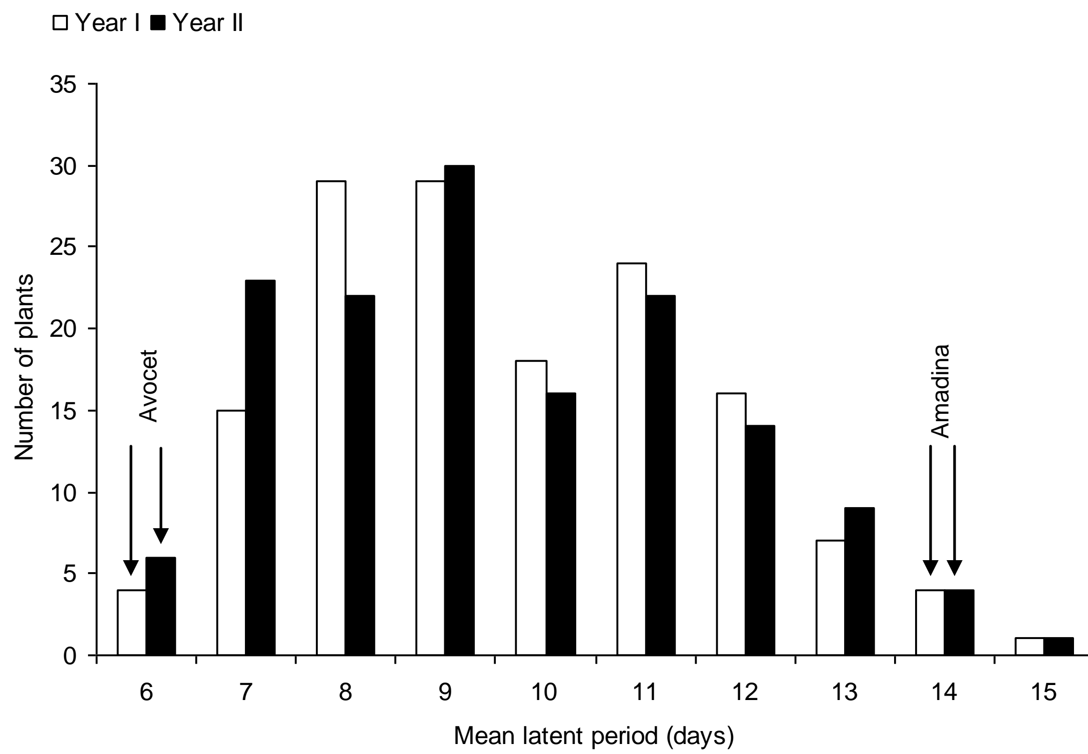


Figure 4.2 Histogram of transformed infection frequency (IF) in the 'Avocet S' x 'Amadina' population

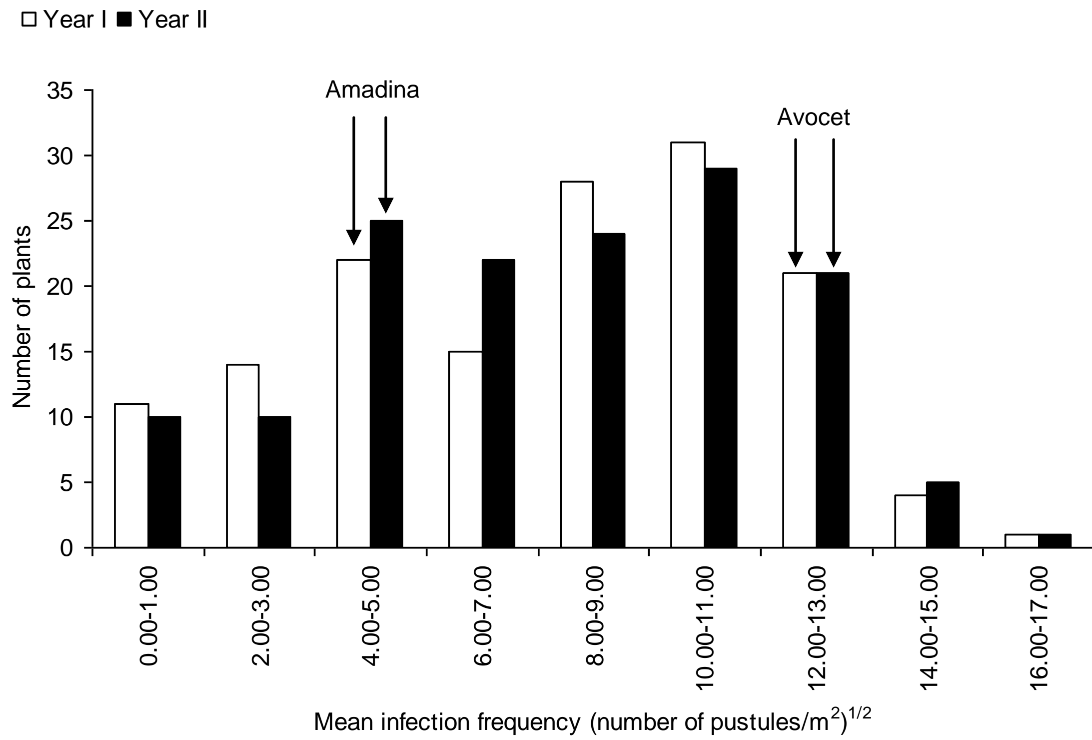


Figure 4.3 Histogram of final disease severity (FS) in the 'Avocet S', 'Amadina population

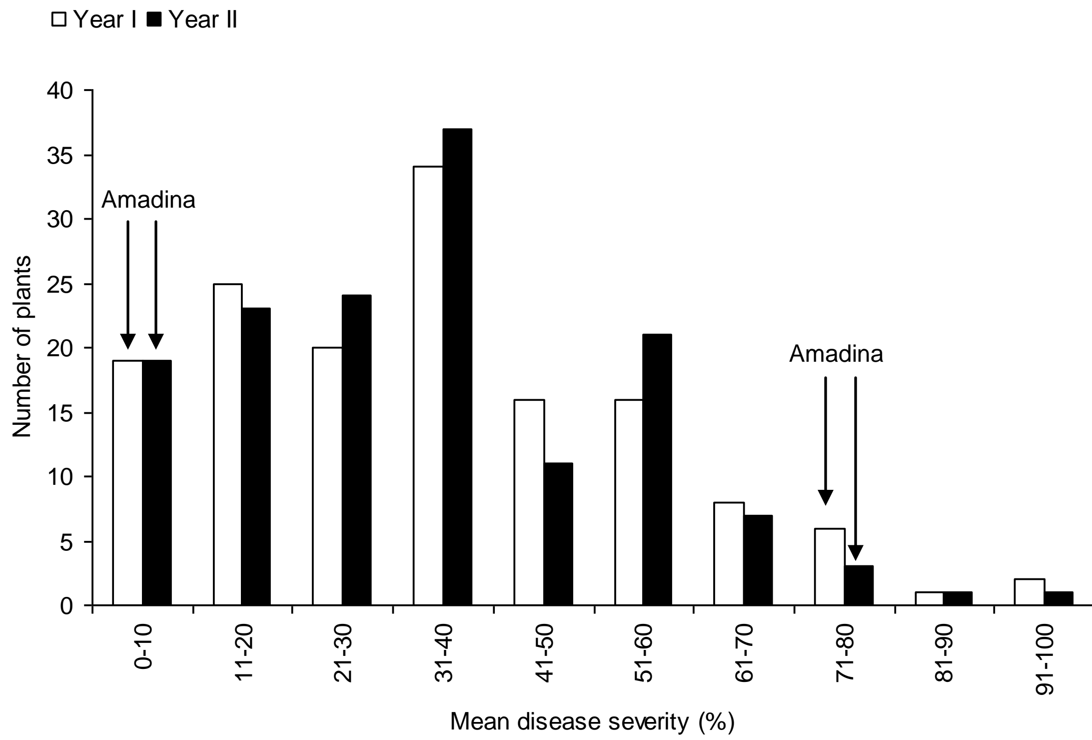


Figure 4.4 Composite interval mapping (CIM) for latent period (LP) in the 'Avocet S' x 'Amadina' population

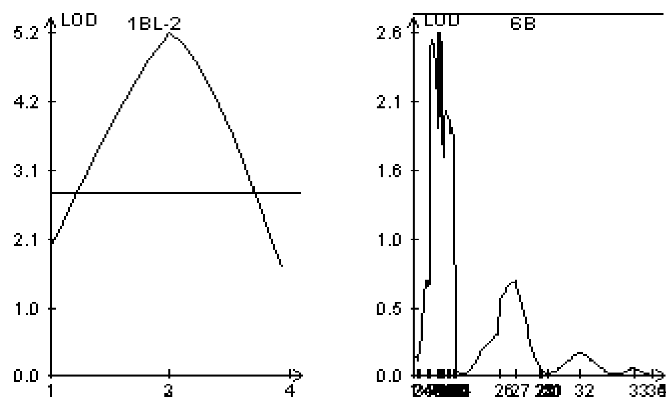


Figure 4.5 Composite interval mapping (CIM) for transformed infection frequency (IF) in the ‘Avocet S’ x ‘Amadina’ population

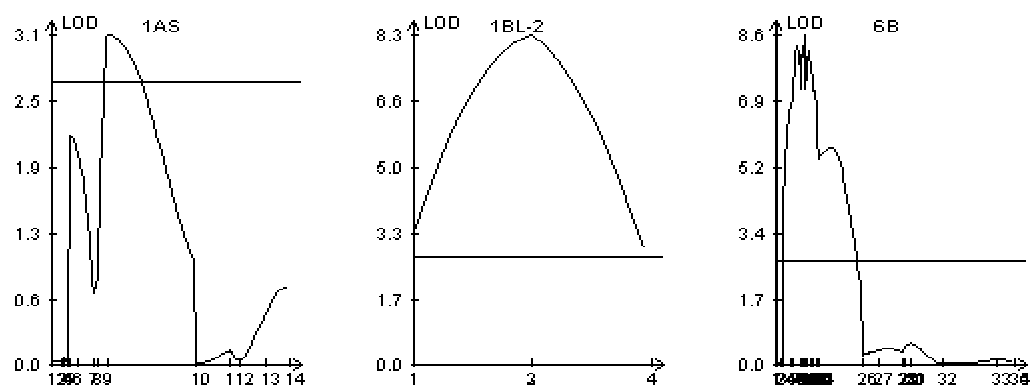


Figure 4.6 Composite interval mapping (CIM) for final disease severity (FS) in the 'Avocet S' x 'Amadina' population

