GENETIC MAPPING OF QUANTITATIVE TRAIT LOCI FOR SLOW-RUSTING TRAITS IN WHEAT

by

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B.S., Yangzhou University, China 2008
M.S., Yangzhou University, China 2011

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Abstract

Wheat leaf rust, caused by *Puccinia triticina*, is an important fungal disease worldwide. Growing resistant cultivars is an effective practice to reduce the losses caused by the disease, and using slow-rusting resistance genes can improve the durability of rust resistance in the cultivars. CI13227 is a winter wheat line that shows a high level of slow-rusting resistance to leaf rust and has been studied extensively. In this research, two recombinant inbreed line (RIL) populations derived from CI13227 x Suwon (104 RILs) and CI13227 x Everest (184 RILs) and one doubled haploid (DH) population derived from CI13227 x Lakin with 181 lines were used to identify quantitative trait loci (QTLs) for slow leaf rusting resistance. Each population and its parents were evaluated for slow-rusting traits in two greenhouse experiments. A selected set of 384 simple sequence repeat markers (SSRs), single nucleotide polymorphism markers (SNPs) derived from genotyping-by-sequencing (GBS-SNPs) or 90K-SNP chip (90K-SNPs) were analyzed in the three populations. Six QTLs for slow-rusting resistance, *QLr.hwwgru-2DS*, *QLr.hwwgru-7BL*, *QLr.hwwgru-7AL*, *QLr.hwwgru-3B_1, QLr.hwwgru-3B_2,* and *QLr.hwwgru-1D* were detected in the three populations with three stable QTLs, *QLr.hwwgru-2DS*, *QLr.hwwgru-7BL* and Q*Lr.hwwgru-7AL*. These were detected and validated by Kompetitive Allele-Specific PCR (KASP) markers converted from GBS-SNPs and 90K-SNPs in at least two populations. Another three QTLs were detected only in a single population, and either showed a minor effect or came from the susceptible parents. The KASP markers tightly linked to *QLr.hwwgru-2DS* (IWB34642, IWB8545 and GBS_snpj2228), *QLr.hwwgru-7BL* (GBS_snp1637 and IWB24039) and *QLr.hwwgru-7AL* (IWB73053 and IWB42182) are ready to be used in marker-assisted selection (MAS) to transfer these QTLs into wheat varieties to improve slow-rusting resistance in wheat.

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Chapter 1 - Literature Review

Origin and evolution of wheat

As one of the major crops in the world, wheat feeds nearly half of the world's population. It is the third largest crop next to rice and corn, and occupies one-sixth of crop acreage worldwide (Curtis 2002). Compared with other cereals, wheat is superior in its nutritive value. About 20% of the total calories consumed by the human population are supplied by wheat. It is not only the major source of carbohydrates, but also an important protein source for a majority of the world's population (Carver 2009). With the rapid growth of world's population, increasing wheat production is becoming the first priority to meet the need for world's food security (Braun et al. 1998; Rosegrant et al. 1995).

Wheat originated in the southwestern part of Asia about 10,000 B.C. Today, some remains of the earliest crops such as *Triticum boeoticum*, *Triticum dicocoides Korn*, and *Aegilops tauschii* can be found in west and southwest Asia (Gibson and Benson 2002). Emmer and Einkorn grown in the southern Levant and southeastern Turkey, respectively, were the two most primitive wild wheats identified by archeologists and geneticists dating back to 9,600 to 7,500 B.C. (Faustino Carvalho 2015; Heun et al. 1997). Around 9000 years ago, durum wheat (AABB), an emmer mutant, was selected (Landi 1995). Bread wheat, as the major type of commercial wheat grown today, was first found in Iran and derived from a cross between wild goatgrass and cultivated durum wheat about 6700 years ago (Marcussen et al. 2014). In the U.S., wheat was first planted on an island off the Massachusetts coast in 1602, and has now become the principal cereal grain produced in almost every state in the country (Gibson and Benson 2002).

Genetically, wild and cultivated wheat include diploid, tetraploid, and hexaploid species. Of the two diploid wheat species, *Triticum urartu* existed only in wild form and *Triticum monococcum* had both wild and cultivated forms. *Triticum urartu* was considered to be the A genome donor in wheat evolution (Chapman et al. 1976). The tetraploid wheat species include *Triticum timopheevii* (AAGG genome) and *Triticum turgidum* (AABB genome), which had both cultivated and wild forms (Gill and Friebe 2002). The two hexaploid wheat, *Triticum aestivum* (AABBDD genome) and *Triticum zhukovskyi* (AmAmAAGG genome) were only found in cultivated form (Gill and Friebe 2002). There were two branches for polyploid wheat evolution revealed in the previous genetic studies as showed in Figure 1.1: one is the branch between *Triticum turgidum* (AABB genome) and *Triticum aestivum* (AABBDD genome) and the other is between *Triticum timopheevii* (AAGG genome) and *Triticum zhukovskyi* (AmAmAAGG genome) (Huang et al. 2002). Two instances of amphidiploidization were involved in the origin of today's common bread wheat, a hexaploid (AABBDD genome). First was the hybridization between two diploid ancestors, *Aegilops* and *Triticum,* to form allotetraploid wheat (AABB genome) with the modified B genome derived from *Ae. speltoides* (SS genome) (Feldman 1976). The second was a hybridization between tetraploid wheat and *Ae. Tauschii* (D genome donor) to form hexaploid wheat (AABBDD genome) (Kihara 1944).

During domestication, cultivated wheat was first selected for large grain and other desirable traits from a wild wheat population without using modern breeding methods. Two important domestication traits to be mentioned here are non-shattering and free-threshing (Shewry 2009). The non-shattering trait was associated with a mutation at the

Br (brittle rachis) locus (Nalam et al. 2006). Rachis shattering resulted in wheat yield losses as seeds automatically disperse themselves when ripe. Free-threshing was determined by a dominant mutation at *Q* locus together with a recessive mutation at *Tg* (tenacious glume) locus (Dubcovsky and Dvorak 2007; Jantasuriyarat et al. 2004; Simons et al. 2006). Free-threshing is an important trait that changes how the glumes adhere to the grain, changing them from hulled forms to naked forms. Aside from spelt bread wheat, cultivated forms of wheat have a tough rachis. Similarly, early domesticated wheat such as einkorn, emmer, and spelt are hulled, while modern wheat is free-threshing (Shewry 2009). Continuous selection for desirable traits such as spike size, growth habit, plant height, seeds size and disease resistance through domestication and breeding has resulted in modern wheat cultivars (Charmet 2011).

Wheat leaf rust and its impact on wheat production

Leaf rust is an important foliar disease in wheat that can cause significant yield losses. Wheat leaf rust had not been distinguished from stem rust until De Candolle (1815) first showed that leaf rust was caused by a separate fungus named *Uredo rubigo-vera.* When Winter (1882) described the *Puccinia rubigo-vera* in 1882, the names for *Puccinia* species causing wheat leaf rust underwent a series change during different eras. The most important one, *P. recondita*, was introduced by Cummins and Caldwell (1956) and is the oldest valid name for the leaf rust pathogen. It was widely used by mycologists and plant pathologists and is still seen in some contemporary publications. However, Urban and Markova (1994) renamed it *Puccinia triticina* based on its different hosts where they produced their aecial stage. Since then, *P. triticina* has been widely accepted as the name of the pathogen for leaf rust.

Rusts are quickly becoming the most important foliar diseases of wheat, due to the worldwide infestation and rapid evolution of new virulent races that cause significant crop production losses (Roelfs 1992). Among the three types of wheat rust diseases caused by different rust pathogens, leaf rust caused by *P. triticina* is the most widespread. The other two are stem rust, caused by *P. graminis,* and stripe rust, caused by *P. striiformis* (Marasas et al. 2004). Leaf rust occurs on the leaf blades, although leaf sheaths can also be infected as well as glumes and awns under favorable conditions when high inoculum density and extremely susceptible cultivars are available (Roelfs et al. 1992). Leaf rust symptoms begin as small yellow spots on the upper surface of wheat leaves, then develop into reddish-orange colored pustules which can produce thousands of spores that are easily disseminated by wind (Lipps 1914). The optimum temperatures for leaf rust development range from 10 to 30 °C. Leaf rust in wheat influences not only the grain yield but also grain quality since reduced floret set and grain shriveling can be attributed to infection. Furthermore, florets, tillers, and even the plants can be killed by early epidemics of leaf rust (Roelfs et al. 1992). Leaf rust can result in 5-20% of yield losses, but the losses can reach up to 50% in susceptible cultivars under favorable conditions.

As the one most widely distributed among rust diseases, leaf rust can be found almost everywhere wheat grows. In general, leaf rust infection spreads by wind from south to north in the United States. Fall-planted winter wheat in Texas and Gulf Coast states first get infected during September and November, followed by the spreading in the fall season. Infection of leaf rust can survive in most southern and middle areas of U.S. including Texas to Georgia, the Atlantic seaboard to South and North Carolina, and the

Great Plains states (Kolmer et al. 2007; Roelfs et al. 1989). In the warmer area such as Texas and Gulf Coast states, the severe epidemic of leaf rust shows up in March through May (Roelfs et al. 1989). In the less warm area from Oklahoma to eastern Virginia, the highest infection levels were seen from April to mid-May. In the relatively cold region from Kansas to South Dakoda, the maximum severity showed from May to June. On the spring wheat distributed in the Northern parts such as Minnesota, South Dakota, and North Dakota, leaf rust reaches the severity levels until mid to late July when the daytime temperature gets higher in summer (Carver 2009).

Disease cycle

The wheat leaf rust disease cycle usually consists of up to five types of spores developed on host plants (Figure. 1.2). Several plant species such as *Thalictrum* and *Isopyrum spp*. were identified as the alternate hosts on which the leaf rust pathogen completes the sexual cycle. Among the five spore types formed in the life cycle, pycnidiospores and aeciospores are developed on the alternate hosts, while urediniospores, teliospores, and basidiospores are developed on wheat plants (Singh 2010).

Urediniospores are dikaryotic and produced on the wheat host (Anikster et al. 2005). Under suitable temperature of 15 to 25 °C, urediniospores are able to re-infect wheat plants continuously. Two-celled teliospores are formed as the plant matures due to the development of uredinial infection (Anikster 1986). The two haploid nuclei of teliospore produce a diploid nucleus in the early stage of development through a process called karyogamy. The diploid teliospore then undergoes meiosis to form four haploid nuclei cells, called basidiospores (Anikster 1986). The basidiospores are released into the

air and disseminate by wind to an alternate host, followed by infection on epidermal cells and development into flask-shaped organisms called pycnia. Pycniospores and flexuous hypea produced by pycina act as male and female gametes in fusion, respectively. However, because of the heterothallic nature of *Puccina triticina*, Pycniospores and flexuous hypea produced by same pycnium are not sexually compatible. The pycniospores are carried by insects or dew to different pycnia and fuse with the receptive hyphea which restores the dikaryotic mycelium. After that, the mycelium grows and forms an aecium below the pycnium on the lower surface of leaf. Aeciospores are produced within the aecium and spread to the wheat host by wind when the aecium erupts. Consequently, aeciospores germinate and penetrate stomata of host plant followed by the development of asexual urediniospores to complete the wheat leaf rust disease cycle (Bolton et al. 2008).

The sexual cycle of leaf rust depends on the presence of alternate hosts. *Thalicutrum spp*. has been found as the alternate host for wheat leaf rust in Europe and southwest Asia but rarely in North America (Bolton et al. 2008). Additionally, the native American species *Thalictrum* and *Isopyrum spp*. are resistance to basidiospores infection (Jackson and Mains 1921; Saari et al. 1968). The epidemiology of leaf rust is not affected by the sexual cycle in North America (Kolmer 2005). Instead, *Puccinia triticina* can cycle indefinitely at the uredinal stage and re-infect wheat leaves. In different seasons, leaf rust fungus survives in different wheat and cause secondary infection on new leaves under favorable conditions.

Epidemiology

Three factors are highly important for the infection, development and survival of leaf rust: moisture, temperature, and wind. *Puccinia triticina* can survive under similar environmental conditions as wheat (Roelfs et al. 1992). The optimum temperatures for leaf rust spores germination and sporulation are 20 and 25 °C, respectively (Hogg et al. 1969). Wheat plants can get infected with a dew period of three hours or less at a favorable temperature. More severe infections can occur with longer dew periods, though fewer infections appear due to the extreme temperatures above 32°C or below 2°C (Stubbs et al. 1986). Spores spread faster to the neighbor field under the dry and windy conditions. Infections are usually complete within 7-8 hours and urediniospores usually develop 8-11 days after infection. Though urediniospores are only viable for several hours, compared to other spore stages they are easily dispersed by the wind and produced in large amounts, which can cause secondary infections. Leaf rust can spread rapidly due to the high production of urediniospores. Under favorable conditions a uredinium can produce approximately 3000 spores per day over a period of 20 days (Stakman and Levine 1922), and 33% of the generated spores can cause a secondary infection on wheat leaves. If there is no loss in spore numbers during transport to a nearby site, it will be exponentially increased on the wheat, which explains the explosive nature of rust disease (Roelfs et al. 1992).

The most of the severe epidemics occur under two conditions: first, an infection survives during the winter on wheat; second, spring-sown wheat is the recipient of exogenous inoculum and gets infected before heading. When the flag leaf gets infected before anthesis, leaf rust can cause severe epidemics and losses in wheat (Chester 1946).

Leaf rust inoculation and evaluation

Several methods have been described in previous studies for inoculating wheat plants with rust spores including dusting, brushing, and spraying (Roelfs et al. 1992). Talcum powder, mineral oil, and water are three spore carriers commonly used for the inoculum. Different methods are chosen depending on the purpose of each study, population size to be inoculated, availability of the inoculum, and environmental condition for the process (Roelfs et al. 1992). The length of dew period is also an important factor for inoculation. A short dew period may allow spores to germinate but they may fail to infect the plant, thus a long dew period is necessary for successful rust infection.

Among the methods of inoculation, using mineral oil as the spore carrier is more commonly used in greenhouse experiments. After preparation of the mist chamber a mixture of inoculum in mineral oil is sprayed onto the front and back of the plants at the flowering stage from about 6 inches away. The inoculated plants are incubated in the dew chamber for approximately 12 h and transferred to a greenhouse with a 16 h photoperiod at ~25 °C for disease development (Sinclair and Dhingra 1995). Disease notes are taken 7-10 d after inoculation when the pustules erupt. The advantage of the spraying method is that it can be easily applied in a greenhouse, and a large number of plants can be inoculated at the same time. However, the inoculum concentration in the oil is a major concern since different concertration can cause different levels of severity, and the effectiveness of inoculation depends on the condition in dew chambers.

Four methods have been proposed by Roelfs (1985) to assess rust disease including infection type (IT), host receptivity, length of latent period (LP), and duration

of sporulation. For IT assessment in the seedling stage, the standard scoring systems still in use for stem rust and stripe rust are using the scale proposed by Stakman et al. (1962) and Gassner and Straib (1932) (Table 1.1, Table 1.2). A 0-7 scale was first developed by Browder and Young Browder and Young (1975), but was not widely used. Instead, a 0-9 scale developed by McNeal et al. (1971) (Table 1.2) has been used frequently. For adult plant assessment, an IT scale developed by Stakman et al. (1962) has been used. A more commonly used leaf rust scale is the modified Cobb scale (Peterson et al. 1948) for disease severity, which uses a percentage of the leaf area covered by rust pustules. Latent period defined as the number of days from inoculation to the appearance of urediniospores has been used to measure leaf rust resistance. The area under disease progress curve (AUDPC) is an assessment of leaf rust in adult plant stage (Roelfs 1985). It requires continuous assessment of disease severity after inoculation, and sums the accumulated disease to track the speed of rust using the formula AUDPC = $\sum_{i}^{n-1}\left[\left(\frac{y_i+y_{i+1}}{2}\right)(t_{i+1}-t_i)\right]$, where y_i is the rust severity value on date i, t_i the time in days between the dates i and $i + 1$ (Shaner and Finney 1980). The benefit of this assessment is the early detection of potentially severe rusting plants through multiple scoring.

Wheat resistance to leaf rust

Wheat resistance to leaf rust can be categorized as either seedling resistance or adult plant resistance (APR). Seedling resistance is effective throughout all wheat growth stages from seedling to adult plant stages. However, APR normally does not express in seedling stage, but is effective in the adult stage (Dyck et al. 1985).

Seedling resistance is only effective against some of the pathogen races and usually characterized by a hypersensitive response (HR) which might result in cell death during rust infection (Dyck et al. 1985). A series of terminologies have been used to describe seedling resistance including race-specific, vertical or major gene resistance, all of which interact with the pathogen in a gene-for-gene manner (Flor 1942). The resistant (R) gene in the host interacts with the effectors, or avirulence proteins, in the pathogen to trigger the defense response in the plants, called effector-triggered immunity or ETI (Gassmann and Bhattacharjee 2012). Many seedling resistance genes have been identified and used in the breeding programs. However, most of them were overcome by the rapid evolution of virulent races shortly after they were deployed in commercial production.

Adult plant resistance (APR) is commonly characterized by susceptible reaction at the seedling stage followed by increased resistance in post-seedling stages. However, APR can be either race-specific or race-nonspecific. Race-specific APR is similar to seedling resistance except for the different stages at which the resistance occurs. Caldwell (1968) found that plants with race non-specific resistance APR show “longer LP, lower infection frequency, smaller uredinial size, and less spore production per infection site”. APR is quantitatively inherited and always associated with a slow rusting phenotype. For slow rusting, the rust progresses at a retarded rate that results in reduced disease severity against all races of different pathogens, and is synonymous with APR. Although slow rusting resistance does not show HR like race-specific resistance does, pyramiding several such genes together can achieve near immunity in host plants (Charpe et al. 2012; Chhuneja et al. 2011; Revathi et al. 2010). Additionally, selection pressure is greatly

reduced on the pathogen for race non-specific APR, and thus reduces the likelihood of new virulent mutants and evolution of virulence races. Therefore, race non-specific resistance is more durable than race-specific resistance (Bjarko and Line 1988).

Reported leaf rust resistance genes

To date, 72 leaf rust resistance (*Lr*) genes have been named and each of them was mapped to a specific chromosome location (McIntosh et al. 2010). Most of the *Lr* genes were identified from hexaploid wheat including *Lr1*, *Lr2a*, *Lr3*, *Lr10* and *Lr11* (Kolmer 2013), while others were transferred from wheat's relatives, including *Lr21* from *Aegiops. tauschii*, *Lr24* from *Ae. longatum*, *Lr9* from *Ae. umbellulata* and *Lr26* from common rye (Browder 1980). Most of these are race-specific genes such as *Lr9*, *Lr10*, *Lr21*, and *Lr42* (Li et al. 2014). The common characteristics of these genes are that they code for nucleotide binding site (NBS) and leucine-repeat-rich (LRR) proteins (Feuillet et al. 2003) and are very vulnerable to selection. When a virulent race in a rust population increases, race-specific resistance is easily eroded.