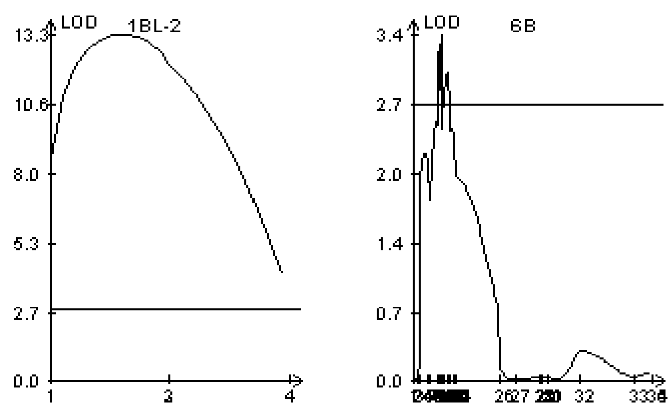
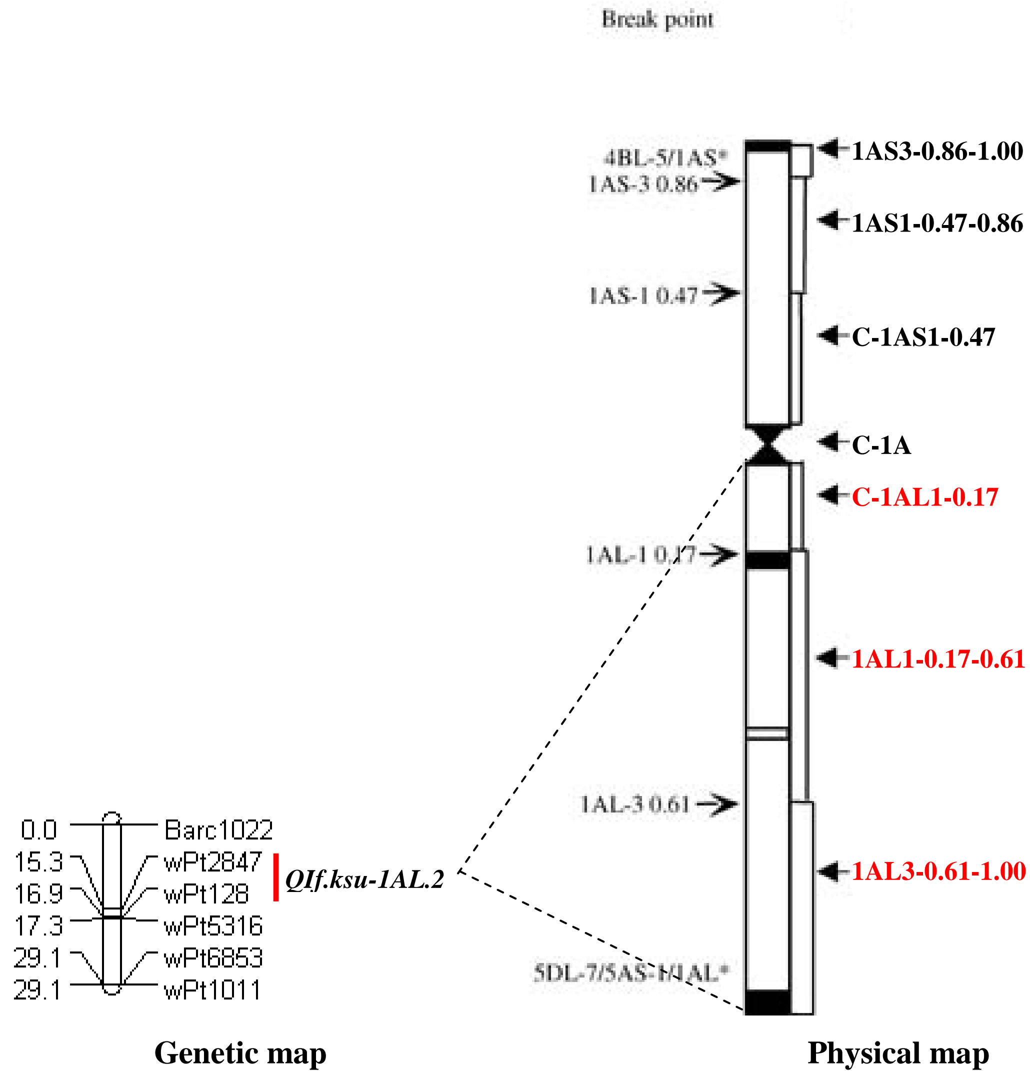
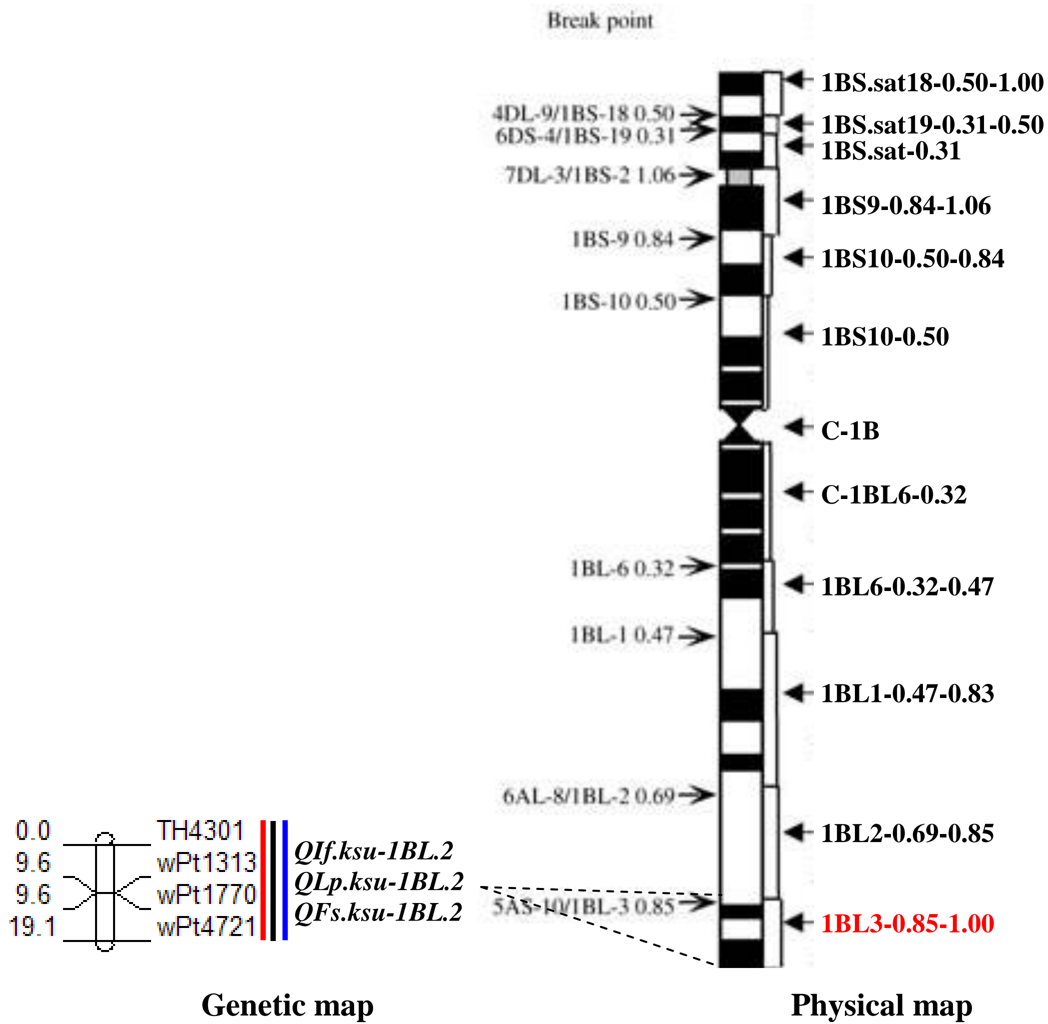


Figure 4.7 Partial Linkage map showing likely genomic location on chromosome 1A conditioning reduced infection frequency detected by multi-environment analyses.



‡The Locus, *QIf.ksu.1AL.2* was detected by the multi-environment analyses of QTLNetwork v2.0. Allele for this locus was contributed by the susceptible parent 'Avocet S'. The region on 1AL.2 had no main effect by itself, but was involved in epistatic interaction with *QIf.ksu-2BS.4* on chromosome 2BS.

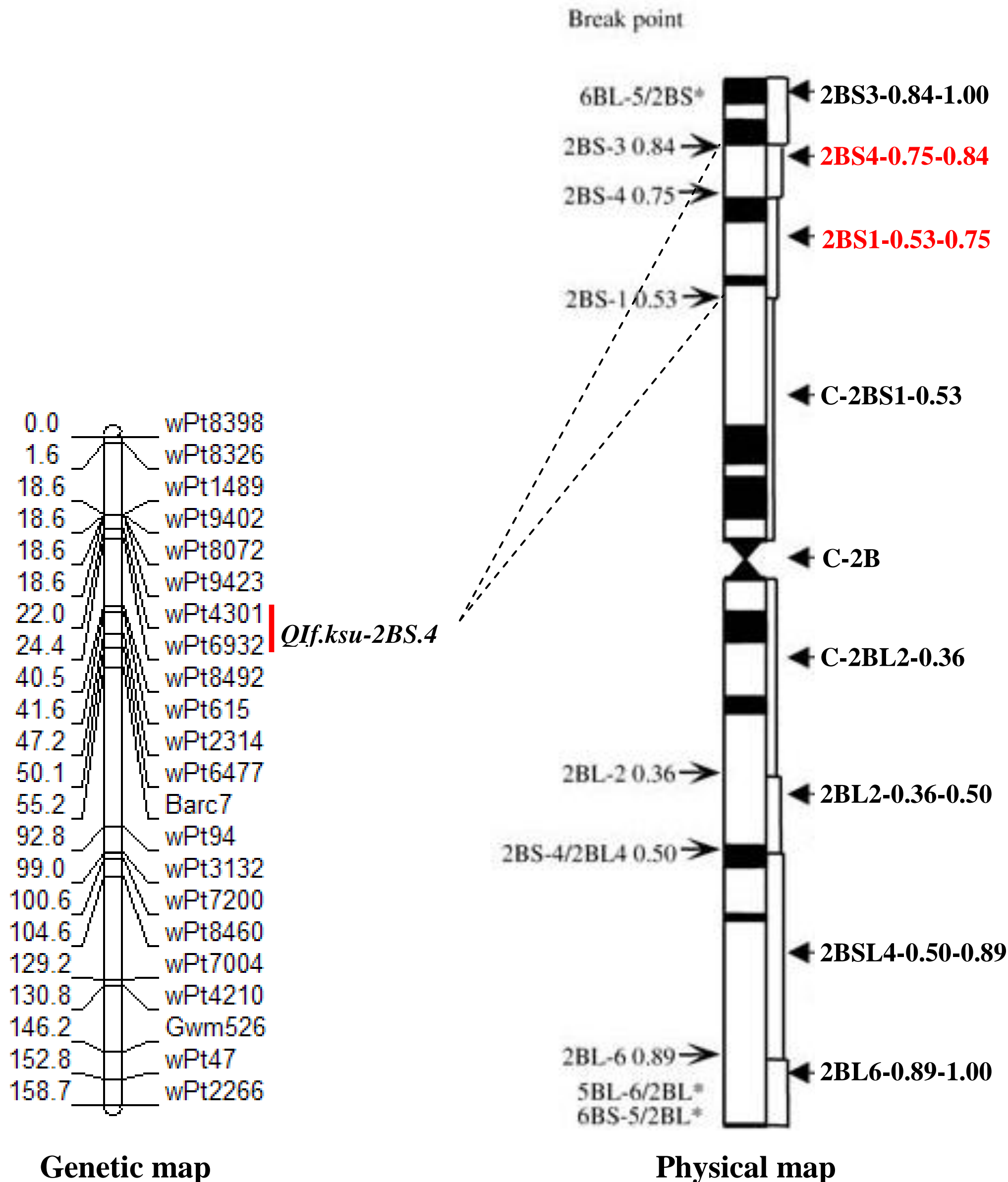
Figure 4.8 Partial Linkage map showing likely genomic location on chromosome 1B conditioning reduced infection frequency, increased latent period, and reduced final disease severity detected by single environment and multi-environment analyses.



†Red bar – indicates QTL for infection frequency (IF); Blue bar – indicates QTL for latent period (LP); Black bar – indicates QTL for final disease severity (FS).

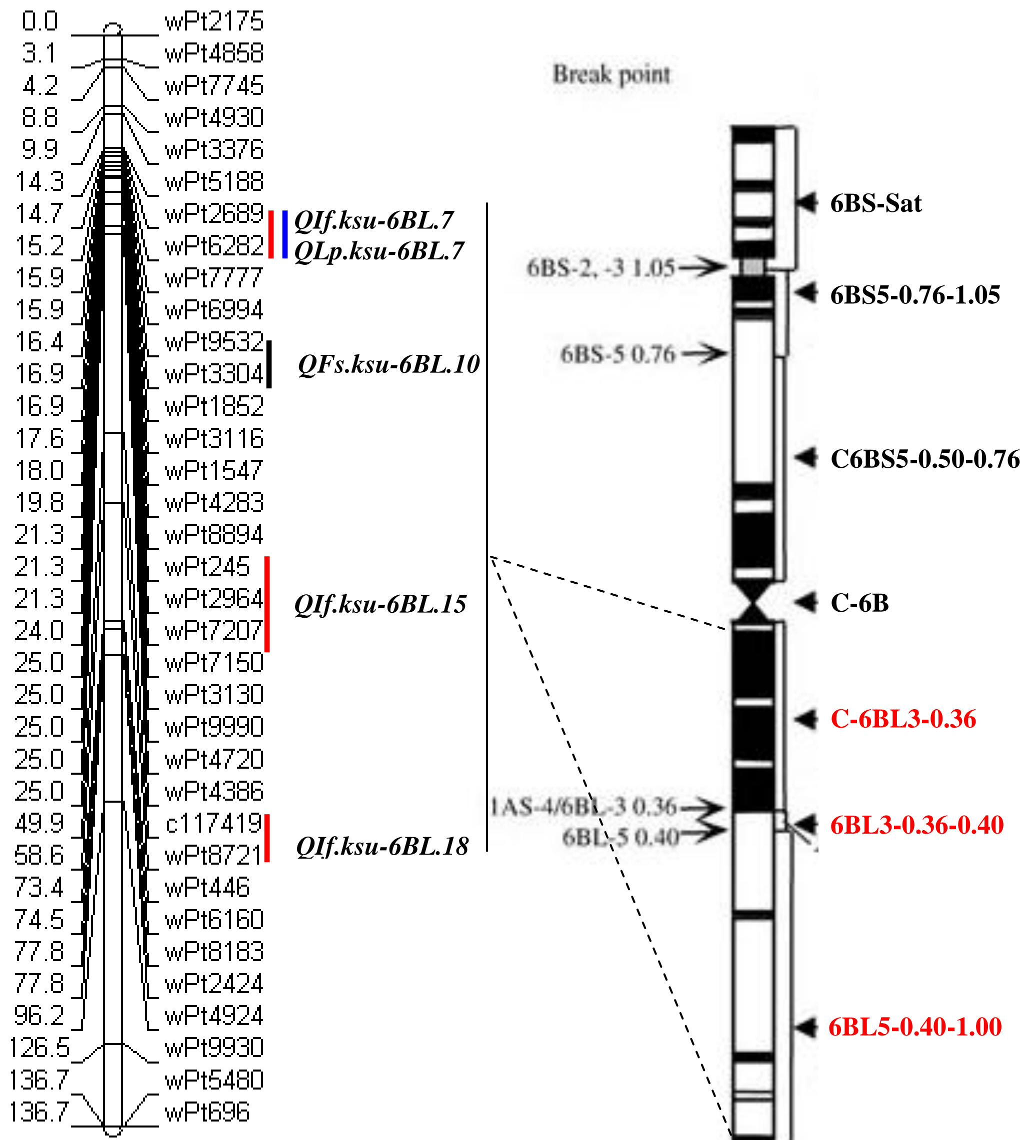
‡The locus *QIf.ksu.1BL.2* (which was the same *QLp.ksu-1BL.2* and *QFs.ksu-1BL.2*) was detected by both single and multi-environment analyses of QTLNetwork v2.0. The locus detected in this study is the *Lr436/Yr29* locus, and the resistance allele was contributed by the resistant parent ‘Amadina’.

Figure 4.9 Partial Linkage map showing likely genomic location on chromosome 2B associated with reduced infection frequency detected by multi-environment analyses.



‡The locus *QIf.ksu-2BS.4* was involved in a digenic epistatic interaction with a locus on the long arm of chromosome 1A. The resistance allele for this locus was contributed by the susceptible parent 'Avocet S'.

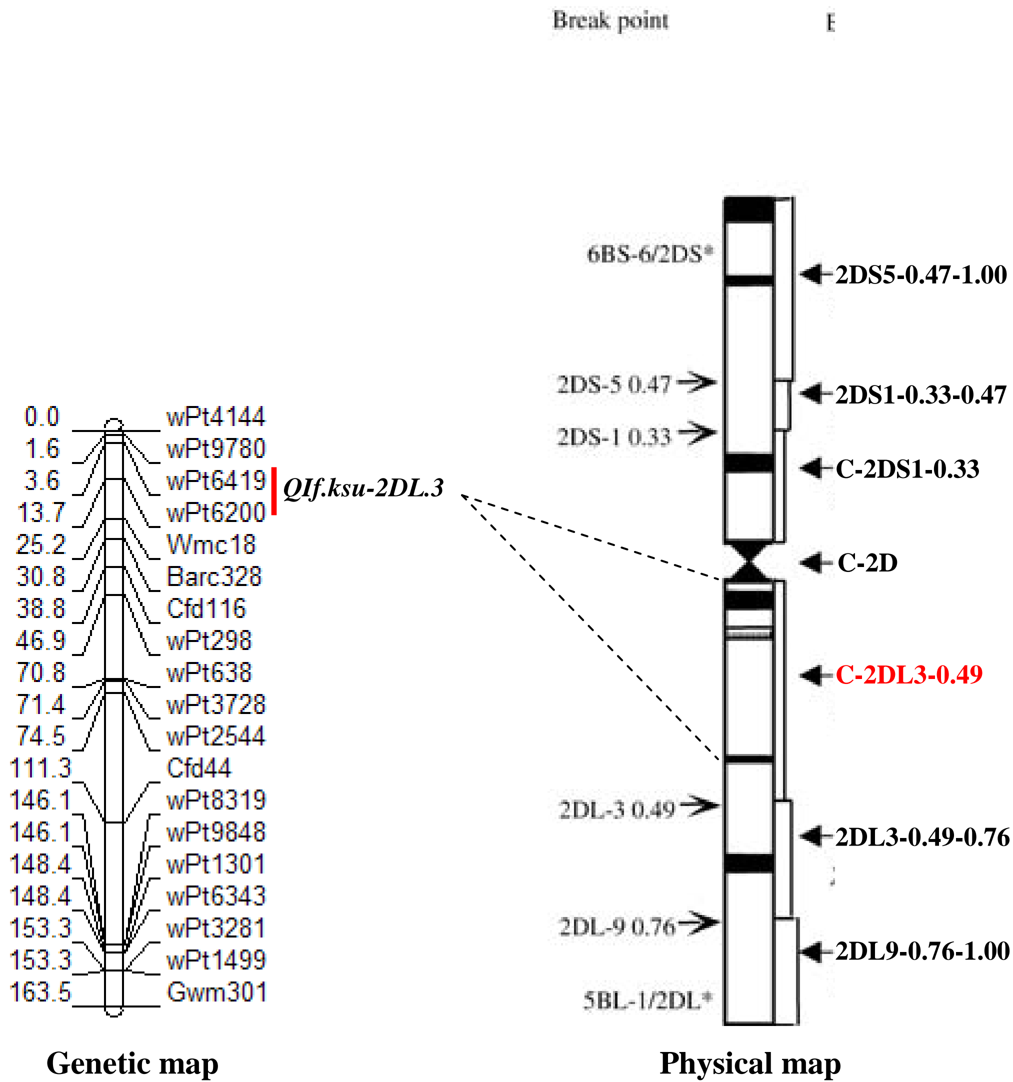
Figure 4.10 Partial Linkage map showing likely genomic location on chromosome 6B conditioning reduced infection frequency, increased latent period, and reduced final disease severity detected by single environment and multi-environment analyses.



[†]Red bar – indicates QTL for infection frequency (IF); Blue bar – indicates QTL for latent period (LP); Black bar – indicates QTL for final disease severity (FS).

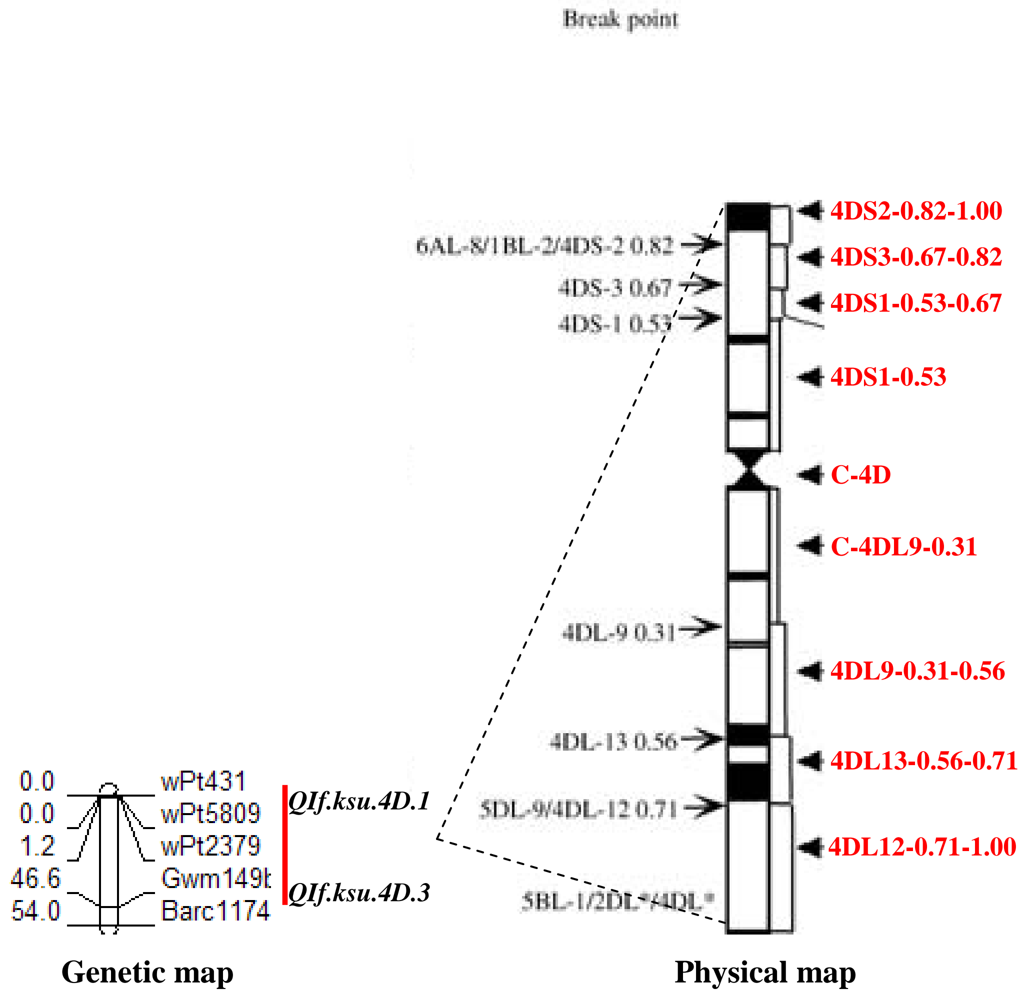
[‡]The loci *QLp.ksu-6BL.7, QIf.ksu-6BL.15, and QFs.ksu-6BL.10* with additive main effects were detected by the multi-environment analysis of QTLNetwork v2.0. The Loci *QIf.ksu-6BL.7* and *QIf.ksu-6BL.18* were involved in epistatic interactions with loci on chromosome 4D. Resistance alleles for *QLp.ksu-6BL.7* (*QIf.ksu-6BL.7*), *QFs.ksu-6BL.10*, and *QIf.ksu-6BL.15* were contributed by the susceptible parent 'Avocet S', whereas resistance allele for *QIf.ksu-6BL.18* was contributed by the resistant parent 'Amadina'.

Figure 4.11 Partial Linkage map showing likely genomic location on chromosome 2D conditioning reduced infection frequency detected by the multi-environment analyses.



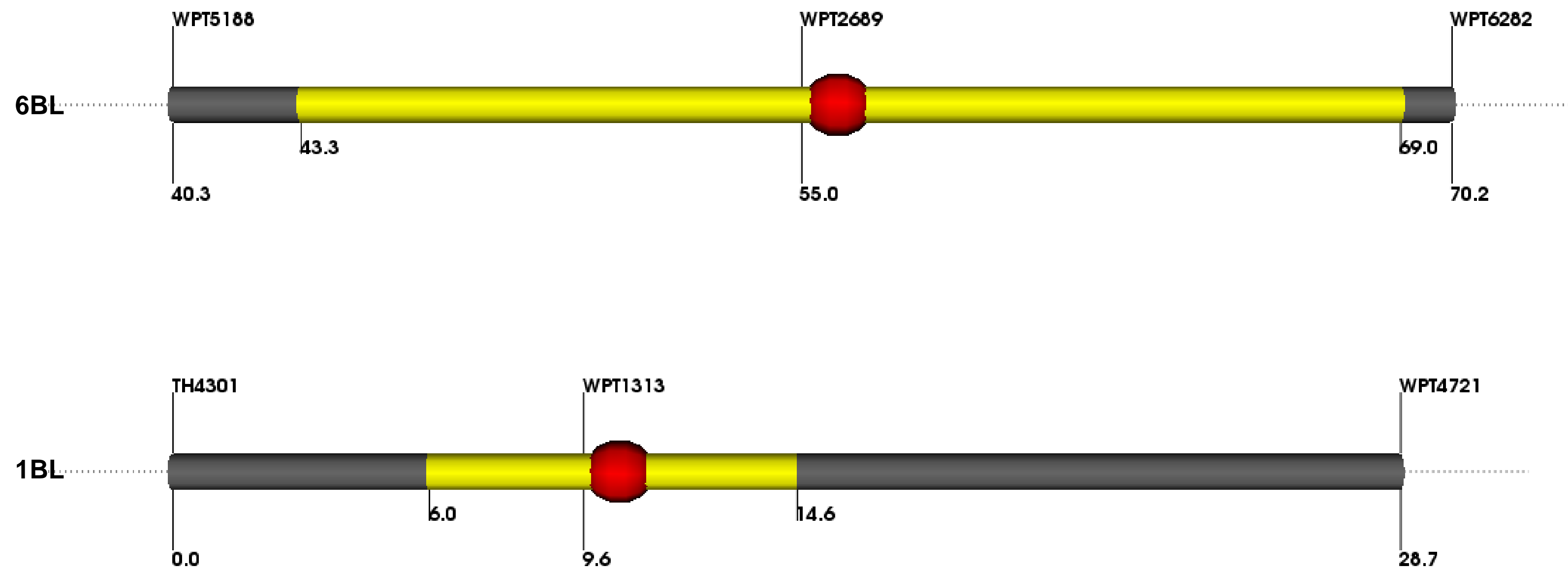
‡The locus *QIf.ksu-2DS.3* was only detected by the multi-environment analyses of QTLNetwork v2.0. Resistance allele for this locus was contributed by the susceptible parent 'Avocet S'.

Figure 4.12 Partial Linkage map showing likely genomic location on chromosome 4D conditioning reduced infection frequency detected by the multi-environment analyses.



‡The locus *QIf.ksu-4D.3* and *QIf.ksu-4D.1* were involved in epistatic interactions with loci, *QIf.ksu-6BL.7* and *QIf.ksu-6BL.18*, on chromosome 6BL. Allele for *QIf.ksu.4D.1* was derived from the susceptible parent 'Avocet', and allele for *QIf.ksu-4D.3* was derived from the resistant parent 'Amadina'. The locus could not be assigned to a chromosome arm due to unavailability of sequence information of the DArT markers, *wPt431*, *wPt5809*, and *wPt2379*.