Some of these *Lr* genes are race non-specific and confer resistance to all known races. Such genes alone may not be able to provide sufficient protection in the host plants, but they can provide durable resistance in the long-term since virulent forms have not yet been detected (Kolmer 2013). Among the 72 leaf rust resistance genes formally cataloged, only 11 have been reported to show APR: *Lr12*, *Lr13*, *Lr22*, *Lr34*, *Lr35*, *Lr37*, *Lr46*, *Lr48*, *Lr49*, *Lr67* and *Lr68* (in Table1.3, Li et al. 2014). Four slow-rusting genes, *Lr34/Yr18/Pm38/Sr57* (Spielmeyer et al. 2005), *Lr46/Yr29/Pm38/Sr57* (William et al. 2003), *Lr67/Yr46/Pm46/Sr55* (Herrera-Foessel et al. 2014) and *Lr68* (Herrera-Foessel et al. 2012), have been reported to be race non-specific APR.

Marker technologies used for rust research

There are two common approaches for mapping quantitative traits loci (QTLs): candidate gene studies with either association or resequencing approaches, and linkage studies such as QTL mapping and genome-wide association study (Tuberosa et al. 2013). DNA markers are widely employed in modern genetic mapping and plant breeding efforts. The high throughput and low cost of marker detection methods drive the evolution of molecular markers (Bernardo 2008).

The throughput of marker detection underwent four levels of technological advances to produce the current ultrahigh-throughput era (Lateef 2015). Restriction fragment length polymorphism (RFLP) markers are representative of low-throughput marker systems mainly invented in 1980s (Botstein et al. 1980), and had been a very popular marker system at 1980s and 1990s due to the advantage of its codominant nature, high reproducibility and locus-specificity (Lander and Botstein 1989). However, the time-consuming and tedious procedure of using RFLPs is a large disadvantage (Edwards and McCouch 2007). To improve throughput, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) markers were developed in the 1990's. Taking advantage of simultaneous detection of several polymorphic genomic regions, RAPDs were widely used in the assessment of genetic variation and studies of relationships among subspecies (Wong 2013). However, RAPDs are dominant markers, and cannot detect allelic differences in heterozygotes. Additionally, the reproducibility was low due to randomly designed primers (Edwards and McCouch 2007), so RAPD was quickly replaced by AFLP due to AFLP's high reproducibility. However, AFLPs are still too laborious to efficiently deploy

in marker analysis and therefore not suitable for breeding application (Meudt and Clarke 2007). SSR markers quickly emerged as the "marker of choice" by breeders for breeding application during the 1990s due to its co-dominance, high polymorphisms and reproducibility (Powell et al. 1996). With the rapid development of next-generation sequencing, single nucleotide polymorphism (SNP) technology has been employed as a major marker system in genetic and breeding applications. Different types of platforms have been developed to meet the demand for high throughput tools, such as Genotyping-by-Sequencing (GBS) (Mammadov et al. 2012) and breeder-friendly Kompetitive allele-specific PCR (KASP).

SNP array platforms

SNPs frequently occur (every 100-300 bp) in plants due to nucleotide substitutions, point mutations, or deletion/insertion during evolution, and can be detected by aligning similar genomic regions among different genotypes (Xu 2010). Due to their abundance and uniform distribution across the whole genome, SNPs are the most desirable markers that provide high-density whole genome scans using either whole genome sequencing, or oligonucleotide microarray (Lateef 2015).

Nowadays, advances in high-throughput and low-cost next-generation-sequencing (NGS) have significantly facilitated SNP discovery. High-density SNP arrays have been developed as an important tool for plant genetic studies and breeding applications (Xing 2014). Successful examples include the rice 44K SNP genotyping array used in the genome-wide association study to identify a number of alleles controlling different traits (Zhao et al. 2011), the 50K maize SNP array used in a nested association mapping to analyze the genetic control of maize kernel composition (Cook et al. 2012), and the

recently developed 9K and 90K wheat SNP arrays used in QTL mapping studies to detect genomic regions targeted by breeders (Cavanagh et al. 2013; Wang et al. 2014). The limitation for application of SNP array is the relatively high cost associated with array analysis (Lateef 2015).

Genotyping-by-Sequencing (GBS)

With the increased throughput and reduced cost in NGS, GBS offers a cost effective approach for simultaneous SNP discovery and genotyping (Sonah et al. 2013). As illustrated in Figure 1.3 (Poland and Rife 2012), GBS library construction includes the digestion of normalized genomic DNA with restriction enzymes and the ligation of barcode adapter to each sample. After PCR amplification and clean-up, the size-selected library is sequenced by Illumina or Ion Proton system. Bioinformatic pipelines are used to interpret GBS sequences to obtain the SNPs.

One of the advantages of GBS is reduced genome complexity via the restriction enzymes in GBS library construction. It allows researchers to reach the important genomic regions that are unreachable by sequence capture approaches (Mir et al. 2013). Additionally, choosing the appropriate restriction enzymes for different species can avoid the repetitive genomic regions, and thus increase efficiency when targeting lower copy regions (Elshire et al. 2011). This procedure has been successfully applied in genetic studies of barley and maize using recombinant inbred populations, where about 25,000 to 200,000 sequence tags were mapped, respectively (Mascher et al. 2013; Romay et al. 2013). GBS can be applied in both species with or without a reference genome. A reference map can be settled around the restriction site for species that lack a complete genome sequence (Elshire et al. 2011). Compared with SNP arrays, GBS approach is

more efficient since it escapes the limitation of fixed arrays, and new species or populations can be studied without the SNP discovery and array design steps. The current challenges for GBS are high rates of missing data with low sequence depth, and difficulty calling SNPs due to lack of bioinformatics knowledge.

Kompetitive Allele Specific PCR (KASP)

Although GBS and chip-based assays are the best choice for multiplexing and high-throughput marker analysis, it is cost-ineffective if a small number of SNPs need to be analyzed in a large set of samples. The KASP genotyping system is a better choice for such a purpose (Mir et al. 2013). KASP is a fluorescence-based genotyping technology suitable for uniplex SNP genotyping. KASP assays are PCR based, and the PCR mix consists of three important components: primer mix, master mix and DNA template (http://www.lgcgroup.com/). The primer mix contains two competitive allele specific tailed forward primers labeled with FAM and HEX dyes, respectively, and one common reverse primer. In the KASP Master mix standard reagents such as *Taq* polymerase, nucleotides and $MgCl_2$ in an optimized buffer solution are used, while the quenched fluorescence resonance energy transfer (FRET) cassette and ROX passive dye are also critical components (He et al. 2014). In the first cycle of PCR reaction, two allele specific primers bind to different DNA templates and elongate to generate corresponding allele specific products. Later, the FRET cassette binds to the allele-specific DNA and releases a fluorescence signal. Either the FAM or HEX signal will be generated if the genotype in the given SNP is homozygous, or a mixed signal will be generated if the genotype is heterozygous (Semagn et al. 2014).

KASP genotyping is a cost-effective, time-saving and flexible genotyping system compared with other uniplex genotyping systems currently available (Semagn et al. 2014). KASP is widely accepted as an effective tool for MAS a number of commercial crops. In maize, a set of 695 highly polymorphic gene-based SNPs were converted into KASP genotyping assay at a 98% success rate (Mammadov et al. 2012). In wheat, KASP genotyping has been used for constructing a linkage map containing several hundred SNPs (Allen et al. 2011). These examples show that the KASP assay is an effective tool for MAS and has the potential to be applied in multiple aspects of genetic studies.

QTL mapping for leaf rust resistance

With the rapid evolution of marker technology, QTL mapping for disease resistance using molecular markers has received great attentions from geneticists and breeders (Broman 2001). QTL mapping is a powerful method to refine information from quantitatively inherited traits of disease resistance. With QTL mapping, researchers can map the genomic location of each genetic factor, dissect the gene action, explore the gene effect and even reveal the direction of gene effects (Pereira et al. 2001).

QTL mapping begins with a segregating mapping population. Population sizes ranging from 70 to 250 lines have been used for preliminary genetic map construction (Mohan et al. 1997). However, a larger population (>150 line) is preferred for QTL mapping (Collard et al. 2005). F_2, backcross (BC), recombinant inbred lines (RILs) and doubled haploid (DH) (Würschum 2012) populations can all be used for QTL mapping. RILs and DH are more commonly used since the same genotypes can be repeatedly evaluated for phenotypes in different years or locations.

QTL mapping methods can be categorized into four levels based on complexity (Mackay et al. 2009). The simplest level is to analyze variance at marker loci to test the simple association between markers and trait values without pre-requirement of map information (Manly and Olson 1999). This method suffers when the markers are widely spaced and there is appreciable missing data in marker genotypes. Simple interval mapping (SIM) is more complicated and involves intensive computation. After map construction, it tests for the presence of a QTL every 2 cM between adjacent markers (Carbonell et al. 1992). Thus, the estimation of the QTL locations and effects are more accurate. However, the limitation of SIM is that it cannot separate effects of linked QTLs. To overcome the shortcoming of SIM, composite interval mapping (CIM) (Jiang and Zeng 1995) and multiple QTL mapping (MQM) methods (Jansen 1993) were developed to reduce the background noise for QTL detection by combining interval mapping with multiple regression. It tests for QTL presence at multiple analysis points across each inter-marker interval. Meanwhile, it includes the effect of background markers as cofactors at each point. The most complex level is using the Bayesian method to construct a multi-QTL model (Sillanpää and Arjas 1998) and apply it to test QTL combinations, positions, and effects. This method would take a lot of computation time, especially for a large number of markers. Among all the methods, CIM is the most commonly used one for QTL mapping in biparental populations.

Several factors may affect the power of QTL mapping including marker density, population size, trait heritability, and the quality of genotypic data. Marker density is a big concern in QTL mapping, as marker space less than 10 cM may have little effect on mapping results, and marker space more than 20 cM may reduce the power of QTL

detection to some extent (Collard et al. 2005). NGS technology lifts number of markers as a limiting factor, because thousands of markers can be generated in each GBS run, whereas population size is still a limitation for QTL detection. For a large number of markers developed, larger population size can increase power in QTL detection (Darvasi et al. 1993). Heritability of different traits can also influence QTL detection. The same QTL can have different levels of effects in different environments, and minor QTLs are more sensitive to environments than major QTLs. QTL experiments conducted with replications across multiple locations can increase the power of QTL detection (Collard et al. 2005). Finally, too much missing marker data may cause changes in marker orders and distances in linkage maps (Hackett and Luo 2003). Some suggested approaches to enhance the power of QTL detection and estimation of QTL effects include progeny analysis, selective genotyping, sample pooling and sequential sampling for optimization of experimental designs (Ronin et al. 1999).

Breeding strategies for rust resistance

In order to breed for rust resistance, wheat varieties with durable resistance are the best. Breeders prefer to use resistance genes that are resistant to all races of leaf rust fungus. Although numerous genes conferring resistance to wheat leaf rust have been identified and used in wheat breeding, only a few genes offer durable resistance including *Lr34*, *Lr46*, *Lr67*, and *Lr68*. *Lr34* is present in many parent lines that have been used for breeding commercial varieties around the world, and has been validated in Thatcher, Glenlea, Jupateco R, Opata, Fukuho-komugi, Condor, Cook, Anza, Forno, Bezostaya, Otane and Chinese Spring wheat lines (Soria et al. 2012). *Lr46* was first discovered in cultivar Pavon 76 (PI 519847) and transferred to susceptible cultivars Jupateco 73 and

Avocet (Singh et al. 1998). Also, substitution lines of Lalbahadur by the chromosome 1B of Pavon 76 were developed (Martinez et al. 2001). Another source for *Lr46* was cultivar Parula (PI 520340). Later Kolmer et al. (2012) also reported adult plant resistance gene, *Lr46*, in winter wheat line CI13227. Recently, *Lr67* was found in wheat accession PI 250413 as a new resource for adult plant resistance and was transferred into Thatcher in order to produce the backcross line RL6077 (Thatcher*6/PI250413) (Hiebert et al. 2010). *Lr68*, formerly designated *LrP*, was first described in CIMMYT's spring bread wheat Parula developed at CIMMYT in 1981 (Herrera-Foessel et al. 2009), and likely originated from the Brazilian cultivar Frontana. Some of these sources of resistance have been used in commercial production to reduce the losses caused by leaf rust epidemics. They also serve as breeding parents to improve wheat durable resistance.

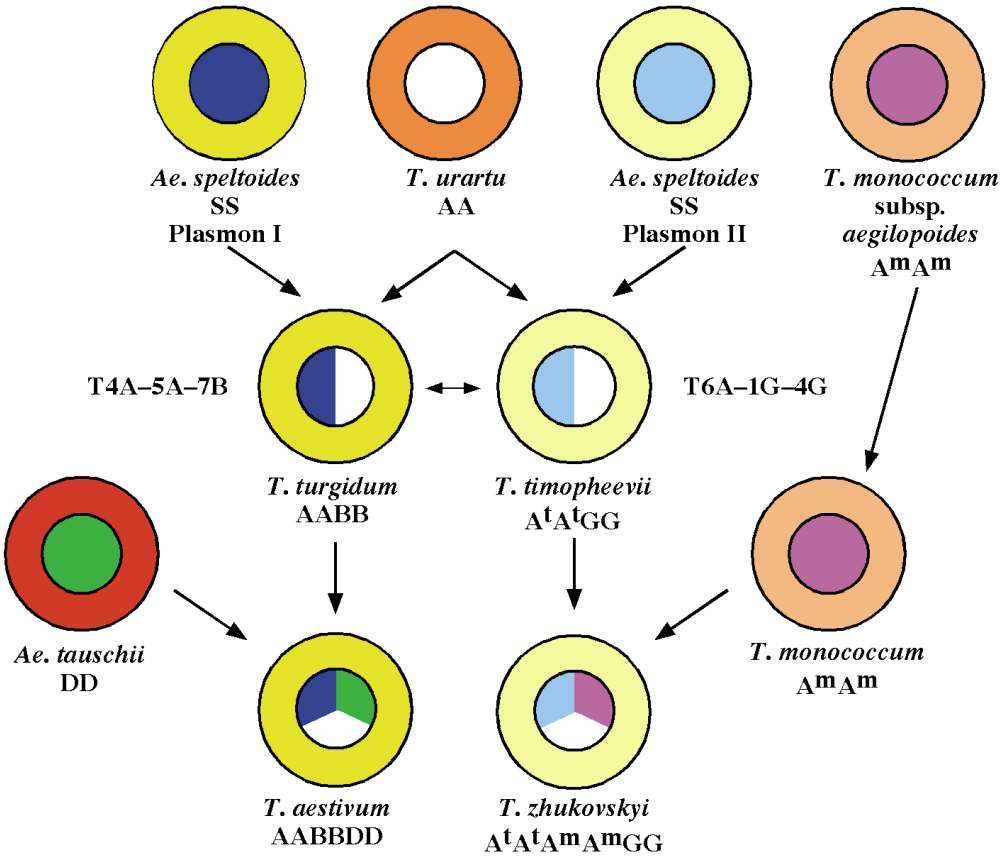
Many race-specific adult plant resistance genes have been deployed to provide resistance to leaf rust (*Puccinia triticina*) of wheat (Revathi et al. 2010). Although effective initially, the resistance can be overcome shortly due to the rapid evolution or mutation of the pathogens to produce new virulent races. Thus, pyramiding multiple resistance genes in one variety is an attractive strategy to delay the breakdown of race specific resistance genes and could efficiently prolong lifespan of resistant cultivars (Charmet 2011; Chhuneja et al. 2011; Revathi et al. 2010). Some race-nonspecific resistance gene combinations such as the '*Sr2* complex' for stem rust resistance (Singh et al. 2006) and the '*Lr34* complex' for leaf rust resistance have shown long-term durability (Roelfs 1985). *Lr13* and *Lr16* (Samborski and Dyck 1982), *Lr2a* and *Lr16* (Kloppers and Pretorius 1997), *Lr13* and *Lr34* (Ezzahiri and Roelfs 1989), *Lr27* and *Lr31* (Singh and McIntosh 1984) have been reported to have an additive effect in combinations. Kloppers

and Pretorius (1997) compared the resistance level in single-gene lines which contain *Lr13*, *Lr34*, and *Lr37* with the combination of three genes, *Lr13* + *Lr34*, *Lr13* + *Lr37*, and *Lr34* + *L37*. The results demonstrated higher levels of resistance in the combination lines *Lr13* + *Lr37* and *Lr34* + *Lr37* than in the lines with a single gene. Due to the successful stories of *Lr34* in providing durable resistance to rusts for more than 50 years, pyramiding several race non-specific resistance genes has attracted great attention in breeding.

Breeding for durable leaf rust resistance is a challenging task in wheat breeding. In order to exploit the benefits of durable resistance conferred by slow-rusting genes, more information on the inheritance of slow-rusting genes, slow-rusting components, and development of user-friendly markers for marker-assisted breeding is mandatory.

Figures and Tables

Figure 1.1 The evolution of wheat

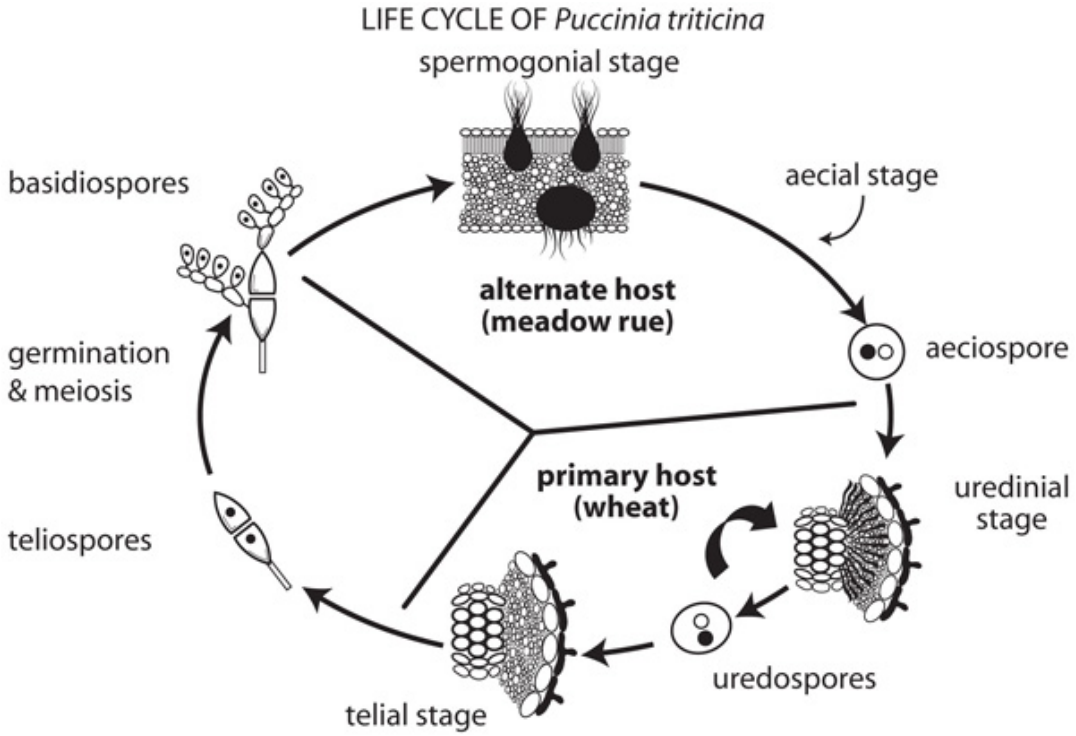


Species	Genome	Nucleus	Cytoplasm
Aegilops speltoides (Plasmon I)	SS		
Triticum urartu	AA		
Aegilops speltoides Plasmon II)	SS		
Triticum monococcum ssp. *aegilopoides*	A^mA^m		
Aegilops tauschii	DD		

Source:

Wheat Genetic and Genomic Resource Group (WGGRC), Kansas State University (https://www.k-state.edu/wgrc/Extras/evolve.html)

Figure 1.2 Leaf rust life cycle showing both primary and alternate hosts.