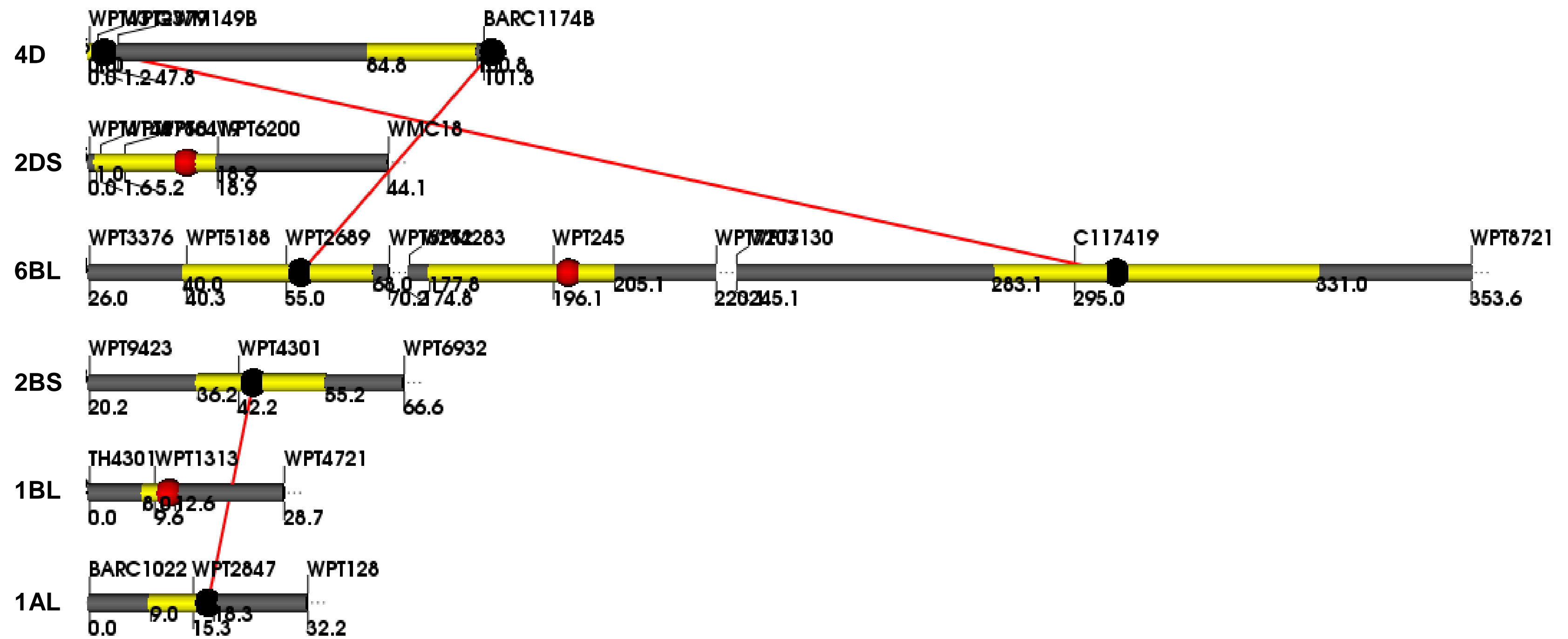
Figure 4.13 The genetic architecture of genomic regions (QTL) associated with latent period (LP) generated with QTLNetwork 2.0.



[†] - Additive main effect; - No additive main effect; - Epistasis; - QTL support interval.

[‡]QTL with additive effect were detected on chromosomes 6BL and 1BL (i.e. the *Lr46/Yr29* locus). There was no digenic epistatic interaction between the two QTL.

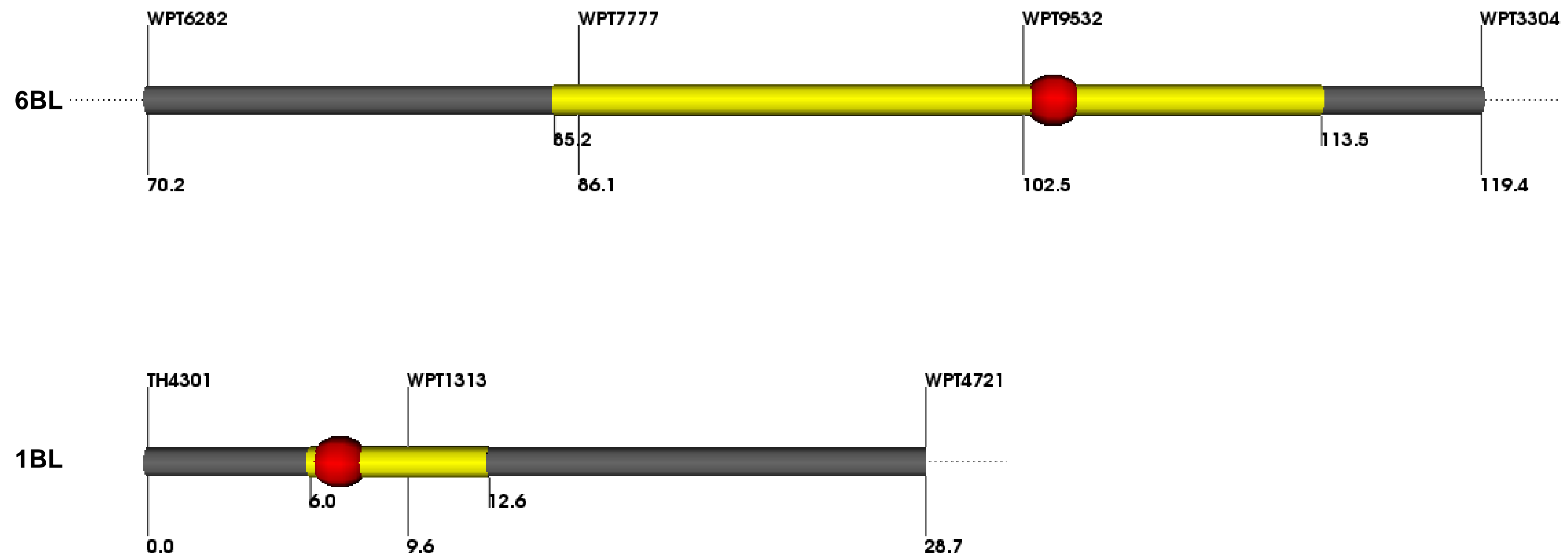
Figure 4.14 The genetic architecture of genomic regions (QTL) associated with transformed infection frequency (IF) generated with QTLNetwork 2.0.



[†] - Additive main effect; - No additive main effect; - Epistasis; - QTL support interval.

[‡]QTL with additive effect on chromosomes 2DS, 6BL, and 1BL (i.e. the *Lr46/Yr29* locus) were not involved in epistatic interactions. Digenic epistatic interactions between QTL on chromosomes 4D and 6BL, and QTL on chromosomes 2BS and 1AL were detected.

Figure 4.15 The genetic architecture of genomic regions (QTL) associated with final disease severity (FS) generated with QTLNetwork 2.0.



[†] - Additive main effect; - No additive main effect; - Epistasis; - QTL support interval.

[‡]QTL with additive effect were detected on chromosomes 6BL and 1BL (i.e. the *Lr46/Yr29* locus). There was no digenic epistatic interaction between the two QTL.

Table 4.1 Summary of latent period (LP), infection frequency (IF), and final disease severity (FS) reactions for the 'Avocet S' x 'Amadina' population

	Latent period (days)		Infection frequency $[(\text{number of pustules/m}^2)]^{1/2}$		Final disease severity (%)	
	Env. I	Env. II	Env. I	Env. II	Env. I	Env. II
Avocet	6	6	12	13	80	80
Amadina	14	14	4	4	5	5
Population mean	10	10	8	8	36	35
Range low	6	6	0	0	0	0
Range high	15	15	17	17	100	100

Scores are given for the parents, population means, and highest and lowest scoring lines in each replication.

Table 4.2 Estimates of genetic (σ^2_G) and genetic x environment ($\sigma^2_{G \times E}$) variances from random effect model of latent period (LP), infection frequency (IF), and final disease severity (FS) for the 'Avocet S' x 'Amadina' population

Covariance parameters			
	Latent period (days)	Infection frequency $[(\text{number of pustules/m}^2)]^{1/2}$	Final disease severity (%)
σ^2_G	3.4705****	14.3747****	394.58****
$\sigma^2_{G \times E}$	0.4563****	0.1121*	9.5324*
h^2	91.8%	98.7%	95.3%

NS = not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. h^2 = heritability estimate.

Table 4.3 Frequency distribution of 'Avocet S' x 'Amadina' population for mean latent period (LP), infection frequency (IF), and final disease severity (FS) responses categories based on 2 environments of greenhouse data

Number of RILs with response				χ^2 value[a]			
Trait	PTR[b]	PTS[c]	Other	2 genes	3 genes	4 genes	5 genes
Latent period	8	5	137	102.63****	21.58****	2.5[NS]	2.46[NS]
Transformed	11	10	129	77.77****	9.71**	0.36[NS]	15.48***
Infection frequency							
Final severity	3	3	144	126.96****	35.28****	9.91*	1.3[NS]

Significantly different from expected ratios at *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; NS - not significantly different from expected ratios; [a] Expected ratios for X^2 tests; [b] Parental-type resistant; [c] Parental-type susceptible.

Table 4.4 Pearson correlation coefficients (*r*) among mean latent period (LP), transformed infection frequency (IF), and final disease severity (FS) data for 2 environments

	LP		Transformed IF		FS	
Trait/Run	Env. I	Env. II	Env. I	Env. II	Env. I	Env. II
LP						
Environment I	1.00	0.85****	-0.65****	-0.64****	-0.60****	-0.59****
Environment II		1.00	-0.68****	-0.67****	-0.60****	-0.59****
Transformed IF						
Environment I			1.00	0.97****	0.62****	0.64****
Environment II				1.00	0.58****	0.59****
FS						
Environment I					1.00	0.91****
Environment II						1.00

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

Table 4.5 PCR-based DArT markers and primer sequences for putative QTL associated with latent period (LP), infection frequency (IF), and final disease severity (FS)

Marker set	Sequence of primers 5'-3'	PCR amplification conditions	Amplified marker fragment size	Chrom location
wPt1313-F wPt1313-R	GTA CTC AGC GGG CTT CAG TG GGC TAG TTT ATG AGG CGG TTT C	94°C 5 min; 40 cycles (94°C – 1 min.; 61°C – 1 min.; 72°C – 2 min); 72°C – 10 min	206 bp	1BL
wPt5188-F wPt5188-R	TCT GAT GTC TGT TTC ACA TC CAT TGC TGT GGA TAA TGT CA	94°C 5 min; 40 cycles (94°C – 1 min.; 60°C – 1 min.; 72°C – 2 min); 72°C – 10 min	222 bp	6BL
wPt2689-F wPt2689-R	TCT TCC CAC AAG CAA CCA CCA AAT TAC CCA AGG TCC AC	94°C 5 min; 40 cycles (94°C – 1 min.; 60°C – 1 min.; 72°C – 2 min); 72°C – 10 min	220bp	6BL
wPt7777-F wPt7777-R	CAA GTG TTG GGT ACT GCT GAC T AAT GAG GAT GGT GTG TGT GG	94°C 5 min; 40 cycles (94°C – 1 min.; 60°C – 1 min.; 72°C – 2 min); 72°C – 10 min	400bp	6BL
wPt3304-F wPt3304-R	TGG ATG GAT CTG GAA CTG AAC CTT GGG CAA CTG CGT AGA TA	94°C 5 min; 40 cycles (94°C – 1 min.; 60°C – 1 min.; 72°C – 2 min); 72°C – 10 min	400bp	6BL
wPt3116-F wPt3116-R	TGA TGG ATG TTG CTG AGG AG AGC CGA CAC GGA TGA TAA	94°C 5 min; 40 cycles (94°C – 1 min.; 60°C – 1 min.; 72°C – 2 min); 72°C – 10 min	380	6BL

Table 4.6 Single environment QTL analysis for latent period (LP), infection frequency (IF), and final disease severity (FS) in the 'Avocet S' x 'Amadina' population

Trait/Flanking interval‡	Chromosome location	Environment I			Environment II		
		A§	SE(A)§	H^2(A)¶	A§	SE(A)§	H^2(A)¶
LP							
wPt1313†-*wPt4721*	1BL	+0.77****	±0.15	0.1493	+0.76****	±0.16	0.1432
wPt2689†-*wPt6282*	6BL	-	-	-	+0.61****	±0.15	0.0936
Transformed IF							
wPt1313†-*wPt4721*	1BL	-1.75****	±0.27	0.2088	-1.61****	±0.27	0.1927
wPt245†-*wPt7207*	6BL	-1.39****	±0.27	0.1369	-1.36****	±0.27	0.1402
FS							
wPt1313†-*wPt4721*	1BL	-11.77****	±1.46	0.3051	-	-	-
th4301†-*wPt1313*	1BL	-	-	-	-10.81****	±1.38	0.2902
wPt9532†-*wPt3304*	6BL	-	-	-	-7.06****	±1.33	0.1520

‡Flanking interval is the interval of testing point, †Marker nearest to the peak of likelihood ratio plot, §A is the additive effect in the testing point, ¶H^2(A) represents the phenotypic variation explained by A, §AA is the additive x additive epistatic interaction, ¶H^2(AA) represents the phenotypic variation explained by AA, - not detected, *P < 0.05; **P < 0.01; ***P < 0.001, ****P < 0.0001. Resistance alleles at intervals *wPt1313-wPt4721* (same as *TH4301-wPt1313*) and *wPt2689-wPt6282* were derived from resistant parent 'Amadina'. Resistance alleles at intervals *wPt245-wPt7207*, and *wPt9532-wPt3304* were derived from the susceptible parent 'Avocet S'.

Table 4.7 Estimated additive (A) and additive x environment interactions (AE) of QTL detected by the mixed linear model approach for latent period (LP), infection frequency (IF), and final disease severity (FS) in the 'Avocet S' x 'Amadina' population

Trait/QTL name	QTL effect							QTL heritability	
	Flanking interval‡	A§	SE (A§)	AE_1§	SE(AE_1§)	AE_2§	SE(AE_2§)	H^2(A)¶	H^2(AE)¶
LP									
QLp.ksu-1BL.2	*wPt1313*†*-wPt4721*	+0.75****	±0.11	NS	±0.01	NS	±0.01	0.1441	0.0001
QLp.ksu-6BL.7	*wPt2689*†*-wPt6282*	+0.56****	±0.11	NS	±0.01	NS	±0.01	0.0864	0.0004
Transformed IF									
QIf.ksu-1BL.2	*wPt1313*†*-wPt4721*	-1.77****	±0.17	NS	±0.01	NS	±0.01	0.2007	0.0003
QIf.ksu-6BL.15	*wPt245*†*-wPt7207*	-1.65****	±0.16	NS	±0.01	NS	±0.01	0.1385	0.0000
QIf.ksu-2DS.3	*wPt6419*†*-wPt6200*	-0.56**	±0.19	NS	±0.01	NS	±0.01	0.0104	0.0001
FS									
QFs.ksu-1BL.1	*th4301*†*-wPt1313*	-11.23****	±1.01	NS	±0.02	NS	±0.02	0.2949	0.0011
QFs.ksu-6BL.10	*wPt9532*†*-wPt3304*	-6.44****	±0.99	NS	±0.02	NS	±0.02	0.1178	0.0007

‡Flanking interval is the interval of testing point; §A is the additive effect in the testing point, §AE_1 and §AE_2 represent QTL x environment interaction effects for environments 1 (run I) and 2 (run II) respectively; †Marker nearest to the peak of likelihood ratio plot; ¶H^2(A) represents the phenotypic variation explained by A, NS - not significant, *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Resistance allele for *QLp.ksu-1BL.2* (same as *QIf.ksu-1BL.2* and *QFs.ksu-1BL.1*), and *QLp.ksu-6BL.7* (same as *QFs.ksu-6BL.10*) were derived from the resistant parent 'Amadina'. Resistance alleles for *QIf.ksu-6BL.15*, and *QIf.ksu-2DS.3* were derived from the susceptible parent 'Avocet S'.

Table 4.8 Estimated non-additive x non-additive epistatic (DD), additive x non-additive (AD), non-additive x non-additive epistasis x environment interaction (DDE), and additive x non-additive epistasis x environment interaction (ADE) effects of QTL detected for transformed infection frequency (IF) data in two environments

				Epistasis effect			Epistasis heritability	
QTL-j††	Flanking interval-i‡	QTL-j††	Flanking interval-j‡	DD†/ AD†/	DDE_1§/ ADE_1§	DDE_2§/ ADE_2§	H^2(DD)¶/ H^2(AD)¶	H^2(DDE)¶/ H^2(ADE)¶
QIf.ksu-1AL.2	*wPt2847*†*-wPt128*	*QIf.ksu-2BS.4*	*wPt4301*†*-wPt6932*	-0.92****	NS	NS	0.0677	0.0002
QIf.ksu-6BL.7	*wPt2689*†*-wPt6282*	*QIf.ksu-4D.3*	*Xgwm149b*†*-Xbarc1174b*	-0.78****	NS	NS	0.0540	0.0000
QIf.ksu-6BL.18	*c117419t*†*-wPt8721*	*QIf.ksu-4D.1*	*wPt431*†*-wPt2379*	-0.97****	NS	NS	0.0594	0.0003

††QTL-i and QTL-j are the QTL of testing points i and j respectively, ‡Flanking interval-i and Flanking interval-j are the intervals of testing points i and j, †DD or AD are the non-additive-by-non-additive or additive x non-additive interactions between testing points i and j, respectively, §DDE_1, or ADE_1 and §DDE_2 or ADE_2 are the non-additive x non-additive or additive x non-additive epistasis x environments 1 (run I), and 2 (run II), ¶H^2(DD) or H^2(AD) represents the phenotypic variation explained by DD or AD, ¶H^2DDE or H^2ADE represent the phenotypic variation explained by DDE or ADE. NS = not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, *$P < 0.0001$. †Marker nearest to the peak of likelihood ratio plot. Resistance alleles for *QIf.ksu-1AL.2* x *QIf.ksu-2BS.4* and *QIf.ksu-6BL.18* x *Qif.ksu-4D.1* were derived from the susceptible parent 'Avocet S', whereas resistance alleles for *QIf.ksu-6BL.7* x *QIf.ksu-4D.3* were derived from the resistant parent 'Amadina'.

Figure 4.16 Pearson-moment correlations for presence of alleles between flanking markers for putatively epistatic loci affecting infection frequency in the ‘Avocet S’ x ‘Amadina’ population

	wPt2847 -1AL	wPt128 -1AL	wPt4301 -2BS	wPt6932 -2BS	*wPt2689 -6BL*	*wPt6282 -6BL*	*Xgwm149b -4D*	*Xbarc1174b -4D*	*c117419 -6BL*	*wPt8721 -6BL*	*wPt431* -4D	*wPt2379 -4D*
wPt2847 -1AL	1.00	0.94****	-0.02^{NS}	-0.01^{NS}	-0.04^{NS}	0.00^{NS}	-0.10^{NS}	-0.08^{NS}	0.02^{NS}	0.09^{NS}	-0.07^{NS}	-0.07^{NS}
wPt128 -1AL		1.00	0.05^{NS}	-0.08^{NS}	-0.03^{NS}	0.01^{NS}	-0.10^{NS}	-0.09^{NS}	0.02^{NS}	0.05^{NS}	-0.13^{NS}	-0.05^{NS}
wPt4301 -2BS			1.00	-0.92****	-0.04^{NS}	-0.04^{NS}	-0.02^{NS}	-0.12^{NS}	0.07^{NS}	-0.04^{NS}	-0.15^{NS}	-0.12^{NS}
wPt6932 -2BS				1.00	0.05^{NS}	0.08^{NS}	-0.01^{NS}	0.13^{NS}	-0.10^{NS}	-0.03^{NS}	0.13^{NS}	0.11^{NS}
wPt2689 -6BL					1.00	0.97****	0.17^{NS}	0.14^{NS}	-0.38****	-0.29**	0.03^{NS}	0.06^{NS}
wPt6282 -6BL						1.00	0.14^{NS}	0.15^{NS}	-0.36****	-0.26**	0.06^{NS}	0.07^{NS}
Xgwm149b -4D							1.00	0.75****	-0.09^{NS}	-0.06^{NS}	0.22*	0.26**
Xbarc1174b -4D								1.00	-0.13^{NS}	-0.13^{NS}	0.26**	0.27**
c117419 -6BL									1.00	0.72****	-0.08^{NS}	-0.09^{NS}
wPt8721 -6BL										1.00	-0.18*	-0.20*
wPt431 -4D											1.00	0.95****
wPt2379 -4D												1.00

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, NS – not significant

Bibliography

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CHAPTER 5 - ERDAS-based method for wheat leaf rust pustule classification and size estimation

Abstract

Leaf rust, caused by *Puccinia triticina* ex Desmaz. f. sp. *triticina*, is one of the most damaging diseases of wheat. Leaf rust resistance breeding has largely shifted from the nondurable race-specific resistance to race-non-specific, slow-rusting resistance. The important components of slow leaf-rusting resistance are prolonged latent period, reduced frequency of infection, and reduced pustule size. Therefore, better understanding of the genetic and molecular basis of slow leaf-rusting depends on accurate determination of the components. We used remote sensing and geographic information systems (GIS) image analysis and analytical operations to discriminate leaf rust pustules from other parts of leaf and to determine pustule size in the slow leaf-rusting resistant CIMMYT wheat breeding line 'Amadina', and the leaf rust susceptible cultivar 'Avocet S'. Three of the main applications of remote sensing and GIS image analysis in this study were leaf area computation, classification of areas where leaf rust pustules developed, and assessment of classification accuracy. The mean size of all pustules in image of flag leaf of 'Amadina' was 0.0653 mm^2, and that of 'Avocet S' was 0.1522 mm^2. This approach provides a means to determine the size of leaf rust pustules in wheat cultivars with different levels of resistance. In addition, this approach has the potential to be effectively used in determination of pustule sizes in a mapping population for identification of genomic regions conditioning the reduced pustule size component.

Introduction

Leaf rust (caused by *Puccinia triticina* Roberge ex Desmaz. f. sp. *triticina*) is globally an important fungal disease that causes significant yield losses in wheat (Knott 1989). Leaf rust resistance breeding has largely shifted from the nondurable race-specific resistance to race-non-specific, slow rusting resistance. Expression of slow leaf-rusting resistance as a reduction in the rate of disease development in the host, despite a compatible host-pathogen interaction is caused by prolonged latent period, reduced infection frequency, and reduced pustule size (Ohm and Shaner 1976).

Understanding of the genetic and molecular basis of this type of resistance depends on accurate determination of each of the components. Das et al. (1993) proposed the following formula for calculating latent period (number of days from inoculation to the appearance of 50% of the pustules): Latent period = $t_1 + [(F/2 - nt_1)(t_2 - t_1)/(nt_2 - nt_1)]$ where F = final number of pustules, t_1 = day before 50% pustules erupted, t_2 = day after 50% pustules erupted, nt_1 = number of pustules erupted at t_1, nt_2 = number of uredinia erupted at t_2. Singh and Huerta-Espino (2003) calculated infection frequency (lower receptivity) by dividing the number of pustules by the surface of a marked leaf area. Lee and Shaner (1985) calculated pustule size by multiplying (length x width x π)/4. The formula proposed by Lee and Shaner is subject to error that might result from bias during selection of pustules for measurement. Furthermore, the formula proposed by Lee and Shaner (1985) cannot accurately determine sizes of each pustule since the shape of leaf rust pustules scattered on a leaf surface can be either circular or oval.

Here we describe the use of a remote sensing object classification approach and geographic information systems (GIS) image analysis and analytical operations to discriminate rust pustules from other parts of the wheat leaf and to determine pustule size and cover.

Plant material

The experiment was conducted at The Plant Sciences Center, Kansas State University. The plant material consisted of a highly leaf rust-susceptible hexaploid wheat, Triticum aestivum L., cultivar 'Avocet S' (WW-119/WW-15//Egret) and slow leaf-rusting resistant hexaploid wheat, cultivar 'Amadina' (Bobwhite/Crow//Buckbuck/Pavon 76/3/Veery#10).

Inoculation

Plants with most secondary tillers at similar stage of anthesis were uniformly and heavily inoculated with urediospores of leaf rust isolate TPBS (PRTUS 55) suspended in Soltrol 170 light oil (Chevron Phillips Chemical Company, The Woodlands, TX). The isolate TPBS has the virulence/avirulence formula: *Lr1*, *Lr2a*, *Lr2c*, *Lr3*, *Lr9*, *Lr16*, *Lr24*, *Lr26*, *Lr3ka*, *LrB*, *Lr10*, *Lr14a*, *Lr21*, *Lr28*, *Lr50*, *Lr23*. Inoculated plants were incubated

overnight in a mist chamber in a greenhouse at 20º C ±3ºC with 100% relative humidity for 14 hrs. After the incubation, plants were transferred to a greenhouse.

Flag leaves were detached after sporulation of the pathogen stopped. The detached leaves then mounted on a graph paper using scotch tape. Pictures of pustules on surface of flag leaf from each F_7 were taken by positioning a Nikon Macro Cool Light SL-1 attached to a Nikon digital camera COOLPIX 4500 (Fig 5.1) in the middle of the detached leaf. The camera settings were: Focus model - Macro close-up; Aperture - ¼; Focus - 9.6. Nikon Macro Cool Light SL-1 enables easy macro photography without the need for special lighting equipment or technique. The SL-1 features 8 extremely bright white LEDs, which illuminate the subject

Pustule discrimination and size estimation

The area of each picture element (pixel) of the scanned leaves was computed by counting the number of pixels within a known unit area on the graph paper on which the leaves were positioned before they were photographed. Width of lines on the graph paper was measured using a magnifying loop with 0.1 mm measurement increments. Number of pixels per line width was determined by zooming in on the line using the Earth Resources Data Analysis Software (ERDAS) ver. 9.1 image processing software (ERDAS, Inc., Narcross, GA). Width of the lines on the graph paper was determined to be 0.2 mm, and the number of pixels per 0.2 mm wide line was 20 (0.01 mm/pixel). The width and length of each pixel was calculated by dividing the line width (i.e. 0.2 mm) by the number of pixels per line width (i.e. 20). Thus from this method it was determined that each pixel represented 0.0001 mm^2 (0.01 mm x 0.01 mm). This unit was later used during the process of computing the area within each leaf rust pustule.