Source: Graphic by Jerry Downs. Adapted from: C.J. Alexopoulus, C.W. Mims, and M. Blackwell. 1996. Introductory Mycology, 4th Ed., John Wiley and Sons, Inc.

Figure 1.3 Schematic overview of steps in genotyping-by-sequencing (GBS) library construction, sequencing, and analysis (Poland and Rife 2012)**.**

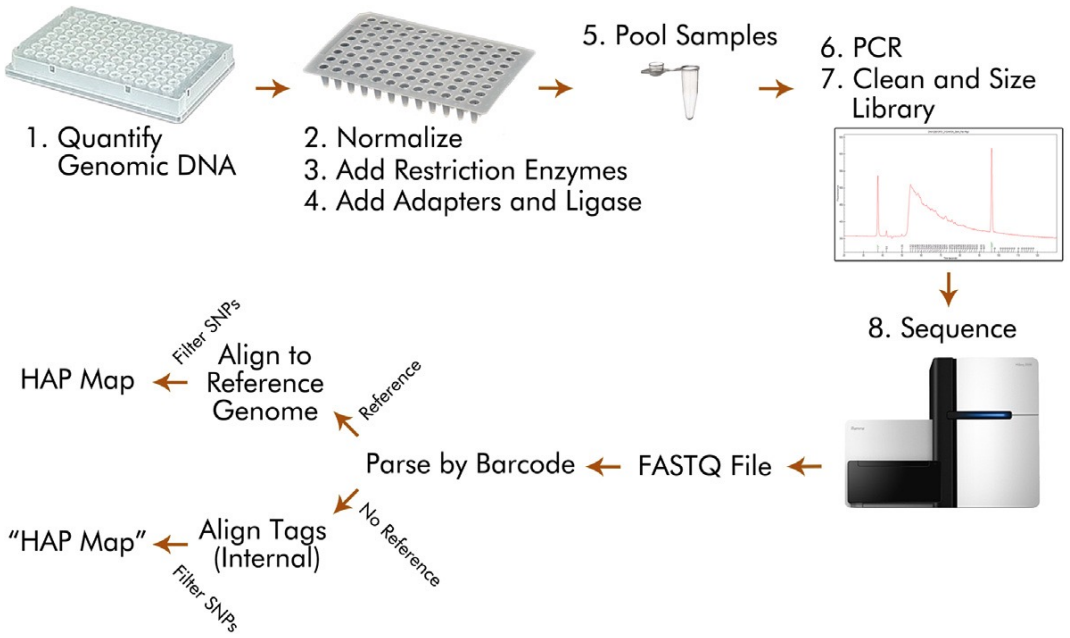


Table 1.1 Host response and infection type descriptions used in wheat stem and leaf rust systems (Roelfs 1984)**.**

Host response (class)	Infection type[a]	Disease symptoms
Immune	0	No uredinia or other macroscopic sign of infection
Nearly immune	;	No uredinia, but hypersensitive necrotic or chlorotic flecks present
Very resistant	1	Small uredinia surrounded by necrosis
Moderately resistant	2	Small to medium uredinia often surrounded by chlorosis or necrosis; green island may be surrounded by chlorotic or necrotic border
Heterogeneous	X	Random distribution of variable-sized uredinia on single leaf
Heterogeneous	Y	Ordered distribution of variable-sized uredinia with larger uredinia at leaf tip
Heterogeneous	Z	Ordered distribution of variable-sized uredinia, with larger uredinia at leaf base
Moderately susceptible	3	Medium-sized uredinia that may be associated with chlorosis
Susceptible	4	Large uredinia without chlorosis

[a] The infection types are often refined by modifying characters as follows: =, uredinia at lower size limit for the infection type; -, uredinia somewhat smaller than normal for the infection type; +, uredinia somewhat larger than normal for the infection type; ++, uredinia at the upper size limit for the infection type; C, more chlorosis than normal for the infection type; and N, more necrosis than normal for the infection type. Discrete infection types on a single leaf when infected with a single leaf when infected with a single biotype are separated by a comma (e.g., 4,; or 2-, 2+ or 1,3C). A range of variation between infection types is recorded by indicating the range, with the most prevalent infection type listed first (e.g., 23 or ;1C or 31N); after Roelfs (Roelfs, 1984).

Table 1.2 Host response and infection type descriptions used in wheat rust system (Gassner and Straib 1932; McNeal et al. 1971)**.**

	Infection type[a]		
Host response (class)	McNeal	Gassner[b]	Disease symptoms
Immune	0	i	No visible infection
Very resistant	1	0	Necrotic/chlorotic flecks, without sporulation
Resistant	2	0	Necrotic/chlorotic stripes, without sporulation
Moderately resistant	3	I	Trace sporulation, necrotic/chlorotic stripes
Light moderate	4	I	Light sporulation, necrotic/chlorotic stripes
Moderate	5	II	Intermediate sporulation, necrotic/chlorotic stripes
High moderate	6	II	Moderate sporulation, necrotic/chlorotic stripes
Moderate susceptible	7	II	Abundant sporulation, necrotic/chlorotic stripes
Susceptible	8	III	Abundant sporulation with chlorosis
Very susceptible	9	IV	Abundant sporulation without chlorosis

[a] McNeal = McNeal et al. (McNeal, Konzak, Smith, Tate, & Russel, 1971), Gassner = Gassner and Straib (Gassner & Straib, 1932).

[b] This scale used for description of seedling infection types only.

Table 1.3 Summary of quantitative trait loci (QTL) or genes for adult plant resistance to leaf rust in wheat (Li et al. 2014)**.**

QTL	Chromosome	Donor	Marker interval	R^2 (%)	Referenece
QLr.sfrs-1BS	1BS	Forno	*Xpsr949–Xgwm18*	10.6	Messmer et al., 2000
QLr.sfr-1BS	1BS	Forno	*Xgwm604–OA93*	28-31.5	Schnurbusch et al., 2004
QLr.cimmyt-1BS.1	1BS	Parula	*Xcmtr03–500*	7-10	William et al., 1997
QLr.cimmyt-1BS.2	1BS	Pastor	*wPT5580–wPT3179*	4.1-6.1	Rosewarne et al., 2012
QLr.pbi-1BS	1BS	Beaver	1BL/1RS	17.3	Singh et al., 2009
Lr46/Yr29/Pm39	1BL	Saar	*Xwmc719–Xhbe248*	49.1	Lillemo et al., 2008
Lr46	1BL	Pavon76	*Xksul27–PAAGMCTA-1*	46.1-53.9	William et al., 2003
Lr46	1BL	Pastor	*cslv46–Xgwm818*	16.7-25.4	Rosewarne et al., 2012
Lr46	1BL	Oligo	*Xwmc44–Xgwm793*	12.9-17.4	Suenaga et al., 2003
Lr46	1BL	Pavon	*Xgwm140, Xgwm259*	46.1-53.9	William et al., 2006
QLr.caas-1BL	1BL	Bainong 64	*Xgwm153.2-–Xwmc44*	16.7–25.4	Ren et al., 2012b
QLr.pser-1BL	1BL	Ning7840	*Xscm9–Xwmc85.1*	12.9–17.4	Li and Bai, 2009
QLr.csiro-1BL	1BL	Attila	*XP84/M78–LTN*	21.1–28.5	Rosewarne et al., 2008
Lr37	2AS	Madsen	*Xcmwg682*	/‡	Helguera et al., 2003
QLr.cimmyt-2AL	2AL	Avocet	*wPT4419–wPT8226*	/‡	Rosewarne et al., 2012
QLr.sfr-2AL	2AL	Forno	*cfa2263c–sfr.BE590525*	9.5–12	Schnurbusch et al., 2004
QLr.ubo–2A	2AL	Lloyd	wPT-386–310911	18.6–30	Maccaferri et al., 2008
Lr48	2BS	CSP44	*Xgwm429b–Xbarc07*	/‡	Bansal et al., 2008
Lr13	2BS	ThLr13	*Xgwm630*	/‡	Seyfarth et al., 2000
QLr.csiro-2BS	2BS	Attila	*Xgwm682–XP32/M62*	/‡	Rosewarne et al., 2008
QLr.cimmyt-2BS	2BS	Pastor	*wPT6278, Yr31*	5.2–9.6	Rosewarne et al., 2012
QLr.ksu-2BS	2BS	W-7984	*Per2 (Lr23?)*	15.7	Faris et al., 1999
Lr35	2BS	Thatcher Lr35	*Xwg996, Xpsr540, Xbcd260*	/‡	Seyfarth et al., 1999
QLr.osu-2B	2BL	CI13227	*Xagc.tgc135–Xcatg.atgc60*	18.8	Xu et al., 2005a
QLrlp.osu-2B	2BL	CI13227	*Xcag.cgat70–Xcatg.atgc60*	16.2	Xu et al., 2005b
QLr.sfrs-2BL	2BL	Oberkulmer	*Xpsr924–Xglk699a*	7.2	Messmer et al., 2000
QLr.cimmyt-2DS	2DS	Avocet	*wPT8319–wPT3728*	3.8–9.8	Rosewarne et al., 2012
QLrlp.osu-2DS	2DS	CI13227	*Xactg.gtg185–Xbarc124*	42.8	Xu et al., 2005b

QLr.hbau-2DS	2DS	Saar	*Xbarc124–Xgwm296a*	12.2–12.5	Zhang et al., 2009
Lr22a	2DS	RL6044	*Xgwm296*	/‡	Hiebert et al., 2007
QLrid.osu-2DS	2DS	CI13227	*Xgwm261, XGCTG.CGCT118*	21.5–26.4	Xu et al., 2005a
QLr.sfr-2DS	2DS	Forno	*gdm35–cfd53*	10.3–14.8	Schnurbusch et al., 2004
QLr.sfr-2DL	2DL	Arina	*glk302–gwm539*	11.4–12.7	Schnurbusch et al., 2004
QLr.ubo-3A	3AS	Lloyd	*311707–Xwmc664*	24.8	Maccaferri et al., 2008
QLr.sfrs-3A	3AL	Forno	*Xpsr570–Xpsr543*	13.5	Messmer et al., 2000
QLr.fcu-3AL	3AL	TA4152-60	*Xcfa2183–Xgwm666*	10-18	Chu et al., 2009
QLr.sfrs-3B	3B	Oberkulmer	*Xpsr919a–Xpsr1101b*	9.3	Messmer et al., 2000
QLr.fcu-3BL	3BL	TA4152-60	*Xbarc164–Xfcp544*	19-20	Chu et al., 2009
Lr12	4A	Exchange	*no data*	/‡	Dyck and Kerber, 1971
QLr.sfrs-4B	4B	Forno	*Xpsr921–Xpsr953b*	10	Messmer et al., 2000
Lr49	4BL	VL404	*Xbarc163–Xwmc349*	/‡	Bansal et al., 2008
QLr.pbi-4BL	4BL	Beaver	*wPt–1708*	12.4	Singh et al., 2009
QLr.cimmyt-4BL	4BL	Avocet	*Xgwm495, Xgwm368*	6.4-8.9	William et al., 2006
QLr.sfr-4BS	4BS	Forno	wm368–gwm540a	10.7	Schnurbusch et al., 2004
Lr67	4D	RL6077	*Xgwm165/Xgwm192*	/‡	Herrera-Foessel et al., 2011
QLr.fcu-4DL	4DL	ND495	*Xgdm61v–Xcfa2173*	7-13	Chu et al., 2009
QLr.sfrs-4DL	4DL	Forno	*Xglk302b–Xpsr1101a*	8.7-19.8	Messmer et al., 2000
QLr.pbi-5AS	5AS	Beaver	*wPt1931–wPt8756*	11.2	Singh et al., 2009
QLr.sfrs-5AS	5AS	Forno	*Xpsr945a–Xglk424*	7.7	Messmer et al., 2000
QLr.cimmyt-5AL	5AL	Avocet	*wPT0373–wPT0837*	5.2-7.4	Rosewarne et al., 2012
QLr.hbau-5BL	5BL	Saar	*XDuPw395–Xgwm777*	4.9-11.2	Zhang et al., 2009
QLr.sfrs-5BL	5BL	Forno	*Xpsr580b–Xpsr143*	14.6	Messmer et al., 2000
QLr.fcu-5BL	5BL	TA4152-60	*Xgdm116–Xbarc59*	7-10	Chu et al., 2009
QLr.sfrs-5DL	5DL	Oberkulmer	*Xpsr906a–Xpsr580a*	9.1	Messmer et al., 2000
QLr.cimmyt-6AL	6AL	Avocet	*Xgwm617*	4.8-6.3	William et al., 2006
QLr.hbau-6AL	6AL	Avocet	*Xgwm617a–Xgwm169*	7	Zhang et al., 2009
QLr.caas-6BS.1	6BS	Bainong 64	*Xwmc487–Xcfd13*	10–10.8	Ren et al., 2012b

QLr.caas-6BS.2	6BS	Jingshuang 16	*Xgwm518–Xwmc398*	8.2–9	Ren et al., 2012b
QLr.fcu-6BL	6BL	TA4152–60	*Xbarc5–Xgwm469.2*	12	Chu et al., 2009
QLr.cimmyt-6BL.1	6BL	Pastor	*wPT6329–wPT5176*	5.4–10.8	Rosewarne et al., 2012
QLr.cimmyt-6BL.2	6BL	Pavon 76	*XpAGGmCGA1*	4.37	William et al., 2006
QLr.ubo-7B.1	7BS	Colosseo	*Xwmc405.1–Xgwm573*	9.7–19.4	Maccaferri et al., 2008
QLr.sfrs-7B.1	7BS	Oberkulmer	*Xpsr952–Xgwm46*	7.6	Messmer et al., 2000
QLr.sfr-7BS	7BS	Arina	*sfr.BE427461–gwm573b*	8.8	Schnurbusch et al., 2004
QLr.osu-7BL	7BL	CI13227	*Xaca.cacg126/Xbarc50*	12.9–20.8	Xu et al., 2005a
QLrlp.osu-7BL	7BL	CI13227	*XBarc182-Xcatg.atgc125*	13.8	Xu et al., 2005b
Lr68	7B	Parula	*Psy1–1–gwm146*	/‡	Herrera-Foessel et al., 2012
QLr.ubo-7B.2	7BL	Colosseo	*Xbarc340.2–Xgwm146*	49.8–76.9	Maccaferri et al., 2008
QLr.sfrs-7B.2	7B	Forno	*Xpsr593c–Xpsr129c*	35.8	Messmer et al., 2000
QLr.sfrs-7B.3	7B	Forno	*Xglk750–Xmwg710a*	12.8	Messmer et al., 2000
QLr.cimmyt-7BL.1	7BL	Parula	*Xcmtg05–50, Xcmti16–1500*	18–30	William et al., 1997
QLr.csiro-7BL.2	7BL	Attila	*Xgwm146, Xwmc273*	/‡	Rosewarne et al., 2008
QLr.cimmyt-7BL	7BL	Pastor	*wPT4342–wPT8921*	3.8–11.5	Rosewarne et al., 2012
QLr.sfr-7BL(Lr14a)	7BL	Forno	*ksuD2–gbxG218b*	15.9	Schnurbusch et al., 2004
QLr.ksu-7BL	7BL	Opata	*Cht1b/Tha1/Cat*	11–42.2	Faris et al., 1999
Lr34/Yr18/Pm38	7DS	Saar	*XwPt3328–XcsLV34*	73.1	Lillemo et al., 2008
Lr34	7DS	Cook	*Xgwm37, Xgwm295*	/‡	Navabi et al., 2005
Lr34	7DS	Forno	*cfd66–gwm1002*	32.6–42.9	Schnurbusch et al., 2004
Lr34	7DS	Fukuho	*Xgwm295.1–Xgwm130*	36.4–45.2	Suenaga et al., 2003
Lr34	7DS	Opata	*Xwg834*	8–24.1	Faris et al., 1999

R^2, percentage of variance explained by the QTL.

/‡, R^2 of this QTL is unknown.

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Chapter 2 - Mapping of Quantitative Trait Loci for Slow-rusting Traits in Winter Wheat CI13227 Using Genotyping-by-sequencing

Abstract

Leaf rust, caused by *Puccinia triticina*, is an important foliar disease of wheat and can be controlled through the use of genetic resistance. Slow-rusting resistance is desirable due to its race non-specific and long-lasting in effectiveness. Winter wheat line CI13227 carries slow-rusting resistance to leaf rust that has been effective for many years. In order to identify quantitative trait loci (QTLs) for slow-rusting resistance against wheat leaf rust, a recombinant inbred line (RIL) population derived from a cross between CI13227 and susceptible cultivar Suwon92 was repeatedly evaluated for slow rusting traits in greenhouse experiments. Genotyping-by-sequencing (GBS) generated 3371 single nucleotide polymorphism (SNP) markers segregating in the RIL population. QTL mapping using these SNPs identified three QTLs, *QLr.hwwgru-2DS, QLr.hwwgru-7BL,* and *QLr.hwwgru-2BL* that were contributed by CI13227 and significantly associated with slow-rusting traits. The chromosome arm 2DS carries a major QTL that was significant for all slow rusting traits measured in both experiments and explained 10.0 to 36.2% of the phenotypic variations for different traits. The QTL on chromosome 7BL was significant for the area under disease progress curve (AUDPC) and Final severity (FS) in both experiments, and explained 8.6 and 15.3% of the phenotypic variation, but only significant in one experiment for latent period (LP). Another QTL on chromosome 2BL was only significant for the traits evaluated in a single experiment. Flanking SNPs closely

linked to the three QTLs were identified and converted to breeder-friendly Kompetitive allele-specific PCR (KASP) markers that can be used in marker-assisted selection to transfer these QTLs to elite breeding lines.