The Adobe Photoshop software cropping tool was used to crop each photograph so that the leaf filled the entire field of view for each picture (Fig 5.2a, b). Once the photographs were cropped, the file extension was converted from “JPG” to “TIF” for import into Earth Resources Data Analysis System (ERDAS) software program that combines remote sensing and GIS analytical capabilities. The areas where leaf rust pustules developed were classified using an Iterative Self-Organizing Data Analysis Technique (ISODATA) unsupervised classification approach (Tou and Gonzalez 1977;

Sabins 1987; Jain 1989). This method was used to find clusters of pixel values in attribute-space and refine the resultant output clusters based on the spatial proximity of pixels within attribute space clusters and the specified maximum number of clusters. The classification approach is deemed unsupervised because the output clusters are not defined beforehand. This approach was used to generate 45 spectral classes that were associated with the leaf rust pustules (Fig 5.3a, b). Mixed classes were then reclassified into 25 classes using a "cluster busting" approach (Fig 5.4a, b) (Jensen et al. 1987). The "cluster busting" approach was accomplished as follows: Pixels belonging to unidentified clusters were extracted from the image by using a binary mask. Unsupervised classification was then run on these pixels to see if they could be reassigned to a meaningful cluster. Newly clustered pixels were added back to the original image. The iterative process was continued until an acceptable level of classification accuracy was obtained.

An ocular approach was used to assess classification accuracy. This was accomplished by overlaying the classification map onto the original image (Fig 5.5a, b) and "flickering" the classification map on and off to determine to what degree the pustule classes aligned with the pustules observed on the original image. The cluster busting approach was continued until the image was classified, and the process no longer yielded improvements in classification accuracy. The ERDAS clumping and sieving algorithms were used to remove small groups of pixels ("salt and pepper" in appearance across the image) from the classification map. This was done to "clean" the map of small mixed pixel classes and reduce noise in the image (Fig 5.6a, b). A visual assessment of the classification map compared to the original image indicated a very high degree of agreement between the two maps and the image.

The pixels are clumped using the four neighbor method (i.e., clumping of pixels at the boundary or edge with different classified). After clumping all pustules, the sieve tool in ERDAS was used to remove clumps less than 100 pixels (Fig 5.7a, b).

Once the classification process was completed (Figure 5.8a, b), the ERDAS generated maps were imported into Arc/GIS raster program (ESRI, Redlands, CA) where each pustule on the map was assigned a unique identifier. The attribute table was exported to Microsoft Excel to calculate the average area of pustule.

Data analysis

The characteristic appearance of pustules observed on 'Avocet S' and 'Amadina' is shown in Figure 5.2. 'Avocet S' displayed susceptible response, i.e., pustules were orange-brown in color, oval to diamond shaped, large in size, and not surrounded by chlorosis and necrosis. On the other hand, 'Amadina' displayed partial resistance response, i.e., pustules were orange-brown in color, circular to oval, small to medium in size, and surrounded by chlorosis. Flecked and necrotic areas were also observed on 'Amadina' leaves.

We used the SAS version 9.2 (SAS Institute, NC) procedure PROC TTEST for paired t-test analyses of the means of pustule size data of the susceptible wheat 'Avocet S' and the slow-rusting resistant wheat 'Amadina. The PROC TTEST tests whether the difference in means between 'Avocet S' and 'Amadina' was zero. Thus the null hypothesis (two sided) is:

Ho $\mu_{difference} = 0$ (The mean difference is 0)

Ha $\mu_{difference} \neq 0$ (The mean difference is not 0)

The paired t-test out put is shown in Table 5.1. The two means differ by 0.1522 – 0.0653 = 0.0869. The paired t-test analysis was performed on the mean of the differences (i.e. 0.3922). The standard error was 0.3139. The paired t-test yielded P = 0.0213, which is statistically significant.

Pustule size is considered one of the important components of slow leaf-rusting trait and influences the area under disease progress curve (AUDPC). Association of small pustule size with slow leaf-rusting was reported in wheat (Ohm and Shaner 1976). Pustule size is also related with sporulation, i.e. bigger pustules produce more spore than small ones (Habtu and Zadoks 1995). The variation in 'Avocet S' and 'Amadina' for pustule size in the present study coupled with the fact that environment and growth stages have less influence on its expression offers the possibility of using pustule size as a reliable component of predicting slow leaf-rusting resistance under controlled conditions in the greenhouse. Mapping of genomic regions conditioning reduced pustule size, and identification of markers closely linked to the genomic regions can open up the possibility of combining different components of slow leaf-rusting resistance into single genetic background by marker-assisted breeding (MAB).

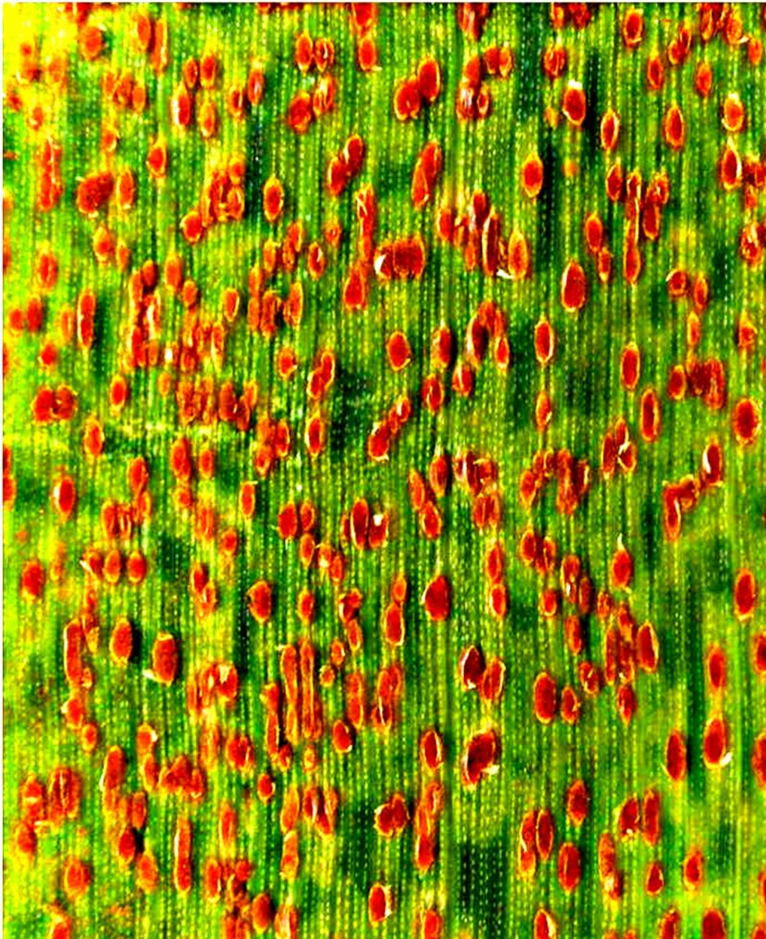
The results in this study show that remote sensing and geographic information systems (GIS) image analysis and analytical operations has the potential to be effectively used for accurate discrimination of rust pustules from wheat leaves, and for estimation of pustule size. The method described in this study is not subject to bias because the sizes of all pustules covered by the camera were determined.

Figures and Tables

Figure 5.1 Nikon Digital camera COOLPIX 4500

Figure 5.2 Cropped images of (a) leaf rust susceptible *cv* 'Avocet S' and (b) slow leaf-rusting resistant *cv* 'Amadina'

(a)



(a)

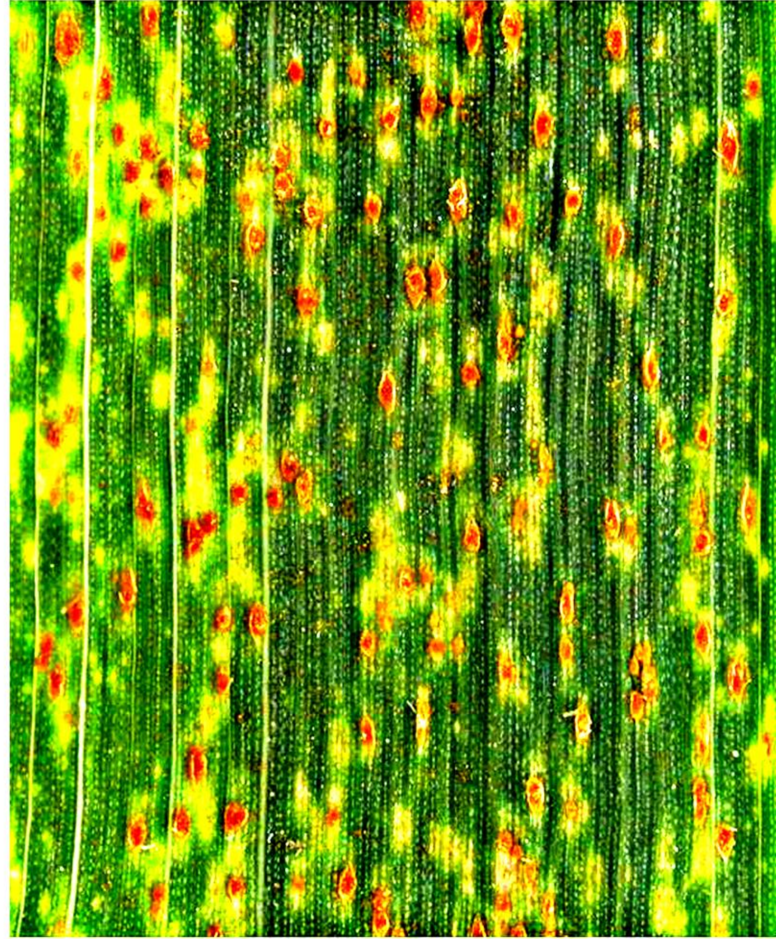
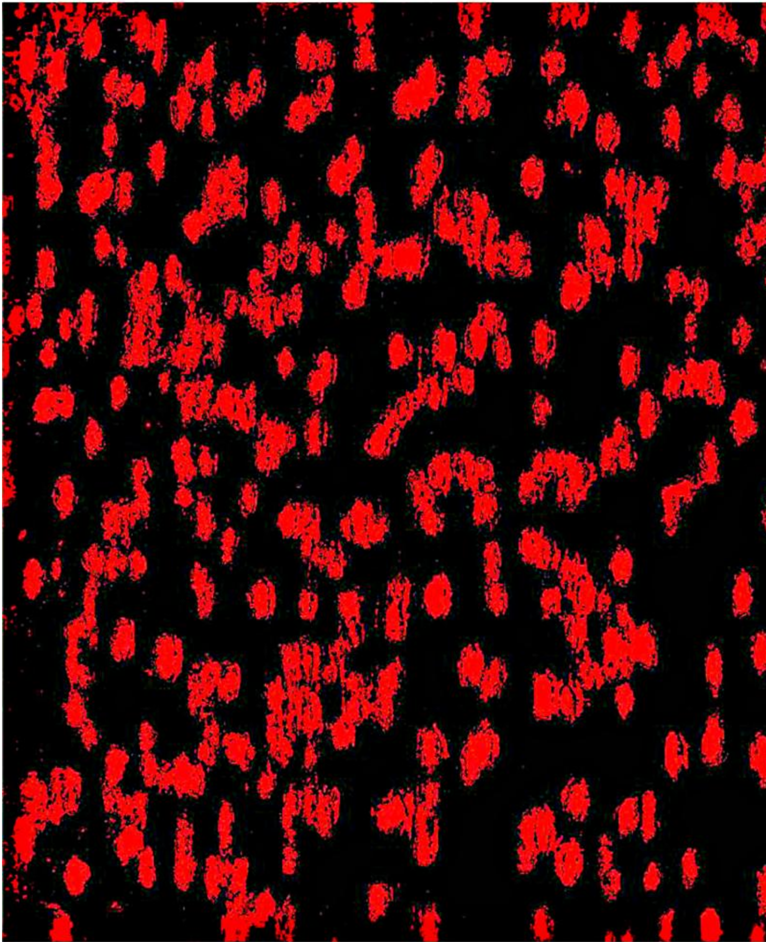
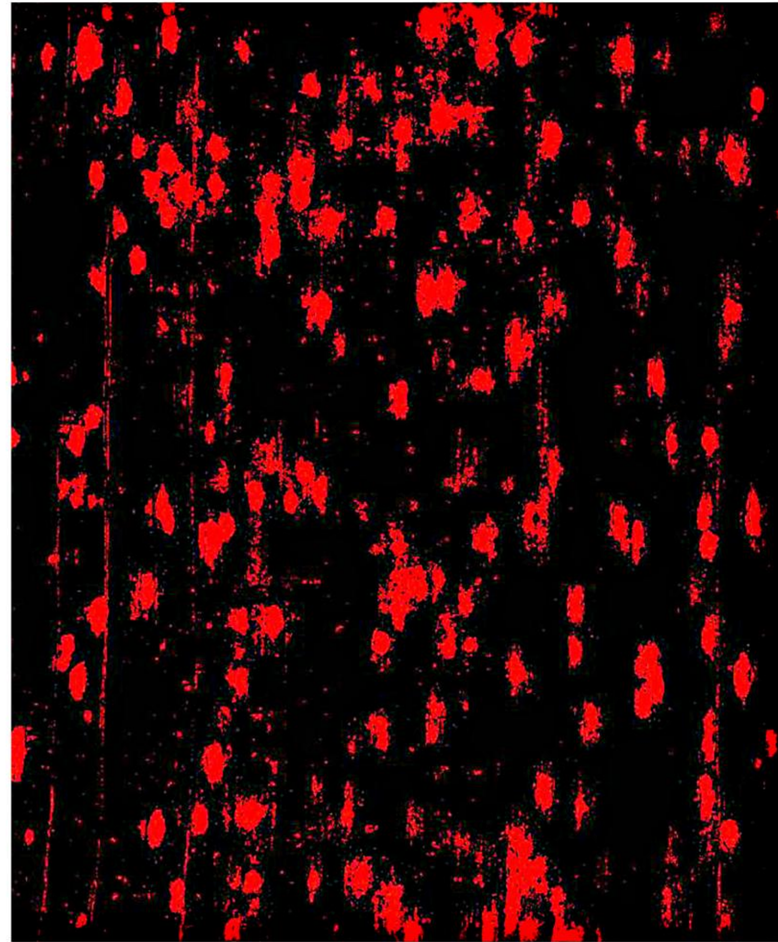