Introduction

Rust has long been one of the most important foliar diseases of wheat (Spielman and Pandya-Lorch 2009), and still poses a major threat to wheat production worldwide (Mammadov et al. 2012). Among all three rusts, leaf rust caused by *Puccinia triticina* is the most important due to its high adaptability and wide geographic distribution (Bolton et al. 2008). Wheat leaf rust can cause significant grain yield losses and is a major constraint to wheat production in many areas. Under favorable conditions, leaf rust can cause yield losses of up to 30-40% (Roelfs 1985). Although rust disease can be managed by application of foliar fungicide, manipulation of host genetic resistance is the most desirable, cost-effective, and environmentally safe method of controlling wheat rusts (Kolmer et al. 2013).

Genetic resistance to leaf rust can be categorized as race-specific and race-nonspecific. The majority of the known resistance genes present in hexaploid wheat (*Triticum aestivum* L.) are race-specific, which is characterized by a hypersensitive response in the host plant infected by a pathogen race that possesses the corresponding avirulence gene (Singh et al. 2011). Examples of cloned race-specific resistance genes are *Lr10* (Feuillet et al. 1997), *Lr21* (Huang et al. 2003) and *Lr1* (Ling et al. 2003), all of which belong to the nucleotide-binding site leucine-rich repeat (NBS-LRR) type resistance gene family. However, high genetic variation in the pathogen and its ability to evolve into new races with added virulence have been the major factors limiting

successful long-term management of leaf rust by race-specific resistance genes. Thus, cultivars that carry durable slow-rusting resistance genes become more popular (Caldwell 1968; Johnson and Law 1975; Parlevliet et al. 1985). Slow-rusting is quantitatively inherited and characterized by a longer latent period, smaller uredinial size, and reduced spore production with lower infection frequency (Caldwell 1968). Among the 72 leaf rust resistance genes that have been reported in wheat, only four race-nonspecific adult plant resistance (APR) genes have been named, including *Lr34/Yr18* on chromosome 7DS (Dyck and Samborski 1979; Singh et al. 2000), *Lr46/Yr29* on chromosome1BL (Martinez et al. 2001; Singh et al. 1998; William et al. 2003), *Lr67/Yr46* on chromosome 4DL (Herrera-Foessel et al. 2011; Hiebert et al. 2010) and *Lr68* on chromosome 7BL (Herrera-Foessel et al. 2012). When present alone, these genes do not provide adequate resistance, but provide near immunity when 4 to 5 such genes are present together in a plant (Singh et al. 2000).

CI13227 is a wheat line that exhibits a high level of slow-rusting resistance (Shaner and Finney 1980). Xu et al. (2005) were able to map various components of slow rusting resistance in a cross of CI13227/ Suwon92 to chromosomes 2B, 2D and 7B. At the time, the best markers available were SSR and AFLP markers. Unfortunately, the older markers resulted in linkage maps that contained many gaps. It is possible that some QTLs failed to be detected or were not precisely mapped in those studies.

The polyploid nature of wheat genome and its abundant repetitive DNA sequences increases the complexity to analyze the genetic variations in wheat. A high-resolution genetic map is required for QTL mapping to narrow down the intervals for causal genes (He et al. 2014). SNP markers have been more common in QTL mapping

studies due to the rapid development of next-generation-sequencing (NGS) technologies and available sequence information for SNP genotyping (Mammadov et al. 2012). Genotyping-by-sequencing that use restriction digestion to reduce the complexity of wheat genome (Poland et al. 2012) has been applied in wheat SNP marker discovery and QTL mapping. GBS also takes advantage of NGS by multiplexing samples using barcodes to reduce the costs of reagents. The efficiency of SNP identification can be increased with available reference genome sequences in different species (Poland et al. 2012; Spindel et al. 2013). In wheat, although complete reference genome sequences are unavailable, some bioinformatics pipelines using incomplete or no reference genome sequences are now available for wheat GBS SNP calling (Mascher et al. 2013).

In this study, we used a recombinant inbred line (RIL) population derived from CI13227 x Suwon92, the same population used in Xu et al. (2005), but an improved set of markers generated by GBS. The objectives of this study were to (1) remap QTLs for slow-rusting traits in CI13227 using a high-density GBS-SNP map, (2) develop KASP markers closely linked to the QTLs for marker-assisted selection in wheat breeding programs.

Materials and methods

Plant materials and experimental design

A mapping population of 104 $F_{6:7}$ RILs was developed from CI13227 x Suwon 92 by single-seed descent. CI13227 is a winter wheat line originally selected from 1976 International Winter Wheat Rust Nursery, and shows a high level of slow leaf-rusting resistance with a long latent period (LP). Its pedigree is Wabash/American Banner//Klein Anniversario (Shaner et al. 1997). Suwon92 is a Korean leaf rust susceptible cultivar

derived from Suwon85 x Suwon13 with a short LP (Shaner and Finney 1980). Both parents and the RILs were evaluated for LP, final severity (FS), and area under disease progress curve (AUDPC) in two greenhouse experiments at Purdue University, West Lafayette, IN, USA in the fall of 1997 and Kansas State University, Manhattan, KS, USA in the fall 2011. All experiments used a randomized complete block design (RCBD) with two replicates.

Seedling rust test

Both parents of the RIL population, CI13227 and Suwon 92, plus Chinese Spring and two cultivars Lakin and Everest from Kansas were grown in a soil mixture in 4.5-cm-diameter pots and tested for reactions to leaf rust inoculation at the seedling stage in the greenhouse. Five seeds per line were planted in each pot with two replications and grown in a greenhouse at 20 ± 3°C. Urediniospores of leaf rust cultures stored at -80°C were heat shocked at 42°C for 6 m before inoculation. Ten-days-old seedlings of all the tested cultivars were inoculated by spraying the seedlings with a suspension of urediniospores in Soltrol 170 isoparaffin light mineral oil (Chevron Phillips Chemical Company LLC, The Woodlands, TX). After the oil had evaporated for 10 min, the inoculated seedlings were incubated for 16-20 h in a dew chamber at 20 ± 2°C. IT was recorded 14 d after inoculation using the 0-4 Stakman scale (Burdon 1987). The seedling test included rust cultures MFPSC, PNMR, ASH11 composite, and three cultures collected from the field in 2010 (Lr10-2.1), 2013(Lr13-1.1), and 2014 (Lr14-1).

Leaf rust resistance evaluated in adult plant stage

In greenhouse experiments, plants were grown at 22 ± 5 °C day/17 ± 2 °C night temperature with supplemental daylight of 12 h. In the greenhouse experiment at Purdue

University, plants were inoculated with urediniospores of *P.triticinia* culture 7434-1-1T when flag leaves fully emerged (Xu et al. 2005). In the Kansas State University, at the flowering stage, 5 plants per replication were inoculated with a suspension of urediniospores of a *P. triticina* isolate selected for virulence to the parents in the seedling test, and incubated overnight in a dew chamber to maintain high moisture for infection. Infected plants were moved to greenhouse benches for rust development at 22 ± 4°C. LP was measured as the number of days from inoculation to when a uredinium ruptured the epidermis (Shaner et al. 1997). Rust severity at 15 d after inoculation was calculated as FS following the modified Cobb scale (Peterson et al. 1948). Meanwhile, rust severity was evaluated at 7, 10, 13, 15 d after inoculation to calculate AUDPC using the formula AUDPC = $\sum_{i}^{n-1}\left[\left(\frac{y_i+y_{i+1}}{2}\right)(t_{i+1}-t_i)\right]$, where y_i is the disease severity collected, and t_i is the day when disease severity is collected (Shaner and Finney 1980).

SSR marker analysis

Genomic DNA of parents with three replications and their RILs was extracted using a modified cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980). A selected set of 384 SSR primers was screened for polymorphism between the parents, and polymorphic SSR markers were used to screen the RIL population. For each SSR reaction, a PCR mix contained 50 ng of genomic DNA, 0.2 mM of each dNTP, 1× ammonium sulfide PCR buffer, 0.1 μM of forward primer, 0.15 μM of reverse primer, 2.5 mM of Mg^{2+}, 0.05 μM of dye-labeled M13 primer and 1 unit of *Taq* polymerase. A touchdown program for PCR amplification started at 95°C for 5 min, followed by five cycles of 45 s at 95°C, 5 min of annealing at 68°C which decreased by 2°C in each subsequent cycle, and 1 min extension at 72°C. In the subsequent five-

cycles, the annealing time was reduced to 2 min with a decrease of 2°C in each subsequent cycle. PCR was continued for an additional 25 cycles of 45 s at 94°C, 2 min at 50°C, and 1 min at 72°C, with a final extension at 72°C for 5 min. PCR products were detected using an ABI Prism 3730 DNA Analyzer, the fragment size was scored using GeneMarker version 1.97 (SoftGenetics, LLC).

GBS library construction and SNPs identification

DNA samples were quantified using the Quanti-iT^{TM} PicoGreen® and concentration of each sample was normalized to 20 ng/µl. Normalized DNA of each sample was digested with two restriction enzymes, *PstI* and *MspI*, according to the protocols of Poland et al. (2012). After digestion, each sample was ligated to barcoded adapters and common Y-adapters, and all the ligated samples were pooled into a single tube for PCR amplification to produce a library. DNA fragments in the library were quantified using Bioanalyzer 7500 Agilent DNA Chip (Agilent Technologies, Inc.) and sequenced using Illumina HiSeq2000 (Illumina, Inc.). Raw data from Illumina Hiseq2000 were first trimmed to 64 bp, and identical reads were grouped into tags. Pairwise alignment of the tags allowed identification of single base pair mismatches as candidate SNPs. SNP calling was conducted using a custom script in Java (www.maizegenetics.net/sourceforge.net/projects/tassel/). Reads with bad quality score (<15) were removed. Sequences from each parent with three replications were clustered, the genotype of each RIL was determined based on the cluster of parents. Because RILs were used in library construction, SNPs with heterozygotes >10 % of total RILs were discarded. SNPs with missing data <20 % were used for mapping.

Genetic map construction and QTL analysis

A linkage map was constructed using SNPs data from GBS (GBS-SNPs) and 90 selected polymorphic SSR markers using the Regression function in JoinMap version 4.1 (Van Ooijen 2006). Recombination fractions were converted into centiMorgans (cM) using the Kosambi function (Kosambi 1943). Composite interval mapping (CIM) in IciMapping 3.3 (http://www.isbreeding.net) was used to identify significant QTLs at a walking speed of 1.0 cM. LOD scores range from 2.65 to 2.87, (p = 0.05) based on the calculation from 1,000 permutations for different traits. Significant QTLs were claimed at LOD score of 2.87 (p = 0.05).

Conversion of SNPs to KASP markers

SNPs closely linked to QTLs were converted to Kompetitive allele-specific PCR (KASP) assay using Primer 3.0 to design primers (http://bioinfo.ut.ee/primer3-0.4.0/). Each assay contains three primers including two forwarded primers and one common reverse primer. Two forward primers were added with FAM and HEX sequences, respectively, as tails. Newly designed KASP assays were evaluated for polymorphisms between the two parents before genotyping the mapping population. KASP assays were performed in a 6-µl reaction volume (3 µl 2X KASP Master Mix, 0.0825 µl KASP primer mix and 3 µl genomic DNA at 25 ng/µl) and data were analyzed in an ABI 7900HT Real-Time PCR System (Life Technology, Grand Island, NY) following the instruction for KASP analysis (http://www.lgcgroup.com).

Results

Linkage map construction

GBS analysis generated a total of 120 million reads in one run of Illumina HiSeq 2000, and 112 million reads met the quality score (≥15) after the initial filtering. After further filtering, a total of 3371, 6336 and 7389 GBS-SNPs were called at <20%, <50%, <80% of missing data, respectively, in the population, and GBS-SNPs with <20% were used for map construction.

Initial analysis mapped 2972 GBS-SNPs and 84 SSR to 47 linkage groups, covering all the 21 chromosomes with the lengths of the individual linkage groups ranging from 12.5 to 253.7 cM. The total map length was 4643.8 cM with an average marker density of 1.6 cM per marker and 10 to 305 markers per linkage group. The B genome had the most mapped markers (46.5%), followed by the A genome (37.2%) and the D genome (16.3%) (Figure 2.1).

Parental seedling reactions to rust inoculation with different isolates

To identify the most effective leaf rust isolate for detection of slow-rusting resistance in CI13227, an initial screening was conducted at the seedling stage using five different isolates. Most of the isolates, except MFPSC (PRTUS 54), showed a resistant reaction (1 to 2+) for CI13227 (Table 2.1). All the parental lines, CI13227 and Suwon92 plus Lakin and Everest tested in this study and a susceptible control, Chinese Spring, showed a susceptible reaction, indicating that MFPSC (PRTUS54) is an appropriate isolate to differentiate adult plant resistance between CI13227 and other cultivars tested.

Resistance at the adult stage in the parents and RIL population plant

In both experiments, CI13227 showed a higher level of slow-rusting resistance to wheat leaf rust than Suwon92, evidenced by lower FS, AUDPC, and longer LP (Table 2.2). The FS, AUDPC, and LP of the RIL population showed continuous distributions, ranging from 14.0 to 95.0% for FS, 24.4 to 585.0 for AUDPC, 5 to 15 d for LP (Figure. 2.2). All traits showed possible transgressive segregation in both greenhouse experiments. Significant correlations between two experiments ($p < 0.0001$) were detected for LP (r = 0.66), FS (r = 0.43), and AUDPC (r = 0.48). Among those traits, LP was negatively correlated ($p < 0.0001$) with FS and AUDPC with correlation coefficients of -0.73 and -0.68, respectively (Table 2.4), indicating that a slow-rusting cultivar usually has a longer LP, but lower FS and AUDPC. Correlation coefficient between AUDPC and FS (r = 0.98, $p < 0.0001$) was very high (Table 2.4), indicating that the two traits are most likely under the same genetic control.

QTL mapping

Composite interval mapping detected three QTLs on chromosomes 2D, 7B, and 2B for at least two of the three traits measured (Table 2.5). One QTL, designated as *QLr.hwwgru-2DS,* was located on the short arm of chromosome 2D based on the SNP markers location blasted to the W7984 reference genome (https://urgi.versailles.inra.fr/blast/?dbgroup=wheat_survey&program=blastn) and was significant for FS, AUDPC, and LP in both experiments (Figure. 2.3). This QTL contributed by CI13227 is flanked by SNPs GBS_snpj1995 and GBS_snpj2228, and explained 10.0 to 38.5% of the phenotypic variation for slow-rusting traits.

A second QTL, designated as *QLr.hwwgru-7BL,* associated with FS, AUDPC, and LP in the fall 1997 experiment and FS and AUDPC in the fall 2011 experiment was localized on the chromosome 7BL based on the linked SSR and SNP markers that were previously mapped on the distal end of 7BL. The peak of this QTL was at a 2.1 cM interval between the SSR marker Xbarc182 and SNP marker GBS_snp1637 (Figure 2.4). The QTL explained 8.6-15.3% of the phenotypic variation for slow-rusting traits and CI13227 contributes the positive allele.

The third QTL for LP, FS, and AUDPC, *QLr.hwwgru-2BL,* was significant only in fall 1997 experiment and was located on the chromosome 2BL based on the SNPs location blasted in the reference map (https://urgi.versailles.inra.fr/blast/?dbgroup=wheat_survey&program=blastn) based on linked markers that were previously mapped on the chromosome arm (Figure. 2.5). This QTL contributed by CI13227 explained 10.2 to 12.5% of the phenotypic variation for the three traits and was located at an interval of 2.4 cM between SNPs GBS_snp2107 and GBS_snp0376.

Verification of GBS-SNPs with KASP assays

To verify the genotypic data generated by GBS and convert the GBS based SNP markers to breeder-friendly KASP assays for MAS in breeding, 28 GBS-SNPs within or around the three QTL regions were used to design KASP assays. Among them, 22 (7 on chromosome 2DS, 10 on 7BL, 5 on 2A) showed polymorphisms between parents and among the RILs, and 18 were remapped to one of the three QTL regions (Table 2.5). Four SNPs were mapped outside the QTL regions after all missing data at these loci were filled by the KASP markers. Comparison between GBS-based SNPs and corresponding

KASP data found that 18 SNPs gave identical genotypes in the population (Figure 2.6), one SNP had an SNP call error in two RIL lines, and three had errors in one RIL lines. Therefore, the average error rate for GBS SNPs determination was 0.22 %.

Effects of QTLs on leaf rust resistance

To investigate the effect of individual QTLs on leaf rust resistance, RILs that carried different allele combinations at the two stable QTLs (*QLr.hwwgru-2DS* and *QLr.hwwgru-7BL*) detected in both experiments from CI13227 were grouped and their allele substitution effects were compared (Figure 2.7). The four possible allele combinations of the two QTLs are AABB, AAbb, aaBB, and aabb, where AA and BB represent the marker alleles linked to the resistance QTLs on 2DS and 7BL from CI13227, whereas aa and bb represent corresponding opposite alleles on the two QTLs on 2DS and 7BL. The closest markers to the two QTLs (GBS_snpj2228 on 2D and GBS_snp1637 on 7B) were selected to represent these QTLs. Two contrasting alleles at each of the two SNPs exhibited a 1:1 segregation ratio in the RIL population. All four genotype combinations could be found in the RIL population. Mean LP and mean AUDPC for the four genotypes ranged from 7.6 to 10.5 d and 204.75 to 304.95, respectively. For LP, the genotypic group with two resistance alleles at *QLr.hwwgru-2DS* and *QLr.hwwgru-7BL* was significantly higher than the genotypic groups with only one of the resistance alleles, and the genotypic group with only resistance allele at *QLr.hwwgru-2DS* was significantly higher than the genotypic group with none of the positive alleles. The same trend was observed for AUDPC (Figure. 2.7), indicating that a combination of both QTLs significantly increased rust resistance. However, LP and AUDPC was not significantly different between the genotypic group with only

QLr.hwwgru-7BL and the group with none of the resistance alleles, suggesting that *QLr.hwwgru-7BL* showed much smaller effect than *QLr.hwwgru-2DS*.

Discussion

In this study, FS, AUDPC and LP were evaluated as slow-rusting traits in the RIL population. Transgressive segregation was observed for all traits, suggesting that both parents contribute alleles to the phenotype. However, we failed to detect any QTL for slow rusting resistance from the susceptible parent Suwon92, all three QTLs mapped were from the resistant parent CI13227. This might be attributed to the small size of the mapping population that prevented capturing alleles contributed from the susceptible parent.

A high-density genetic map was generated using GBS in our study. GBS have been applied to constructing high-resolution maps in sorghum, wheat, rice and barley, and widely used in QTL mapping for different traits and candidate genes identification (Poland et al. 2012; Saintenac et al. 2013; Spindel et al. 2013). One disadvantage of GBS markers for QTL mapping is the large amount of missing data for some markers due to limited sequencing depth. In our study, in order to increase data quality, we use only SNPs with <20% missing data to construct linkage map for QTL detection. Then we converted GBS-SNPs closely linked to the QTLs to KASP-SNPs to verify the GBS-SNPs in the QTL regions. After QTL mapping using the high-density genetic map generated from 2972 SNPs and 80 SSR markers, we re-genotyped the mapping population with KASP markers converted from GBS-SNPs in the QTL regions to minimize the negative effect of missing data on QTLs and correct sequencing errors of GBS-SNPs in the QTL regions. The data from the 18 QTL-linked KASP markers successfully verified GBS-SNP

data after filling missing data, suggesting most GBS-SNPs provide accurate genotypic data for QTL mapping.

The QTL with the largest effect contributed by CI13227 detected in this study is the *QLr.hwwgru-2DS*. This QTL explained up to 38.5% of the phenotypic variation of slow-rusting traits. Raupp et al. (2001) reported the location of a race specific resistance gene, *Lr39*, on 2DS. *Lr39* was located on the distal end of chromosome 2D at 10.7 cM away from a SSR marker Xgwm210. Singh et al. (2004) suggested that the leaf rust resistance gene *Lr41* was the same as *Lr39* located on chromosome 2D. Sun et al. (2009) mapped *Lr41* on the distal end of chromosome 2D and the closest marker, Xbarc124, was 1.0 cM away from *Lr41*, while *QLr.hwwgru-2DS* in the current study was located on the other side of Xbarc124 at a distance of 22 cM. Also, *Lr39* was seedling resistance gene, thus *QLr.hwwgru-2DS* detected in the current study is most likely different from *Lr39.* This study used the same population used by Xu et al. (2005), but different maps. Because a new set of GBS-SNPs were added to the new map on the chromosome 2D, the QTL on the chromosome 2DS was shifted away from the most closely linked SSR marker Xbarc124 previously reported and located more closely to Xbarc95. Also in the current study, the two closest SNPs, GBS_snpj1995 and GBS_snpj1891 to *QLr.hwwgru-2DS* had much higher r^2 values, 16.3% and 25.3%, than Xbarc124 (10.1%) had. Therefore, the SNP markers identified from this study are better markers than Xbarc124 for MAS, assuming that sufficient polymorphisms exist between parents used in breeding.

The *QLr.hwwgru-7BL* detected in this study was flanked by the markers Xbarc182 and GBS_snp1637. Several QTLs associated with leaf rust resistance have

been detected on the chromosome 7B. Xu et al. (2005) reported a minor QTL, *QLr.ous-7BL*, for LP between an AFLP marker XCATG.ATGC125 and Xbarc182. Based on the location of common marker Xbarc182, *QLr.ous-7BL* was the same QTL as *QLr.hwwgru-7BL*. Herrera-Foessel reported a gene, designated *Lr68,* for slow-rusting in a CIMMYT's spring wheat, Parula, and a dominant marker, csGS*,* was reported to be tightly linked to the gene (Herrera-Foessel et al. 2012). We failed to detect polymorphism in our RIL population using *csGS*, thus further studies are needed to determine whether *QLr.hwwgru-7BL* is the same gene as *Lr68*.

The third QTL, *QLr.hwwgru-2BL,* was only significant for slow-rusting traits in 1997 greenhouse experiment. In the previous study, Xu et al. (2005) reported a QTL on chromosome 2BL close to a SSR marker Xbarc167. In our study, the detected QTL is 1.1 cM away from Xbarc167 and located between SNPs GBS_snp2107 and GBS_snp0376 at an interval of 2.4 cM. Based on the location of the common marker Xbarc167, *QLr.hwwgru-2BL* located at the same position as *QLr.osu_2BL.* However, this QTL was only detected in a single greenhouse experiment. It is likely a QTL with minor effect and affected by environmental factors such as plant growth stage when infection was initiated and temperature for wheat growth. It is also possible that the QTL is race-specific. The culture used in the second experiment was selected for being virulent on CI13227 at the seedling stage, but seedling susceptibility was not tested for the culture used in the first experiment. Genes on 2BL include *Lr50*, *Lr58*, and *Qlr.ifa-2BL* (Brown-Guedira et al. 2003; Buerstmayr et al. 2014; Kuraparthy et al. 2007).

AFLP and SSR markers were used in the previous study to detect QTLs in the population. However, direct application of AFLP markers in MAS is not feasible due to

complicated procedure, thus Xu et al. (2005) recommended to use the SSR markers instead. Because some SSR are far from the QTLs, they might reduce the accuracy and effectiveness of MAS. In order to further utilize the QTLs detected in this study, user-friendly markers are critical for MAS. KASP assay is considered as a time-saving and cost-effective genotyping platform for SNP analysis (Semagn et al. 2014), and has been widely used in MAS. In the current study, map resolution was significantly increased in QTL regions by adding GBS-SNPs, tightly linked SNP markers, GBS_snpj2228 and GBS_snpj1891 on chromosome 2DS, GBS_snp1637 on chromosome 7BL, GBS_snp2107 and GBS_snp0376 on chromosome 2BL were successfully converted to KASP assays. Thus they can greatly facilitate the introgression of these QTLs into other cultivars in wheat.

Conclusions

In this study, three QTLs for adult plant resisitance to leaf rust associated with slow-rusting traits were mapped on chromosome 2DS, 7BL and 2BL. These QTLs were all contributed by CI13227 and were in the same locations as QTLs reported previously by Xu et al (2005a,b) for CI13227. *QLr.hwwgru-2DS* is a major APR gene that was associated with slow-rusting resistance. *QLr. hwwgru-7BL* is another APR gene associated with a slow-rusting phenotype. Its phenotype and location appears to be identical to Lr68 as described by Herrera-Foessel et al (2012). QLr.hwwgru-2BL was detected in the first experiment, but not in the second. It is possible that this QTL is spurious or race-specific. If *QTL.hwwgru-2BL* is real, it should be compared to other known genes on 2BL. SNP markers closely linked to the QTL regions were converted to

KASP-SNPs that can be easily used for marker-assisted selection to improve slow rust resistance in wheat.

Figures and Tables

Figure 2.1 Distribution of GBS-SNPs on each chromosome based on linkage map in the recombinant inbred line (RIL) population derived from CI13227 x Suwon92

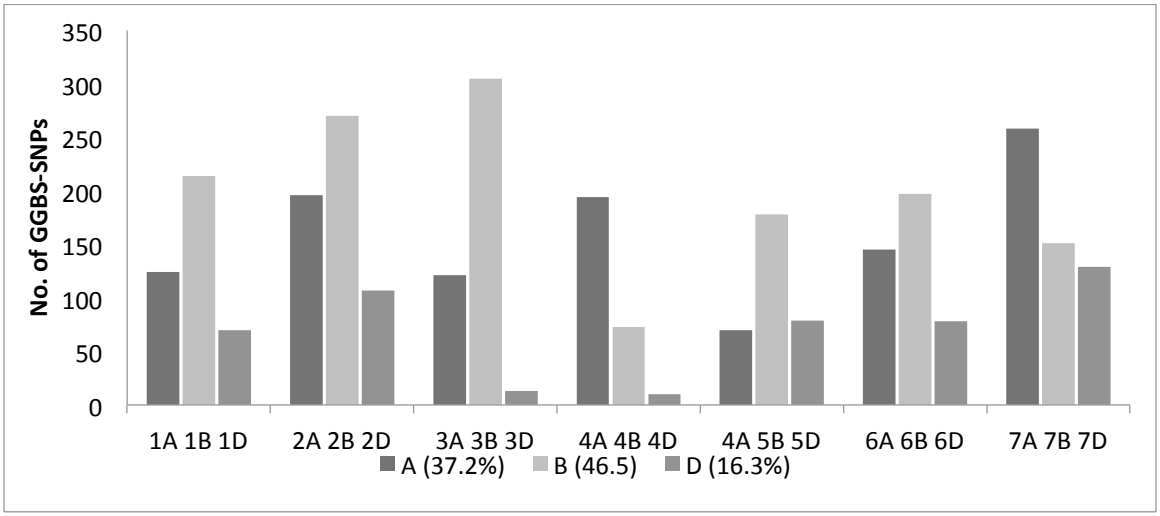
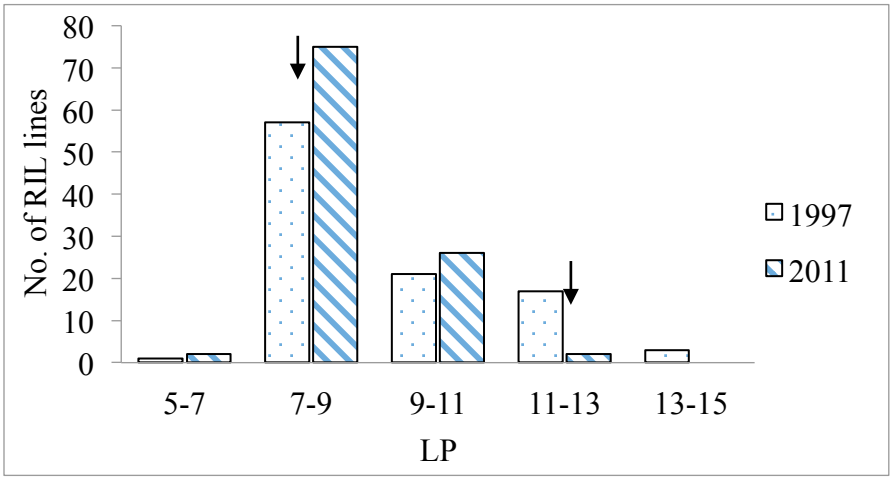
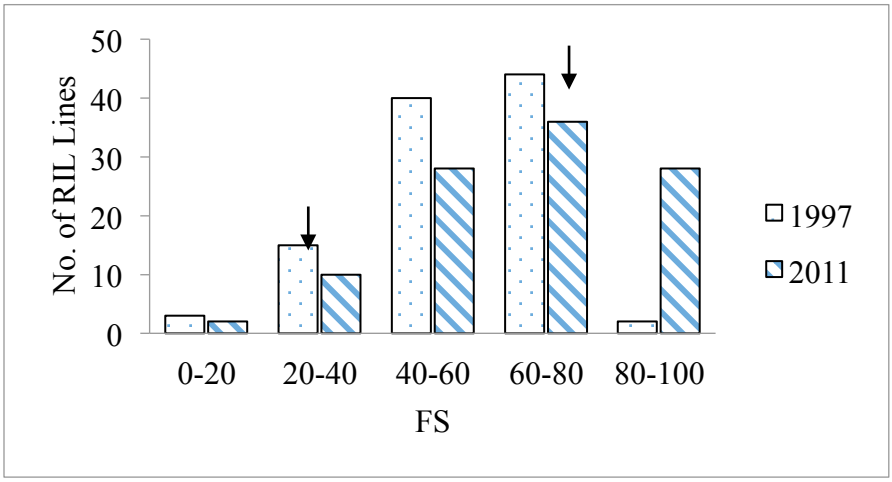


Figure 2.2 Frequency distribution of latent period (LP), final severity (FS), area under disease progress curve (AUDPC) for parents, CI13227 and Suwon92, and their 104 recombinant inbred lines (RILs) measured in the two greenhouse experiments





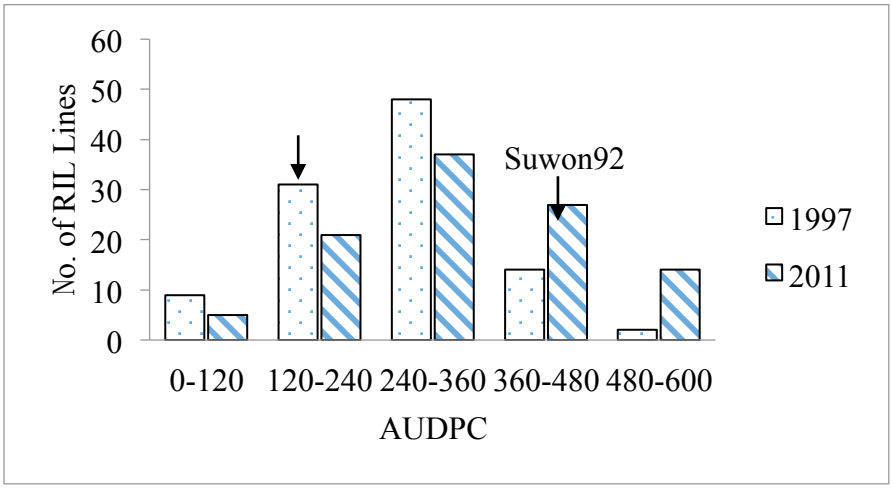


Figure 2.3 Part of the high-density linkage map for chromosome 2D constructed using GBS-SNPs and SSR markers (left) and QTL location of *QLr.hwwgru-2DS* for slow-rusting traits mapped in the recombinant inbred line (RIL) population of CI13227 x Suwon92

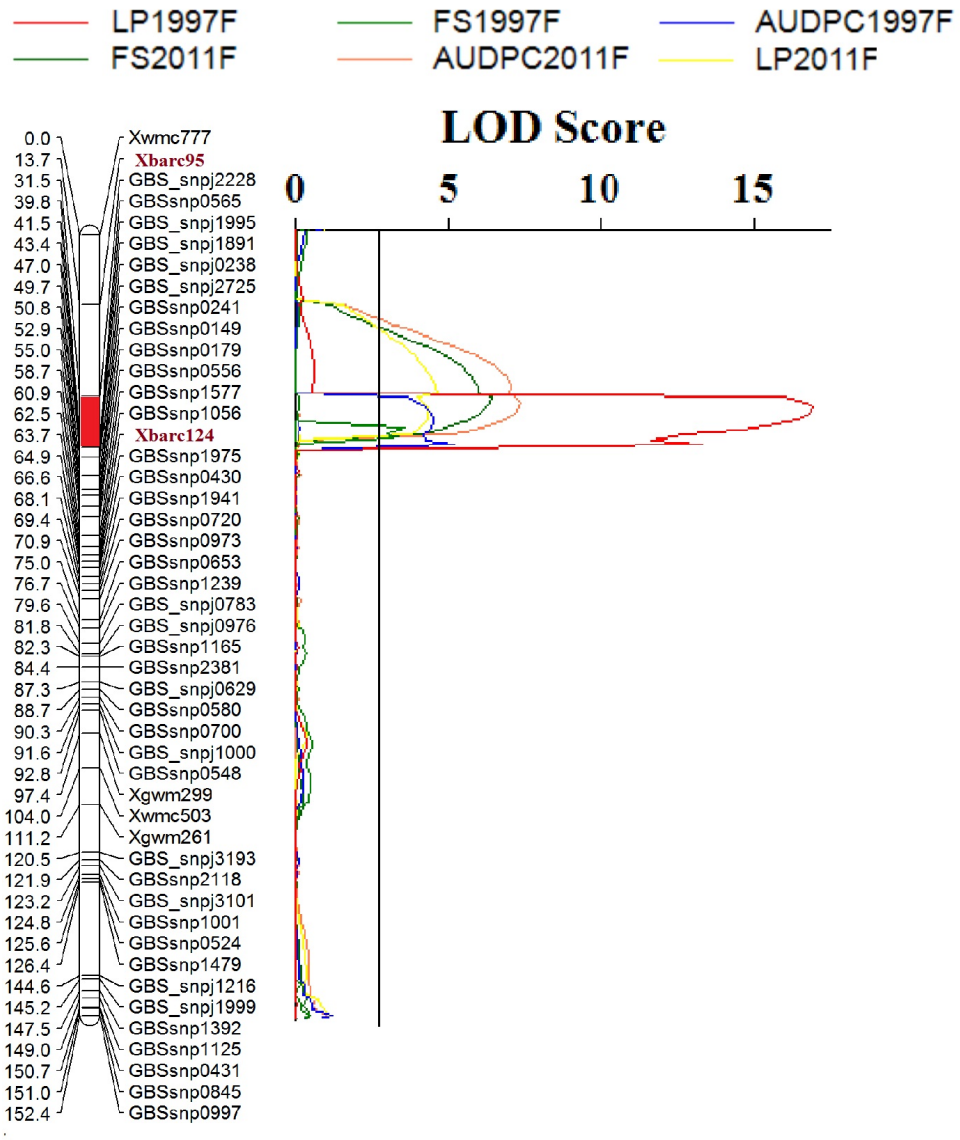


Figure 2.4 Part of the high-density linkage map for chromosome 7B constructed using GBS-SNPs and SSR markers (left) and QTL location of *QLr.hwwgru-7BL* for slow-rusting traits mapped in the recombinant inbred line (RIL) population of CI13227 x Suwon92

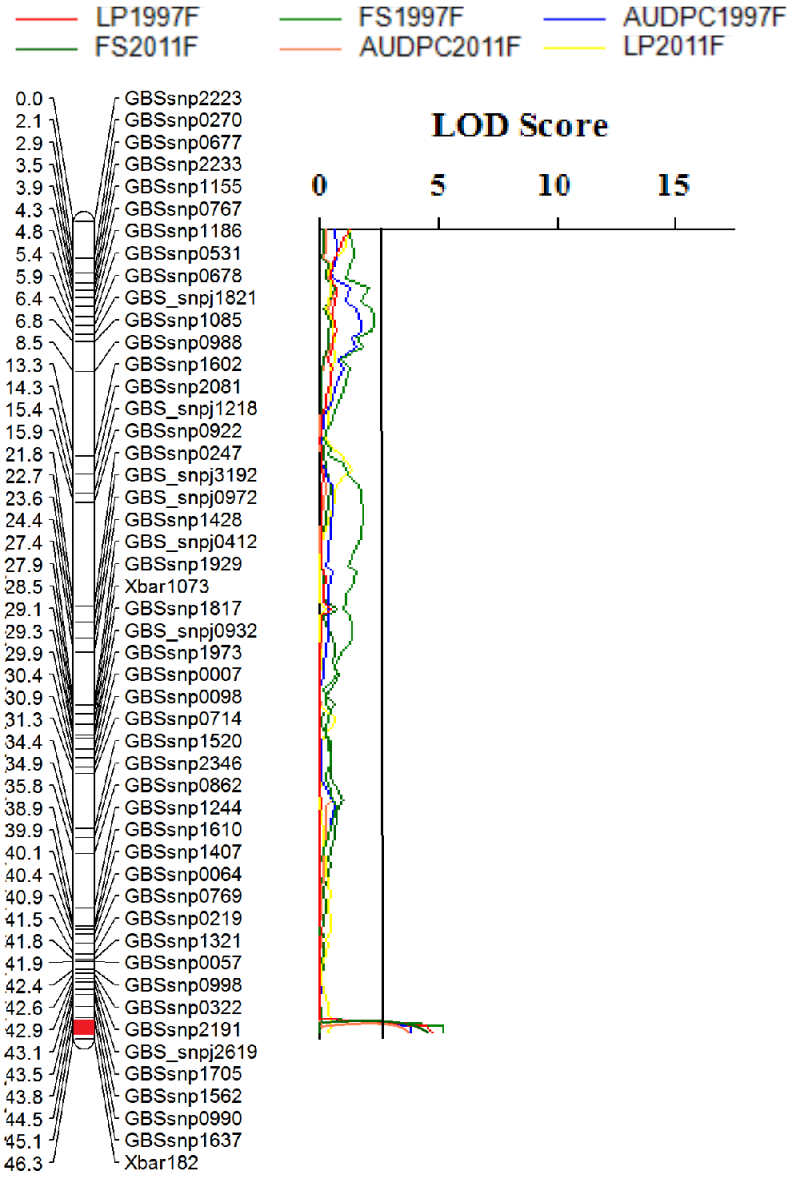


Figure 2.5 Part of the high-density linkage map chromosome 2B constructed using GBS-SNPs and SSR markers (left) and map location of *QLr.hwwgru-2BL* for slow-rusting mapped in the recombinant inbred line (RIL) population of CI13227 x Suwon92