Figure 5.3 Forty-five (45) class unsupervised classification (a) leaf rust susceptible *cv* 'Avocet S' and (b) slow leaf-rusting resistant *cv* 'Amadina'

(a)



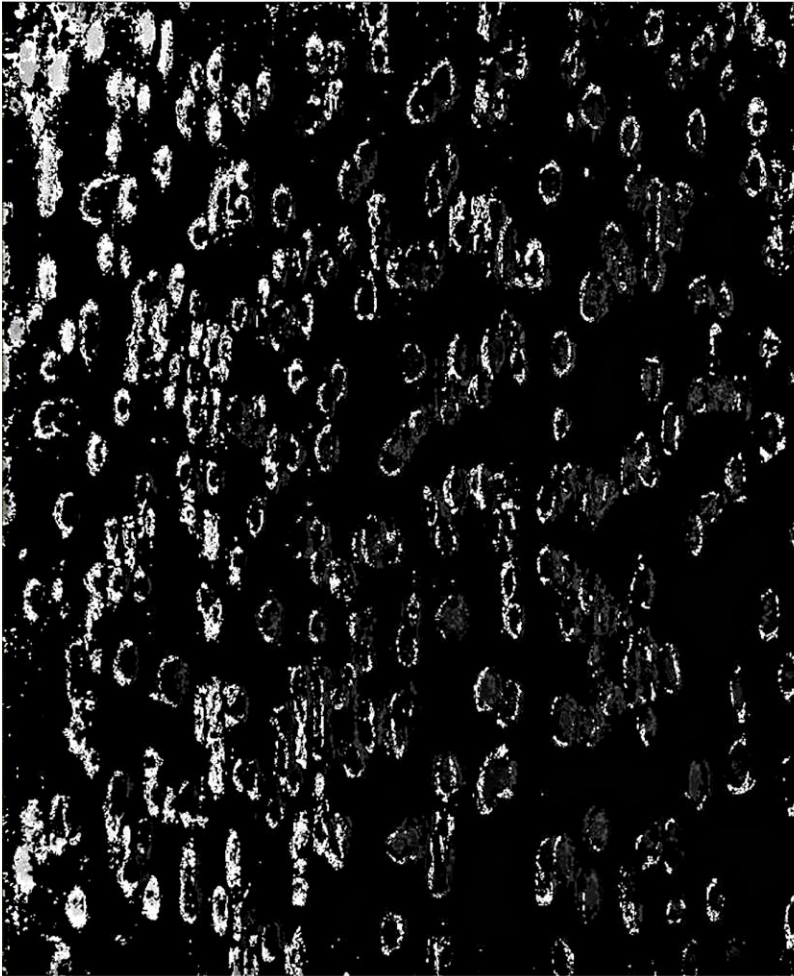
(a)



**Red pixels are pustule classes and mixed classes

Figure 5.4 Twenty-five (25) classes from unsupervised classification (Clusterbusting) of (a) leaf rust susceptible *cv* 'Avocet S' and (b) slow leaf-rusting resistant *cv* 'Amadina'

(a)



(a)

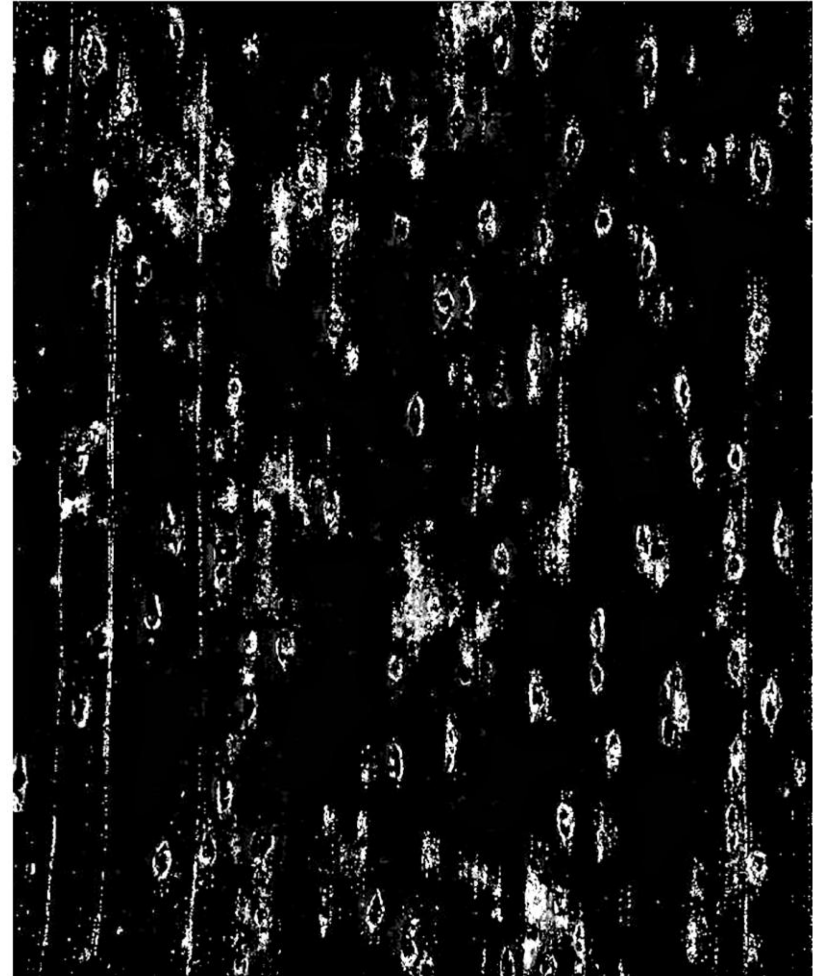
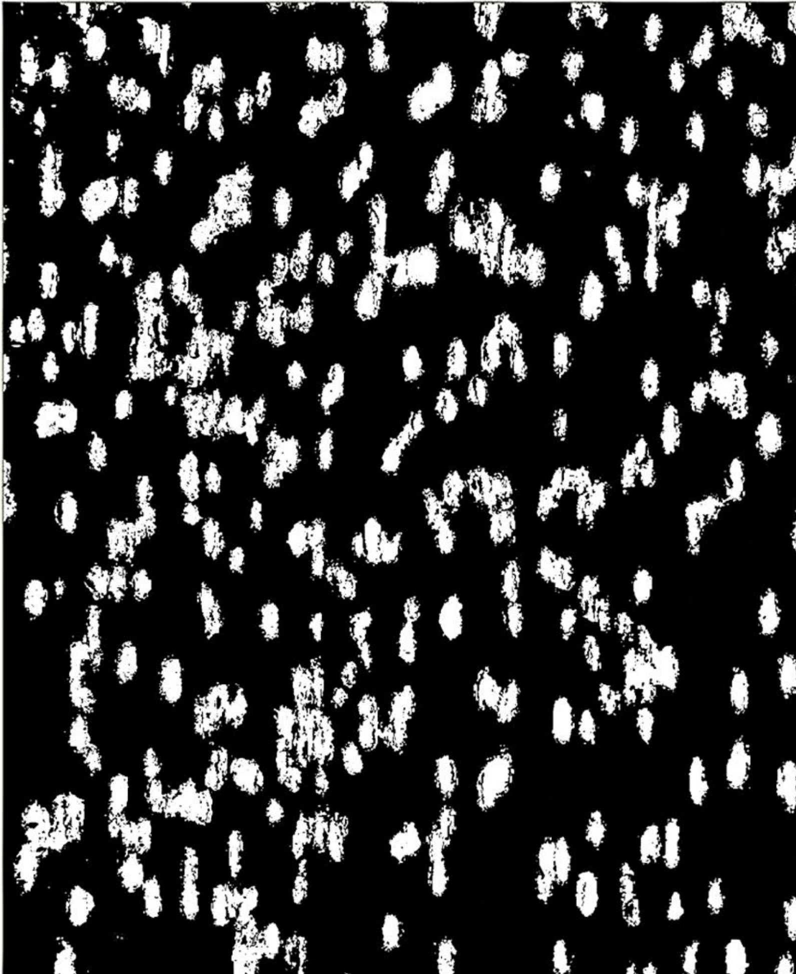


Figure 5.5 Final pustule classes of leaf rust susceptible *cv* 'Avocet S' and (b) slow leaf-rusting resistant *cv* 'Amadina' using overlay function

(a)



(a)