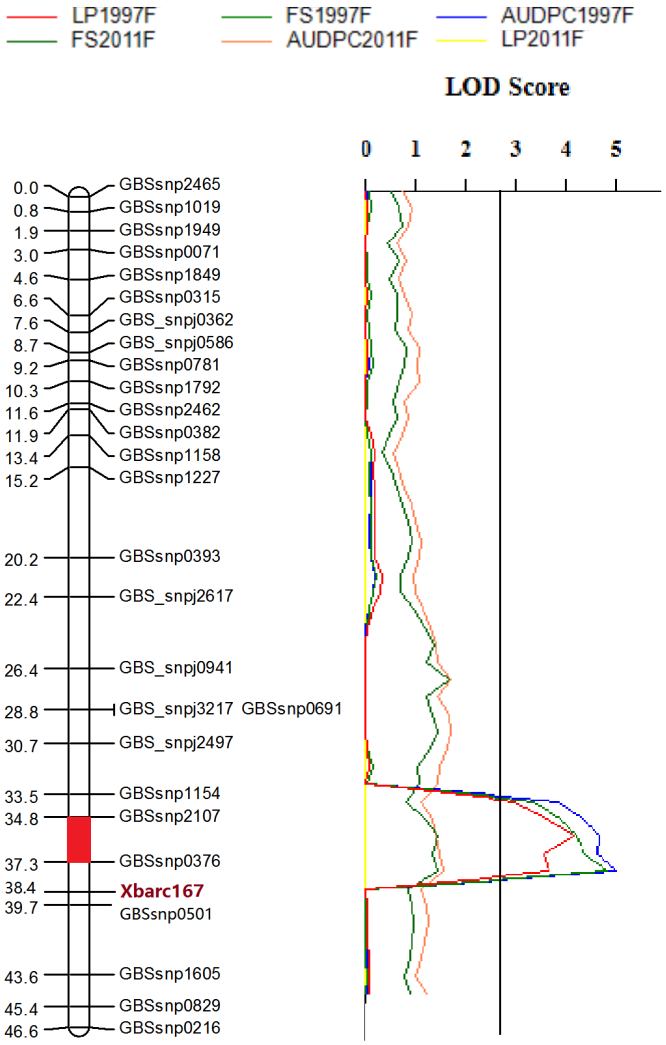
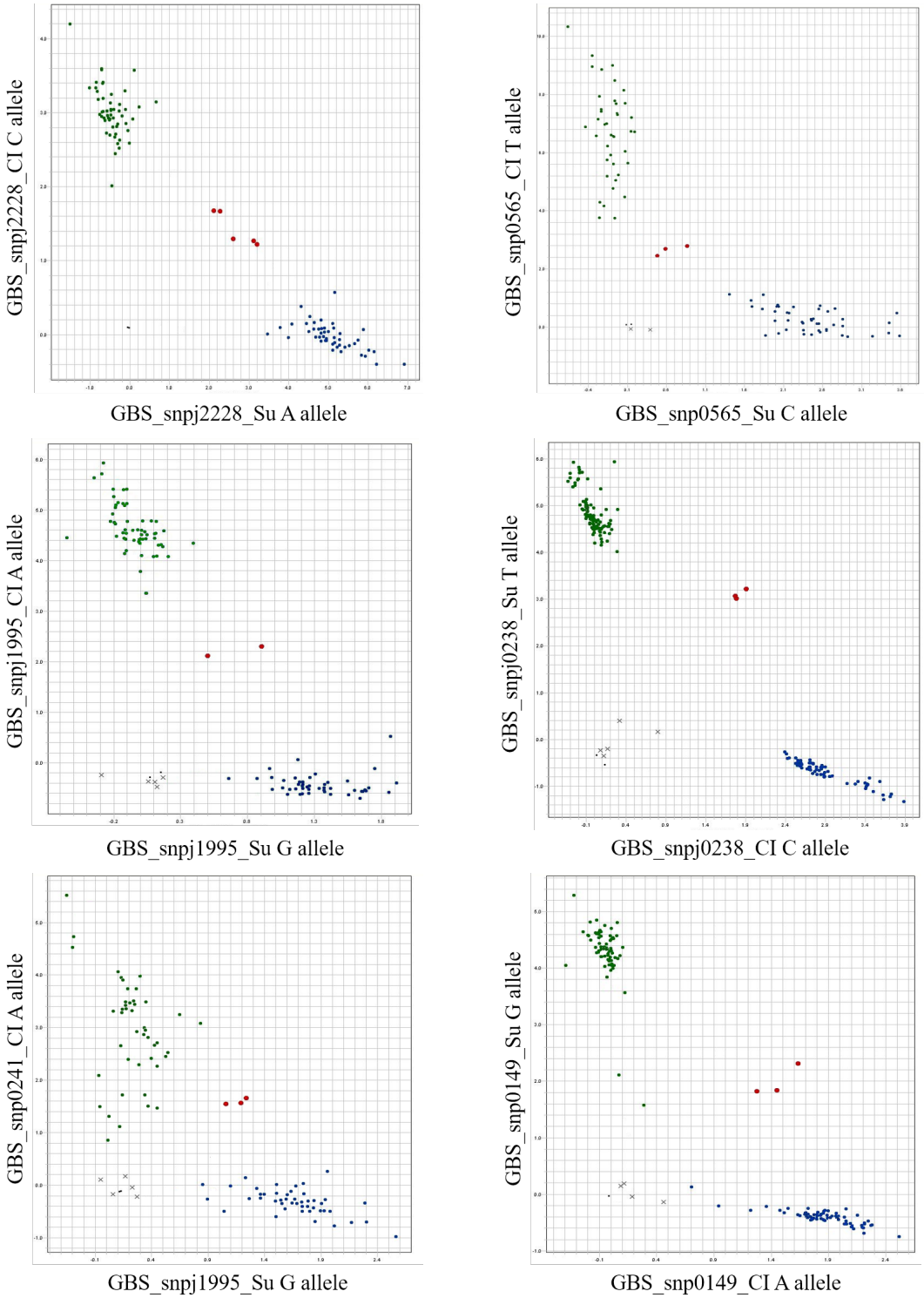
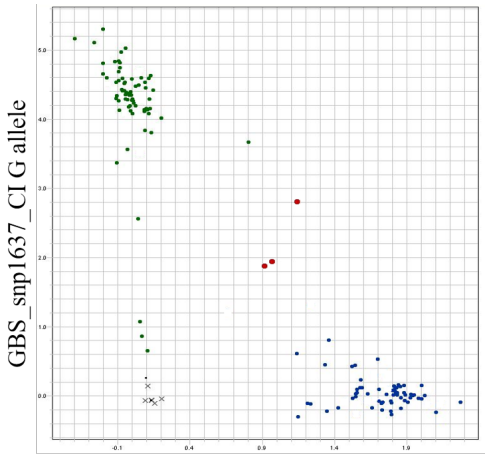
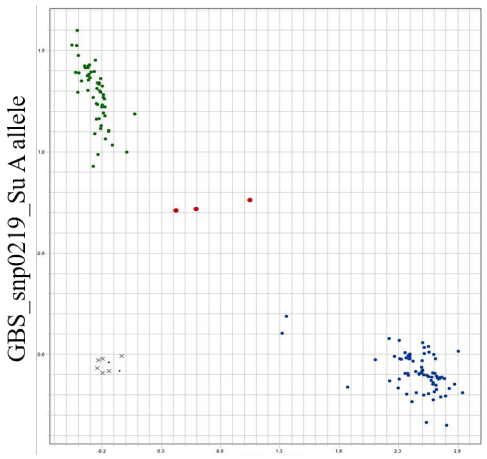
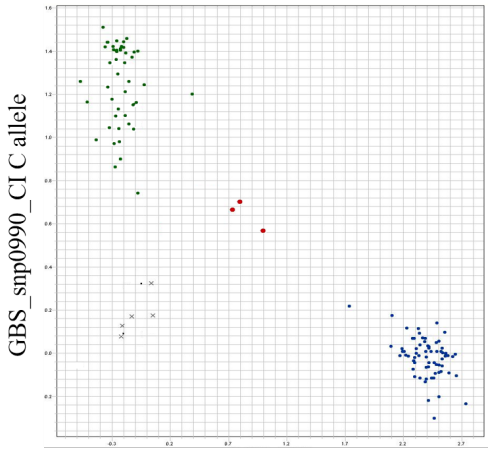


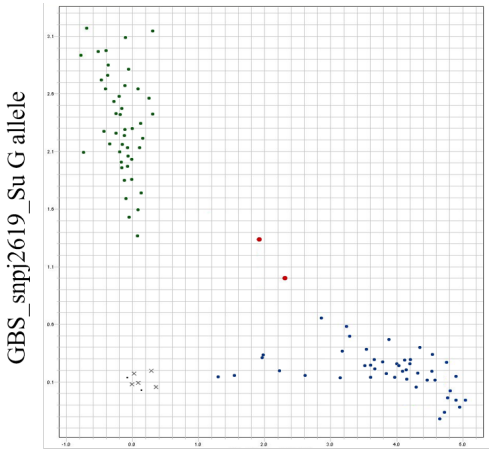
Figure 2.6 A KASPar assay profile to show allelic segregation of SNPs in the RIL population of CI13227 x Suwon92. The blue and green dots show different alleles, and the dark dots indicate the water control.

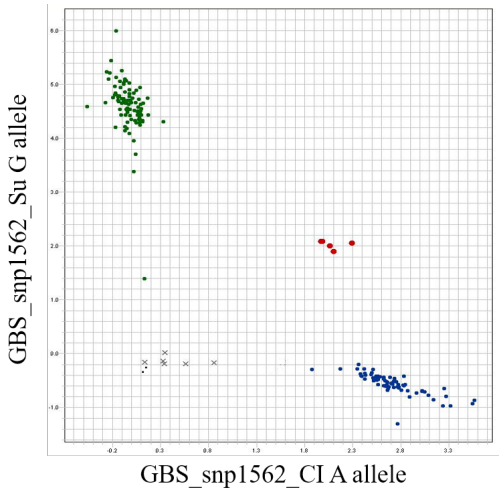


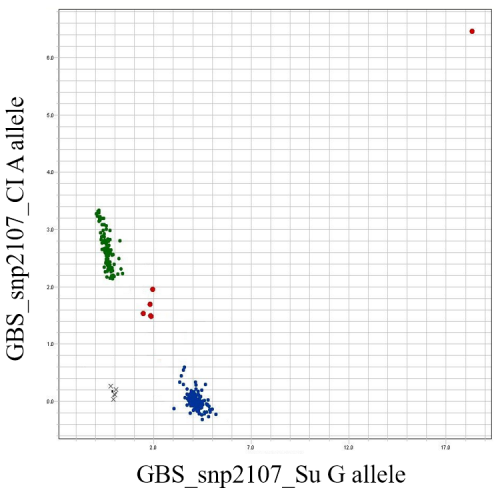

GBS_snp1637_CI G allele
GBS_snp1637_Su A allele

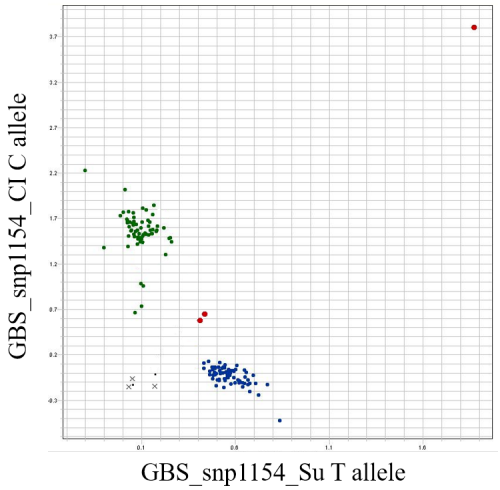

GBS_snp0219_Su A allele
GBS_snp0219_CI G allele

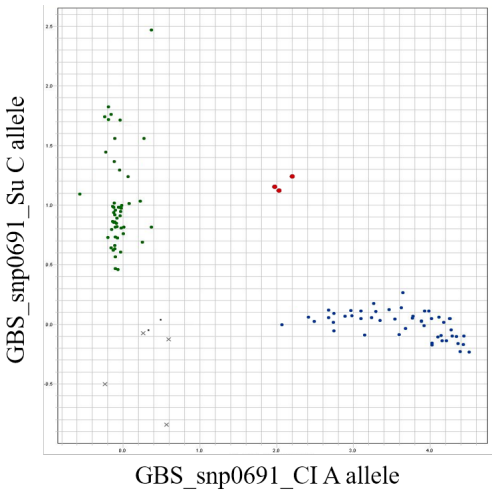

GBS_snp0990_CI C allele
GBS_snp0990_Su T allele

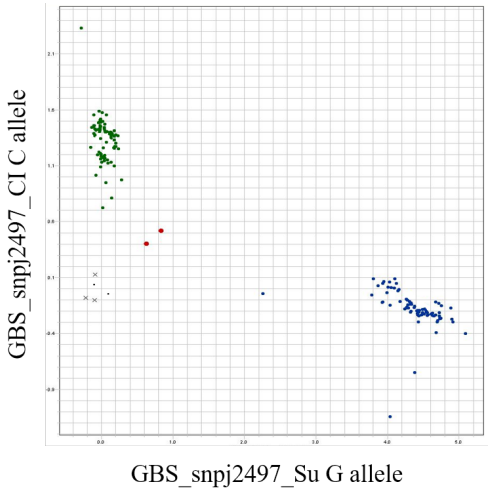

GBS_snpj2619_Su G allele
GBS_snpj2619_CI A allele

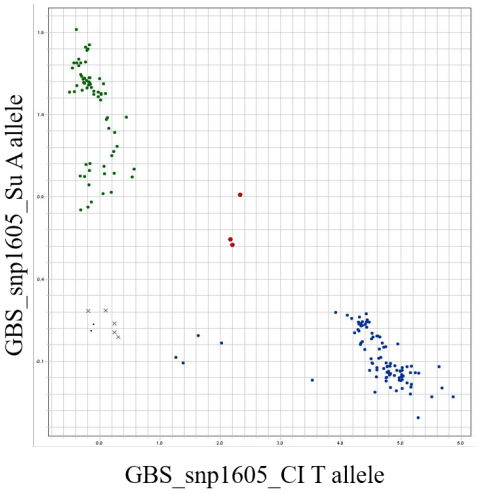

GBS_snp1562_Su G allele
GBS_snp1562_CI A allele

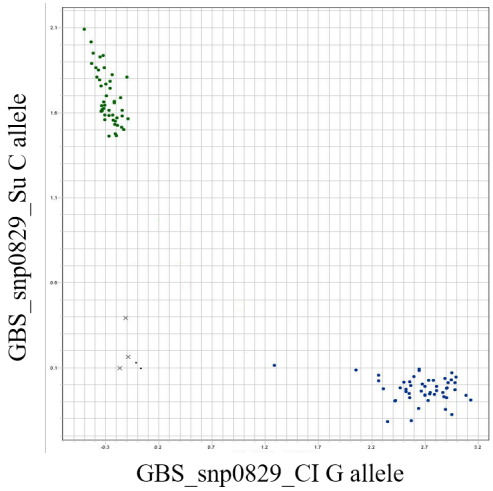

GBS_snp2107_CI A allele
GBS_snp2107_Su G allele


GBS_snp1154_CI C allele
GBS_snp1154_Su T allele


GBS_snp0691_Su C allele
GBS_snp0691_CI A allele


GBS_snpj2497_CI C allele
GBS_snpj2497_Su G allele


GBS_snp1605_Su A allele
GBS_snp1605_CI T allele


GBS_snp0829_Su C allele
GBS_snp0829_CI G allele

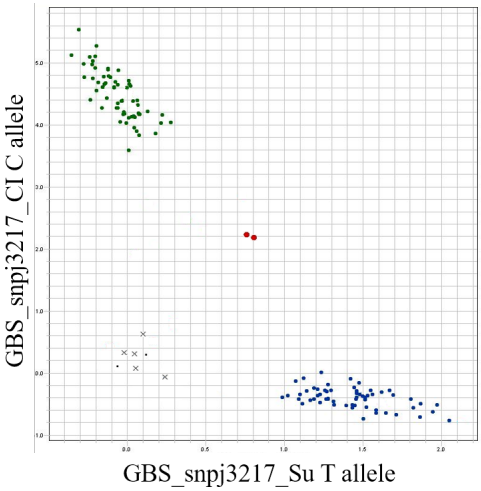
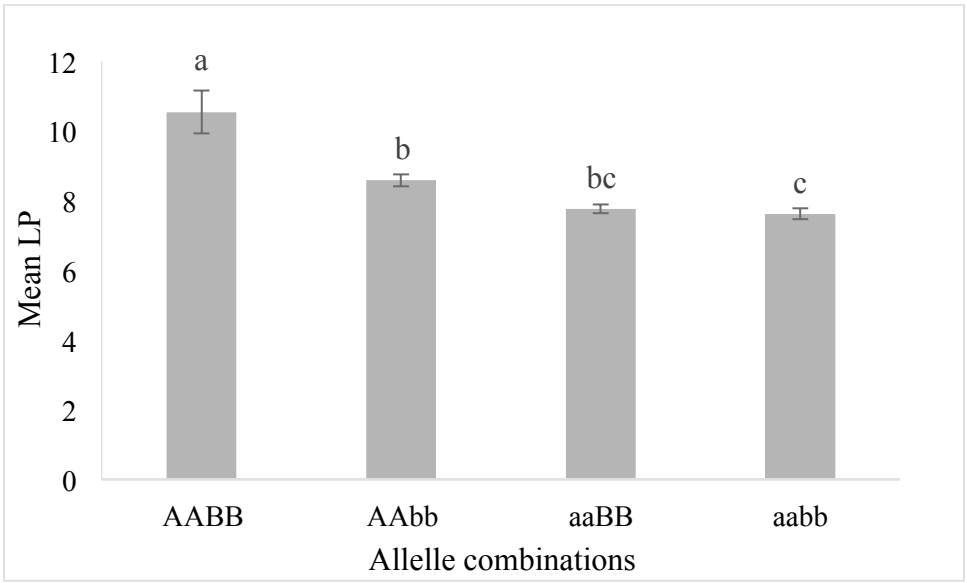

GBS_snpj3217_CI C allele
GBS_snpj3217_Su T allele

Figure 2.7 Quantitative trait loci (QTL) effect for latent period (LP) and area under disease progressive curve (AUDPC) for different allele combinations using Duncan multiple range test comparison at alpha 0.05 probability level: AA and BB refer to the marker alleles linked to resistance QTLs on 2D and 7B in CI13227, and aa and bb refer to corresponding opposite alleles of the two QTLs in Suwon92. Means with the same letters are not significantly different.



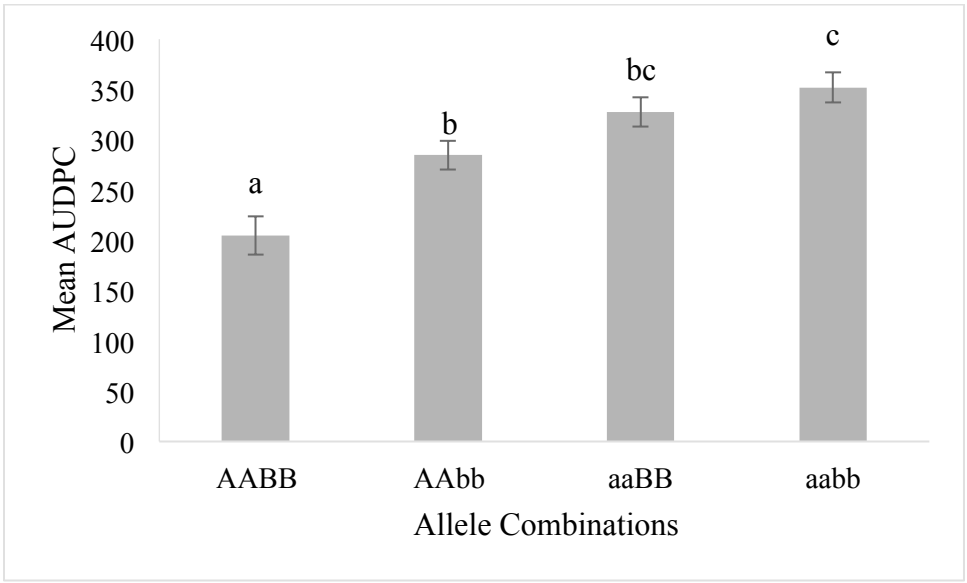


Table 2.1 Infection types (IT) of parental wheat lines, CI13227 and Suwon92, and three other cultivars, Lakin, Everest and Chinese Spring (susceptible check) by *Puccinia triticina* at seedling stage in a greenhouse test

	PNMR	MFPSC (PRTU54)	ASH11 composite	Lr13-1.1	Lr14-1	Lr10-2.1
Chinese Spring	3 +	3 +	3 +	3	3 +	3 +
CI13227	2 ;	3 +	2 + ;	; 1	; 1	2 + ;
Suwon92	3 +	3 +	3 +	3	3	3
Lakin	2 -	3	2 -	1 ;	2	; 1
Everest	2 -	3	2 - ;	1 ;	2	; 1

Table 2.2 Latent period (LP), final severity (FS), and area under disease progress curve (AUDPC) of CI13227, Suwon92, and their recombinant inbred line (RIL) population (n=104) in 1997 fall and 2011 fall greenhouse experiments

	Year[a]	LP day	FS %	AUDPC
CI13227	1997F	12	32.3	107.3
	2011F	11	28.3	102.6
Suwon92	1997	9	75.5	287.9
	2011F	8	68.3	259.9
RILs means	1997	9	54.1	268.5
	2011F	8	64.2	329.7
RILs ranges	1997	7-15	14.0-83.8	24.4-576.7
	2011F	5-12	14.2-95.0	51.5-585.0

[a]1997F=1997 fall, and 2011F=2011 fall

Table 2.3 Correlation coefficients among three slow-rusting traits, latent period (LP), final severity (FS), and area under disease progress curve (AUDPC)

	LP	FS
FS	-0.73***	
AUDPC	-0.68***	0.98***

*** $p < 0.0001$

Table 2.4 Chromosomal locations, marker intervals, determination coefficients (R^2), additive effects and logarithm of the odds (LOD) values for significant quantitative trait loci (QTLs) identified in the CI13227 x Suwon92 recombinant inbred line (RIL) population

Trait	Chromosome	QTL	Flanking Markers	Interval (cM)	LOD	R^2 (%)	Add
LP1997F	2D	*QLr.hwwgru-2DS*	GBS_snpj1995 - GBS_snpj2228	6.8	3.16	8.63	-0.32
FS1997F	2D	*QLr.hwwgru-2DS*	GBS_snpj1995 - GBS_snpj2228	6.8	3.15	8.86	4.95
AUDPC1997F	2D	*QLr.hwwgru-2DS*	GBS_snpj1995 - GBS_snpj2228	6.8	4.44	12.24	67.97
LP2011F	2D	*QLr.hwwgru-2DS*	GBS_snpj1995 - GBS_snpj2228	6.8	5.59	17.74	-0.37
FS2011F	2D	*QLr.hwwgru-2DS*	GBS_snpj1995 - GBS_snpj2228	6.8	12.37	38.51	11.49
AUDPC2011F	2D	*QLr.hwwgru-2DS*	GBS_snpj1995 - GBS_snpj2228	6.8	10.27	36.42	76.13
LP1997F	7B	*QLr.hwwgru-7BL*	Xbar182 - GBS_snp1637	2.1	4.4	12.01	-0.55
FS2011F	7B	*QLr.hwwgru-7BL*	Xbar182 - GBS_snp1637	2.1	4.94	13.51	7.15
AUDPC2011F	7B	*QLr.hwwgru-7BL*	Xbar182 - GBS_snp1637	2.1	3.93	9.99	27.67
FS1997F	7B	*QLr.hwwgru-7BL*	Xbar182 - GBS_snp1637	2.1	5.3	15.73	6
AUDPC1997F	7B	*QLr.hwwgru-7BL*	Xbar182 - GBS_snp1637	2.1	5.01	15.32	33.35
LP1997F	2B	*QLr.hwwgru-2BL*	GBS_snp2107 - GBS_snp0376	2.4	4.22	10.8	-0.34
FS1997F	2B	*QLr.hwwgru-2BL*	GBS_snp2107 - GBS_snp0376	2.4	4.7	12.47	6.59
AUDPC1997F	2B	*QLr.hwwgru-2BL*	GBS_snp2107 - GBS_snp0376	2.4	4.14	10.19	48.19

Table 2.5 List of Kompetitive allele-specific PCR (KASP) primers designed based on sequences from GBS-SNPs

Primer name[a]	Chromosome	Primer sequence (5'-3')
GBS_snpj2228_CI	2D	GCAGTGAACTTGGC
GBS_snpj2228_Su		GCAGTGAACTTGGA
GBS_snpj2228_R		AGCGTGCTAAGGAGT
GBS_snp0565_CI	2D	CGAGCTTATAAACAGGTAGCGAT
GBS_snp0565_Su		CGAGCTTATAAACAGGTAGCGAC
GBS_snp0565_R		TTTAGTCCCACCTCGCCT
GBS_snpj1995_CI	2D	TGCAGCCTCATAGAAA
GBS_snpj1995_Su		TGCAGCCTCATAGAAG
GBS_snpj1995_R		CCTTCCTTGGTGTCGT
GBS_snpj0238_CI	2D	GCAGACGCGGCGGC
GBS_snpj0238_Su		GCAGACGCGGCGGT
GBS_snpj0238_R		CTGCTTCGTGCCCTGTG
GBS_snp0241_CI	2D	TGCAGCCCCACCTCGTTA
GBS_snp0241_Su		TGCAGCCCCACCTCGTTG
GBS_snp0241_R		GAACCGCTCTTCCGATCTC
GBS_snp0149_CI	2D	AGTTCAGACCTCAGAGTA
GBS_snp0149_Su		AGTTCAGACCTCAGAGTG
GBS_snp0149_R		GGACGTCCACCCAGGTCT
GBS_snp1637_CI	7B	ACTCTCGCAGCAGGCCAG
GBS_snp1637_Su		CTCTCGCAGCAGGCCAA
GBS_snp1637_R		ACCGATGGCCACGAGAGT
GBS_snp0219_CI	7B	TAAACGGCAGCCATCTCG
GBS_snp0219_Su		TAAACGGCAGCCATCTCA
GBS_snp0219_R		GCGGTAGGGTTGTACATGCT
GBS_snp0990_CI	7B	CTGCAGTTGGTCGTC
GBS_snp0990_Su		CTGCAGTTGGTCGTT
GBS_snp0990_R		CTCGGTCAGGATCAGGTTCT
GBS_snpj2619_CI	7B	TGCAGTACCATCAAATCCAA
GBS_snpj2619_Su		TGCAGTACCATCAAATCCAG
GBS_snpj2619_R		GAACCGCTCTTCCGATCTC
GBS_snp1562_CI	7B	CTGCAGCACCACCA
GBS_snp1562_Su		CTGCAGCACCACCG
GBS_snp1562_R		CGCGCGCCCATTAG
GBS_snp2107_CI	2B	GTCAGCAATGGGTCAGCGA
GBS_snp2107_Su		TCAGCAATGGGTCAGCGG
GBS_snp2107_R		GCCGACATTTGGCACC
GBS_snp1154_CI	2B	TCGCCGCCGTCCACC
GBS_snp1154_Su		CTCGCCGCCGTCCACT
GBS_snp1154_R		GGCGACGACGGAAGG

GBS_snp0691_CI	2B	GCCTCGGCCGCCA
GBS_snp0691_Su		GCCTCGGCCGCCC
GBS_snp0691_R		GTACCACTCTGGTGCACTCC
GBS_snpj2497_CI	2B	CAGCAACAACATTGTTGTAGAGAC
GBS_snpj2497_Su		CAGCAACAACATTGTTGTAGAGAG
GBS_snpj2497_R		CACCGCGATCCTGACTAAAG
GBS_snp1605_CI	2B	CAGACTAATATTGCCCCATCTTTCGT
GBS_snp1605_Su		CAGACTAATATTGCCCCATCTTTCGA
GBS_snp1605_R		CAAATATAGGACGGCTAATCATGACTGAT
GBS_snp0829_CI	2B	CCTCCTTTCGACGTTTACTCG
GBS_snp0829_Su		CTCCTCCTTTCGACGTTTACTCC
GBS_snp0829_R		CGAGAGGAGAACAGTGAACGAGAA
GBS_snpj3217_CI	2B	CTGCAGTTTCAGC
GBS_snpj3217_Su		CTGCAGTTTCAGT
GBS_snpj3217_R		TGCATACGGTGATACGGAT

[a]CI refers to forward primer with CI13227 alleles, Su refers to forward primer with Suwon92 alleles, and R refers to reverse primer

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Chapter 3 - Mapping of Quantitative Trait Loci for Slow-rusting Traits in wheat Using SNP-chip based Genotyping

Abstract

In this study, in order to identify quantitative trait loci (QTLs) for slow-rusting traits in a germplasm line CI13227, a linkage map with 6415 single nucleotide polymorphism (SNP) markers derived from wheat 90K-SNP assays and 84 simple sequence repeat (SSR) markers were constructed using a doubled haploid (DH) population from CI13227 × Lakin. Four QTLs were identified for four slow-rusting traits on chromosomes 2D, 7B, 7A and 3B. The QTL on 2D is a major QTL that was significant for all four traits measured in both experiments and explained 11.2 to 25.6% of the phenotypic variations for different traits. The QTL on the chromosome 7B was significant for area under disease progress curve (AUDPC) in both experiments, and explained 8.1 and 19.3% of the phenotypic variation, but only significant in one experiment for final severity (FS), infection type (IT) and latent period (LP). The other two QTLs on chromosomes 7A and 3B showed a minor effect on some of the traits evaluated in a single experiment. Flanking SNPs closely linked to the four QTLs were identified and were converted to breeder-friendly Kompetitive allele-specific polymorphism (KASP) markers that can be used in marker-assisted selection to transfer these QTLs to elite breeding lines.

Introduction

A high-density genetic map is essential for QTL mapping and anchoring the genes into an accurate genomic regions (Li et al. 2014). More recently, high-density SNP chips have been developed for various of crops and animals (Bindler et al. 2011; Sim et al. 2012; Song et al. 2013; Wiedmann et al. 2008) and successfully used for genetic studies. The recent development of a wheat SNP array comprised of approximately 90,000 SNPs provides high-density marker coverage in the wheat genome, and thus has been widely utilized in wheat QTL map and genome-wide association studies. In this study, a doubled haploid (DH) population derived from CI13227 x Lakin, genotyped with a 90K-SNP chip was used for mapping and QTL detection. The objectives of this study were to 1) identify QTLs for slow-rusting resistance in CI13227 using 90K-SNPs, 2) identify SNP markers tightly linked to the QTLs, and 3) convert chip-based SNPs into breeder-friendly Kompetitive allele specific PCR (KASP) markers so that they can be used in MAS to improve APR in wheat.

Materials and Methods

Leaf rust evaluation

A doubled haploid (DH) population with 181 lines was used to construct a linkage map for QTL mapping. DHs were developed from a cross between Lakin (Arlin/KS89H130) and the resistant wheat line CI13227 (Wabash/American Banner//Klein Anniversario). CI13227 is the same resistance parent as used in Chapter 2, whereas Lakin is highly susceptible parent to leaf rust developed in Kansas.

Both parents and their DH lines were evaluated for LP, FS, AUDPC and IT in greenhouse experiments at Kansas State University, Manhattan, KS in fall 2012 and

spring 2013 using a randomized complete block design with two replications. About 5 plants per replication were inoculated with uredinospores of *P. triticina* isolate MFPSC (PRTUS 54) after heading. The inoculation method was the same as described in Chapter 2. LP, FS and AUDPC were evaluated based on the same criteria as in Chapter 2. Additionally, at 10 to 12 d after inoculation the infected plants were evaluated for IT using a 0 to 9 scale (Roelfs 1985).

Molecular marker analysis

Two-week old leaf tissue was harvested from the parents and DH lines for DNA extraction using the cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson 1980). SNPs genotyping was performed using the wheat 90K-SNP assays developed by Illumina Inc. (San Diego, CA) and assembled by the International Wheat SNP Consortium (Cavanagh et al. 2013). The genotyping assay was conducted at the USDA Small Grains Genotyping Laboratory in Fargo, ND and SNP genotype was determined using GenomeStudio v2011.1 (Illumina, San Diego, CA). Additionally, a selected set of 384 SSR primers was screened for polymorphism between the parents, and polymorphic SSR markers were used to screen the DH population.

Linkage map construction and QTL identification

A genetic map was constructed using 90K-SNPs and 84 SSR markers with the same method of Chapter 2.

Conversion of SNPs to KASP markers

SNPs that were closely linked to QTLs were converted to Kompetitive allele-specific PCR (KASP) assays as described in Chapter 2.

Results

Leaf rust resistance in the parents and DH population

CI13227 had significantly lower IT, FS, AUDPC, and longer LP than the susceptible parent Lakin (Table 3.1). The DH lines showed continuous distributions for all four traits measured (Figure. 3.1, Table 3.1), indicating the quantitative nature of leaf rust resistance. Transgressive segregation was observed for all traits in both experiments (Figure. 3.1), suggesting both of the parents contribute resistance QTLs.

Correlation coefficients for IT (r = 0.62), AUDPC (r = 0.61), FS (r = 0.61), and LP (r = 0.41) were highly significant ($P < 0.01$) between the two experiments. Among those traits, correlation coefficients between AUDPC and FS (r = 0.96, $p < 0.0001$) were the highest (Table 3.2), indicating the two traits are most likely under the same genetic control. LP was negatively correlated ($p < 0.0001$) with AUDPC, FS, and IT with correlation coefficients of -0.91, -0.89 and -0.63 respectively, indicating that a slow-rusting cultivar is usually characterized as having a longer latent period, but lower AUDPC, FS and IT than these in a susceptible cultivar. IT was significantly correlated ($p < 0.0001$) with AUDPC, FS and LP with relatively lower correlation coefficients of 0.71, 0.69 and -0.63, respectively, suggesting that different gene(s) may involve in controlling IT and LP.

Linkage map construction

Together with 84 SSR markers, 5570 out of 6415 polymorphic SNPs analyzed in the DH population were mapped in a linkage map. The map consists of 44 linkage groups with at least five makers per group, and represents all 21 chromosomes at a total length of 4670.1 cM and an average interval of 0.84 cM between markers. Each chromosome had 1

to 4 linkage groups. The B genome had the most mapped markers (41.0%), followed by the A genome (34.7%) and D genome (24.3%) (Figure 3.2). Chromosome 2B had the most markers (434) at a density of 0.52 cM per marker, and chromosome 4D had the least at a density of 3.72 cM per marker.

QTL analysis

Composite interval mapping detected four QTLs on chromosomes 2D, 7B, 7A, and 3B for at least two of the four traits measured (Table 3.3). One QTL on the chromosome 2D was significant for AUDPC, FS, LP, and IT, designated as *QLr.hwwgru-2DS* (Figure. 3.3), in both experiments. This QTL is contributed by CI13227, located between SNPs IWB34642 and IWB8545, and explained 11.2 to 25.6% of the phenotypic variation for slow-rusting resistance.

A second QTL associated with FS and AUDPC in the fall 2012 experiment and LP, IT, and AUDPC in the spring 2013 experiment was localized on the chromosome arm 7BL based on the SSR and SNP markers linked to the QTL, thus it is designated as *QLr.hwwgru-7BL*. The peak of this QTL was at a 1.8 cM interval between SNPs IWB9496 and IWB24039 with LOD values of 3.5-8.2 in the two experiments (Figure 3.4). The QTL explained 6.1-13.9% of the phenotypic variation for different traits and CI13227 contributes the positive allele.

A third QTL for LP and FS that was significant in spring 2013 experiment was on chromosome 7A and designated as QLr.*hwwgru-7A* (Figure. 3.5). This QTL is located between SNPs IWB42182 and IWB73053 with an interval of 10.2 cM. This QTL is also contributed by CI13227, and explained 6.6 and 11.2% of the phenotypic variation for the two traits, respectively.

The fourth QTL showed a minor effect on FS, IT, and AUDPC in the fall 2012 experiments. This QTL was located at a 0.4 cM interval of chromosome 3B based on the location of the flanking SNP markers IWB35536 and IWB5899 (Figure. 3.6). However, this QTL is contributed by susceptible parent Lakin, and explained 4.2 to 5.4% of the phenotypic variation for the three traits.