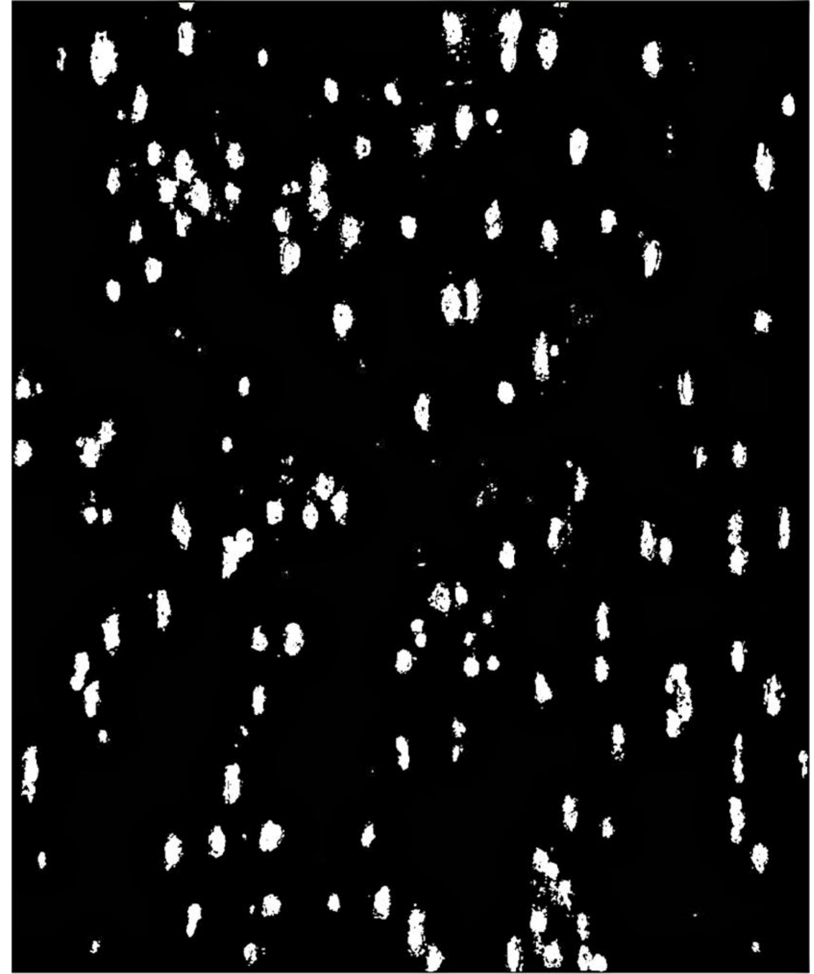
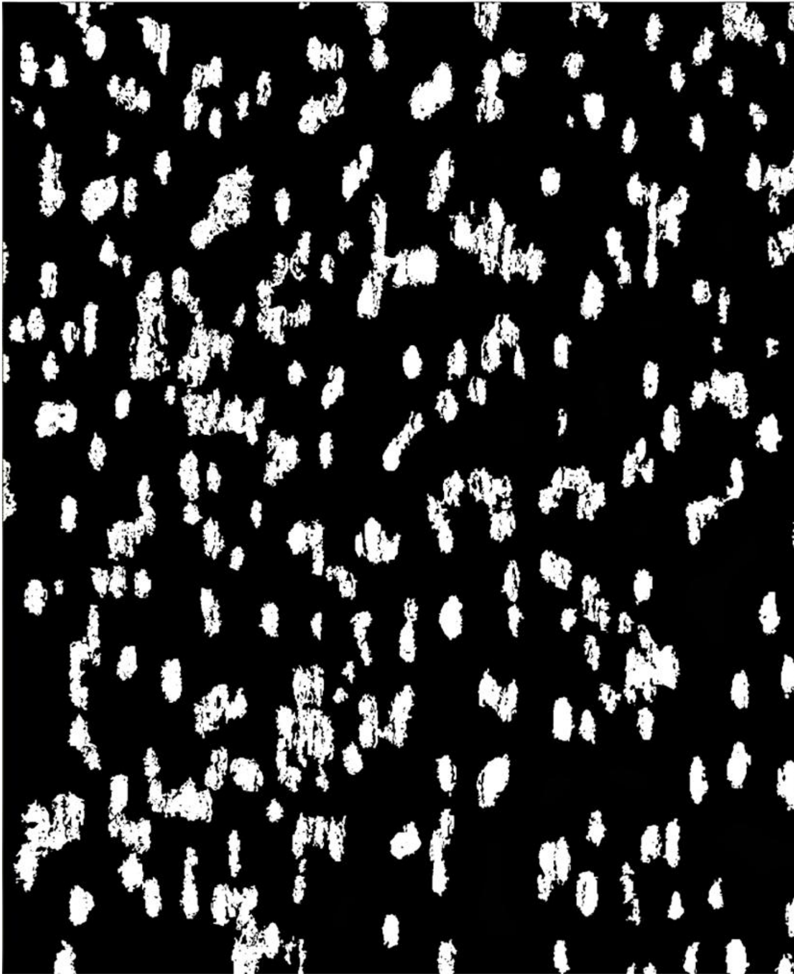
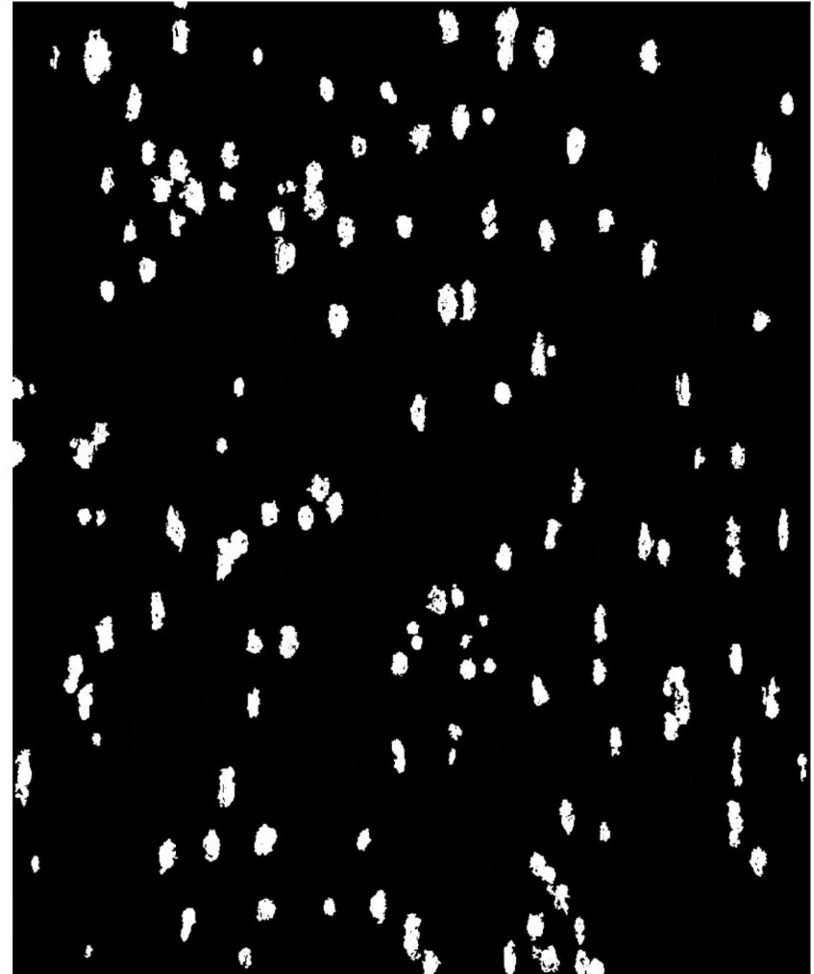


Figure 5.6 Final pustule classification of leaf rust susceptible *cv* 'Avocet S' and slow leaf-rusting resistant *cv* 'Amadina' images using sieve function

(a)

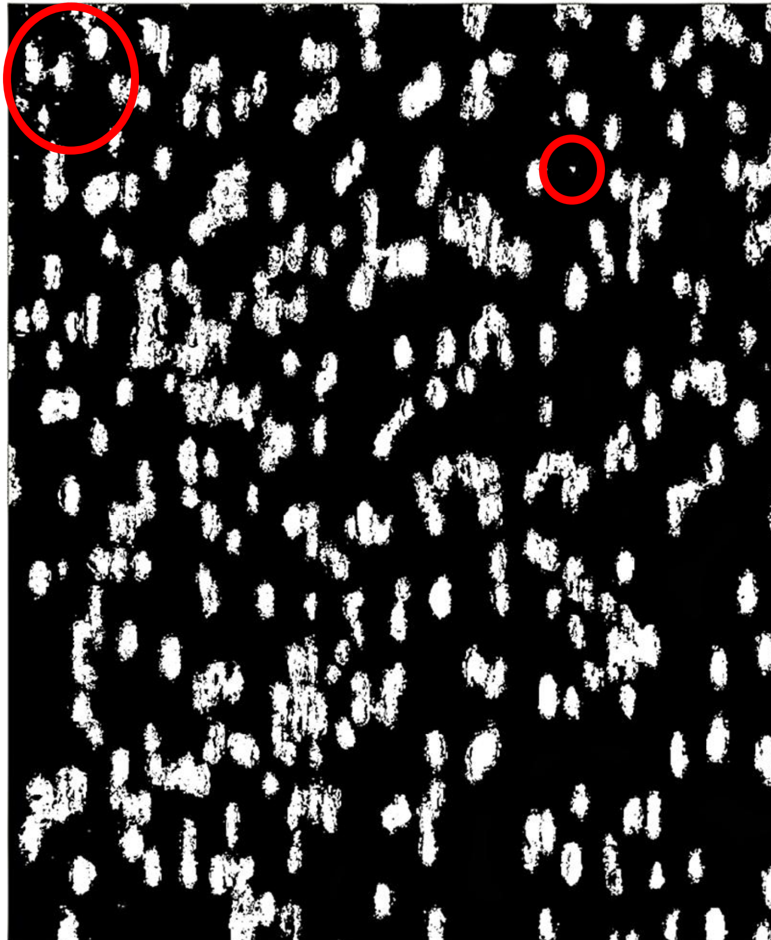


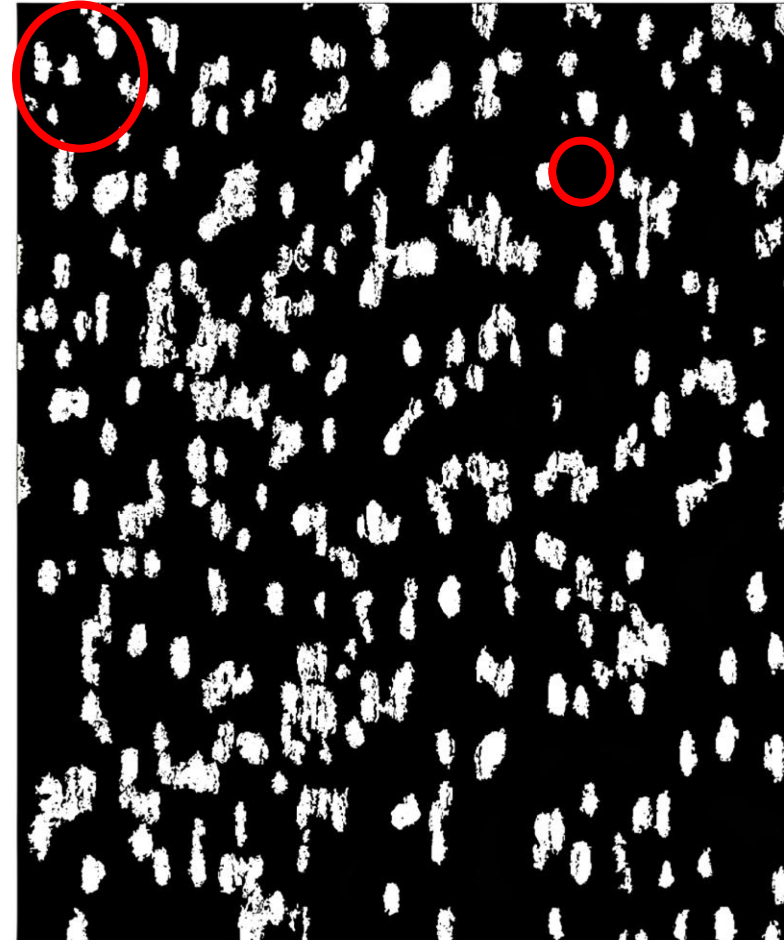
(a)



**Clumps that contained less than 100 pixels were removed from final image

Figure 5.7 (a) Overlay and (b) sieve image of leaf rust susceptible *cv* 'Avocet S'





**Red circles indicate areas where random pixels that are actual pustules have been removed from image using clump and sieve method

Figure 5.8 Final pustule classifications of (a) leaf rust susceptible *cv* 'Avocet S' and (b) slow leaf-rusting resistant *cv* 'Amadina'

(a)

(a)

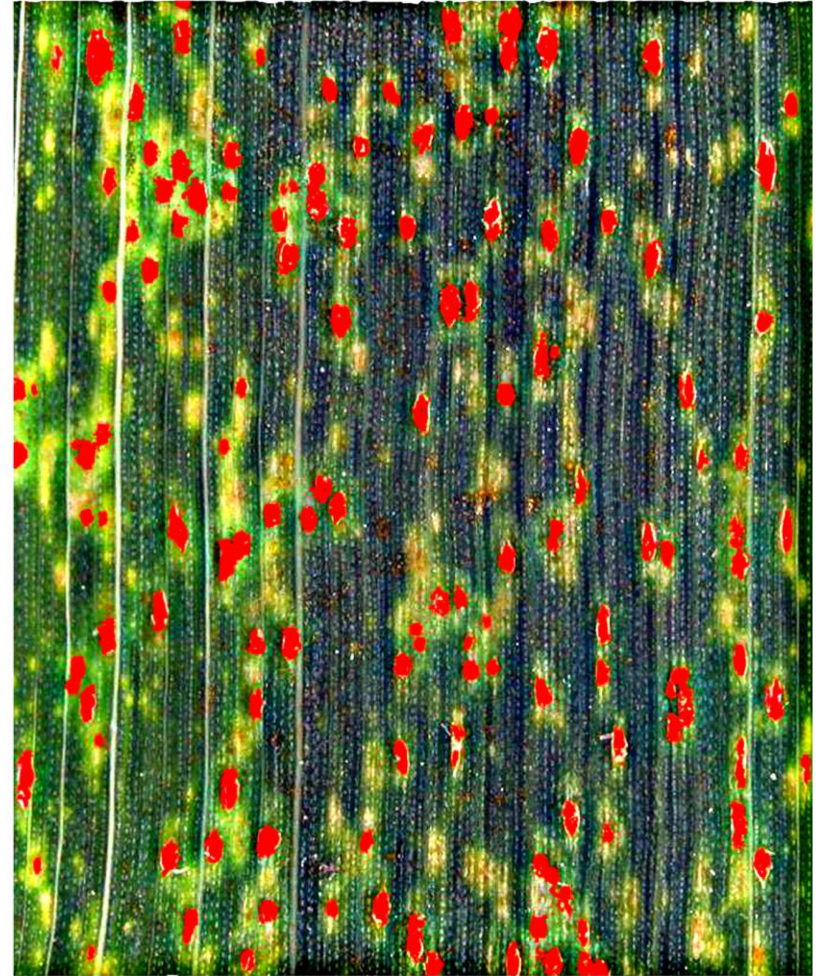


Table 5.1 The TTEST Procedure

Statistics								
	Mean pustule size		T-Tests					
Difference	Avocet S	Amadina	Mean difference	Std Dev	Std Error	DF	t-value	Pr > | t |
Cultivar – Pustule size	0.1522	0.0653	0.0869	0.3557	0.3139	3	4.43	0.0213

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