Effects of QTLs on leaf rust resistance

To investigate the effect of individual QTLs on leaf rust resistance, DHs carry different allele combinations for the three QTLs on 2D, 7B, and 7A were grouped and the mean latent period and AUDPC of two-year data for each combination was compared. The eight possible allelic combinations of the three QTLs are AABBCC, AABBcc, AAbbCC, AAbbcc, aaBBCC, aaBBcc, aabbCC, and aabbcc, where AA, BB, and CC represent the resistance marker alleles from CI13227 at QTLs on 2DS, 7BL and 7A, respectively, and aa, bb and cc represent corresponding susceptible alleles at the three QTLs from Lakin, respectively. The closest markers to the three QTLs (IWB34642 on 2DS, IWB24039 on 7BL, and IWB73053 on 7A) were selected to represent these QTLs. Two contrasting alleles at each of the three SNPs exhibited a 1:1 segregation ratio in the DH population. All eight genotype combinations could be found in the DH population. Mean AUDPC for the eight genotypic groups of 181 DHs ranged from 95.8 to 316.57 (Figure 3.7). The mean AUDPC for the genotypic group with one of the resistance QTL *QLr.hwwgru-2DS* and *QLr.hwwgru-7BL* respectively was significantly lower than the genetic group with none of QTL. The genetic groups with two of the resistance QTL (*QLr.hwwgru-2DS* and *QLr.hwwgru-7BL, QLr.hwwgru-2DS* and *QLr.hwwgru-7A*) had a significantly lower mean AUDPC than genotypic group with only one resistance QTL.

The genetic groups with three of the resistance QTL had a significantly lower mean AUDPC than the genetic groups with two resistance QTLs (*QLr.hwwgru-2DS* and *QLr.hwwgru-7A, QLr.hwwgru-7BL* and *QLr.hwwgru-7A*). Thus *QLr.hwwgru-2DS* had the largest effect on leaf rust resistance, *QLr.hwwgru-7BL* second, and *QLr.hwwgru-7A* the smallest. The mean AUDPC of the groups with resistance alleles on 2DS plus an additional QTL (QTL on 7BL or 7A) were consistently lower than those with susceptible alleles at one QTL.

KASP design and verification

To verify the genotypic data generated by the 90K-SNP array and convert the array-based SNP markers to breeder-friendly KASP-based SNPs for MAS in breeding, 28 array-based SNPs within or around the four QTL regions were used to design KASP primers. Among them, 11 (3 on chromosome 2DS, 2 on 7BL, 2 on 7A and 2 on 3B) showed polymorphisms between parents and among the DHs, and 8 of them were remapped to one of the four QTL regions (Table 3.4). Three SNPs were mapped outside the QTL regions after all missing data at these loci were filled by the KASP markers. Comparison between array-based SNPs and corresponding KASP data found that eight SNPs showed identical genotypes in the DH population, two SNPs had a SNP call error in one DH line, and one had errors in five DH lines. Therefore, the average error rate for SNP determination was 0.25%.

Discussion

In this study, the QTLs *QLr.hwwgru-2DS* and *QLr.hwwgru-7A* were significant for LP, AUDPC and FS. Strong correlations showed among three components of slow-rusting, -0.91 between LP and AUDPC，-0.89 between LP and AUDPC and 0.96

between AUDPC and FS (Table 3.2), suggested that the two major QTLs had a pleiotropic effect on the LP, AUDPC and FS, which agrees with previous reports (Das et al. 1993). Autocorrelations may exist among the three parameters because AUDPC was calculated based on LP and rust severities over time during disease development including FS. Both the correlation and QTL data suggested that the AUDPC, FS, and LP were under the same genetic control and reflected different aspects of the same process, slow-rusting. However, the correlation between IT and other traits is relatively low, since IT is the character of race specific resistance. Compared with other slow rust resistance traits, IT may be less reliable to reflect the process of slow-rusting although it can reflect the resistance level of APR. Hence among the four slow-rusting traits, LP, FS, and AUDPC are more reliable traits for slow-rusting.

The QTL with the largest effect on the four slow-rusting traits identified in the current study is *QLr.hwwgru-2DS* contributed by resistance parent CI13227*,* which was mapped between SNPs IWB8545 and IWB34642 and explained 11.2 to 25.6% of the phenotypic variation for the four traits. Previously, several QTLs associated with leaf rust have been mapped on chromosome 2DS. Later Sun et al. (2009) mapped *Lr41* on the distal end of chromosome 2D Lr39/Lr41 was an seedling resistance gene mapped on the distal end of chromosome 2D by Raupp et al. (2001) and Singh et al. (2004). Later Sun et al. (2009) mapped *Lr41* on the distal end of chromosome 2D and the closest marker, Xbarc124, was 1.0 cM away from *Lr41* (Figure. 3.8A). While in the current study, *QLr.hwwgru-2DS* was located on the other side of Xbarc124 at a distance of 17.2 cM (Figure. 3.8C). Additionally, unlike *Lr39/Lr41* reported as a seedling resistance gene, *QLr.hwwgru-2DS* in our study confers adult plant resistance. These indicates that

QLr.hwwgru-2DS detected in the current study is different from *Lr39*. Hiebert et al. (2007) reported a leaf rust resistance gene *Lr22a* conferred APR, which was closely linked with a SSR marker Xgwm296 on chromosome 2DS (Figure. 3.8D). One of the flanking markers, IWB8545, on chromosome 2DS in our study was located at 2.0 cM away from Xgwm296 (Figure. 3.8C). However, *Lr22a* was originally found in a synthetic hexaploid wheat RL5404 derived from the cross of Tetra Canthach (AABB) x *Ae.tauschii* (DD) in early 1970s (Rowland and Kerber 1974) and was introgressed to the common wheat after that. The QTL *QLr.hwwgru-2DS* identified in our study was from CI13227, a line originally selected from the 1976 International Winter Wheat Rust Nursery (IWWRN) due to its long LP. Whereas slow-rusting resistance in CI13227 was probably derived from Wabash, a landrace from Indiana, that was collected in 1940s (Clark et al. 1926). Hence based on the time course when these two genes were discovered, we can infer that *QLr.hwwgru-2DS* in common wheat appeared far before *Lr22a* was introgressed into wheat, and *QLr.hwwgru-2DS* might be a different gene than *Lr22a*. Xu et al. (2005), using recombinant inbred line population derived from CI13227 x Suwon92, detected the same QTL on 2DS with Xbarc124 as the closest marker (Figure. 3.8B). In the current study, the two closest linked SNPs, IWB8545 and IWB34642, had much higher r^2 values, 19.3% and 22.3%, than Xbarc124 had (10.1%). Therefore, the SNP markers identified from this study are better markers for MAS, assuming that sufficient polymorphisms exist between parents used in breeding.

Several QTLs associated with leaf rust resistance have been detected on chromosome 7B. Herrera-Foessel et al. (2008) reported a race specific leaf rust gene, *Lr14a,* in durum wheat on chromosome 7BL. Xu et al. (2005) reported a minor QTL,

QLr.ous-7BL, for LP between an AFLP marker XCATG.ATGC125 and a SSR marker Xbarc182 (Figure. 3.9B). Later, Herrera-Foessel et al. (2012) reported a gene, designated *Lr68,* for slow-rusting in a CIMMYT's spring wheat, Parula, and a dominant marker, *csGS,* tightly linked to the gene was identified. In the current study, *QLr.hwwgru-7BL* was located on the similar chromosome position and explained 6.1 to 13.9% of the phenotypic variation in CI13227 x Lakin population. To test if *QLr.hwwgru-7BL* is the same one as *Lr68*, the marker *csGS* was analyzed in the CI13227 x Lakin DH population. The results showed that *csGS* explained r^2 = 11% of the phenotypic variation for LP and located closely (0.3 cM) to one of the flanking markers IWB9496 in our study (Figure. 3.9A), indicating *QLr.hwwgru-7BL* in CI13227 and *Lr68* might be the same gene. However, in the previous study (Herrera-Foessel et al. 2012), one linked marker *cs7BLNLRR* was a cleaved amplified polymorphic (CAP) marker, which had to be digested by specific enzyme after PCR, and the other is a dominant sequence-tagged sites (STS) marker, *csGS,* which cannot separate the heterozygotes from susceptible homozygotes in the population. In the current study, we converted two flanking markers IWB9496 and IWB24039 to KASP assays for *QLr.hwwgru-7BL.* These two markers are breeder-friendly and closer to the gene, thus should be good markers for MAS.

The third QTL contributed by the resistance parent CI1327 in this study between SNPs IWB42182 and IWB73053 with an interval of 10.2 cM was on chromosome 7AL. A few studies reported adult plant resistance genes detected on 7AL recently. Tsilo et al. (2014) detected a QTL located on the chromosome 7AL and explain 8.1% of the phenotypic variance of adult plant resistance close to SSR marker Xbarc92. The QTL peak detected in our study was 90 cM away from Xbarc92. Another important QTL on

chromosome 7AL has been reported by Babiker et al. (2015) is a new gene for UG99 resistance from wheat landrace PI374670 for stem rust. This gene located on 7AL and designated as *QSr.abr-7AL* between two KASP markers (KASP_IWB13813 and KASP_IWB30995) derived from 90K-SNPs. Based on the blaste result of common makers from the 90k-consense map, the QTL detected in our study was located far away (> 100 cM) from the flanking markers of *QSr.abr-7AL.* Thus, the QTL verified in our study is a new QTL. The KASP markers, IWB73053 and IWB42182, could be used for further breeding.

The minor QTL detected in our study is located on chromosome 3B contributed by the susceptible parent Lakin. Chu et al. (2009) reported *QLr.fcu_3BL* Located on 3BL with two flanking markers Xbarc164 and Xfcp544. In our study, based on the blast results of two flanking marker IWB35586 and IWB5899 with the released 90k-consensus map, the QTL detected in our study was located on the short arm of chromosome 3B which is a different locus from *QLr.fcu_3BL.* Another important QTL reported by Mago et al. (2011) on chromosome 3BS was *Sr2/Lr27.* However, the SSR marker Xgwm493 closely linked to *Sr2/Lr27* was located 34 cM away from the QTL detected in our study. Thus, it is likely that the minor QTL detected in our study located on chromosome 3B is a new QTL and the flanking markers IWB35586 and IWB5899 could be converted to KASP markers and applied in further breeding.

Conclusions

LP, FS, AUDPC and IT are four traits to be used to evaluate slow leaf-rusting resistance. By evaluating these traits in the CI13227 x Lakin DH population, we identified four QTLs for slow rust resistance with *QLr.hwwgru-2DS* and *QLr.hwwgru-*

7AL as two new major QTL for slow-rusting. The introgression of the four QTLs, especially *QLr.hwwgru-2DS and QLr.hwwgru-7BL* into commercial cultivars bears considerable agronomic importance. The flanking markers for all the four QTLs have been converted to breeder-friendly KASP markers, and are readily to be used in MAS for transferring these QTLs to adapted cultivars in the breeding program.

Figures and Tables

Figure 3.1 Frequency distributions of final severity (FS), area under disease progress curve (AUDPC), latent period (LP), and infection type (IT) for the parents, CI13227 and Lakin, and their 181 doubled haploid (DH) lines measured in the two greenhouse experiments

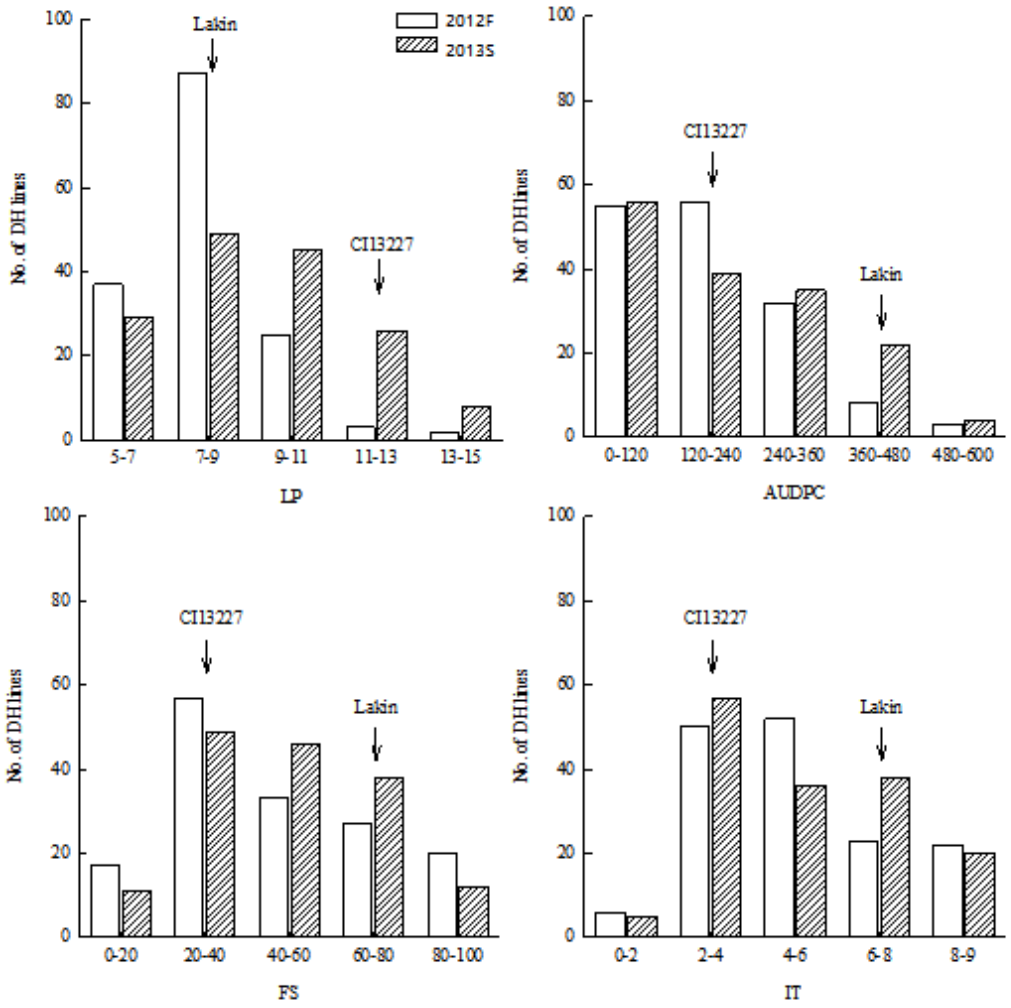


Figure 3.2 Distribution of 90K-SNPs on each chromosome in the doubled haploid (DH) population derived from CI13227 x Lakin

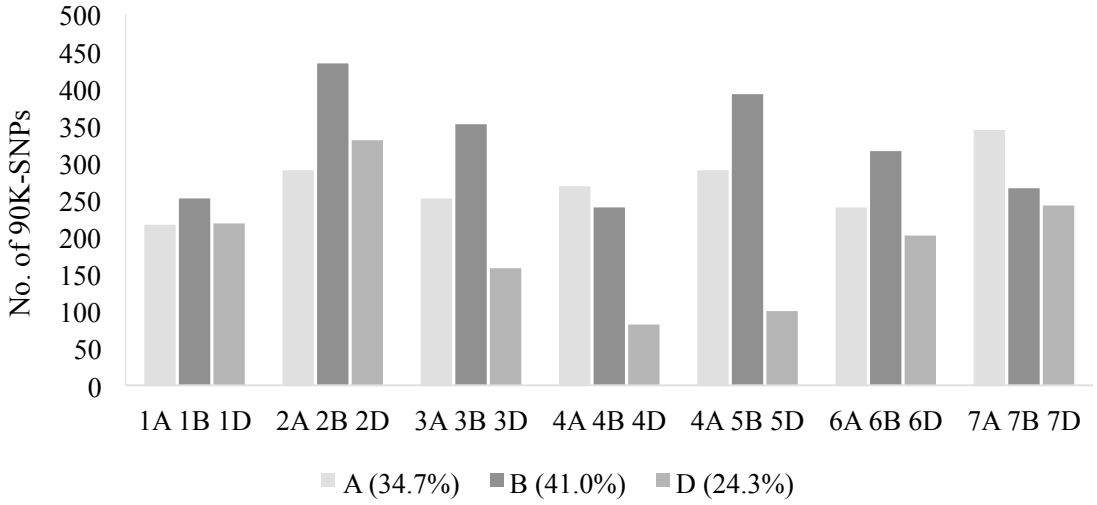


Figure 3.3 Part of the high-density linkage map for chromosome 2D constructed using wheat 90K-SNP arrays and SSR markers (left) and map location of *QLr.hwwgru-2DS* for slow-rusting mapped in the doubled haploid (DH) population of CI13227 x Lakin (right)

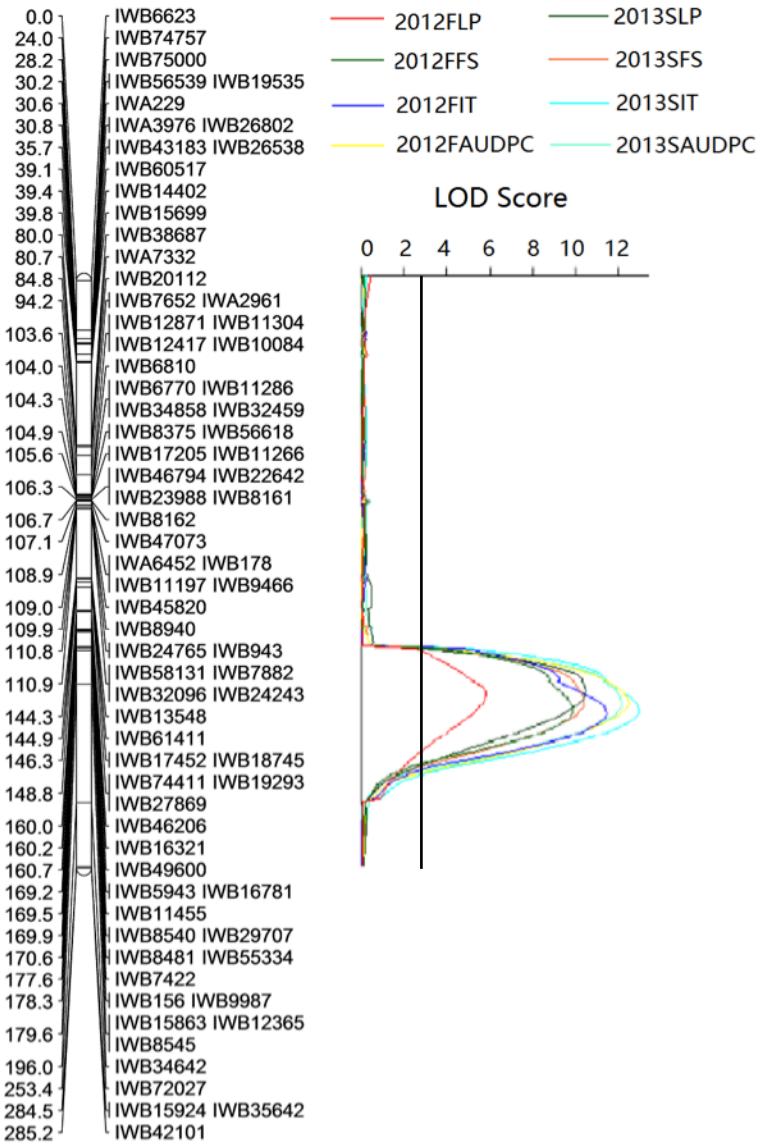


Figure 3.4 Part of the high-density linkage map for chromosome 7B constructed using wheat 90K-SNP arrays and SSR markers (left) and map location of *QLr.hwwgru-7BL* for slow-rusting mapped in the doubled haploid (DH) population of CI13227 x Lakin (right)

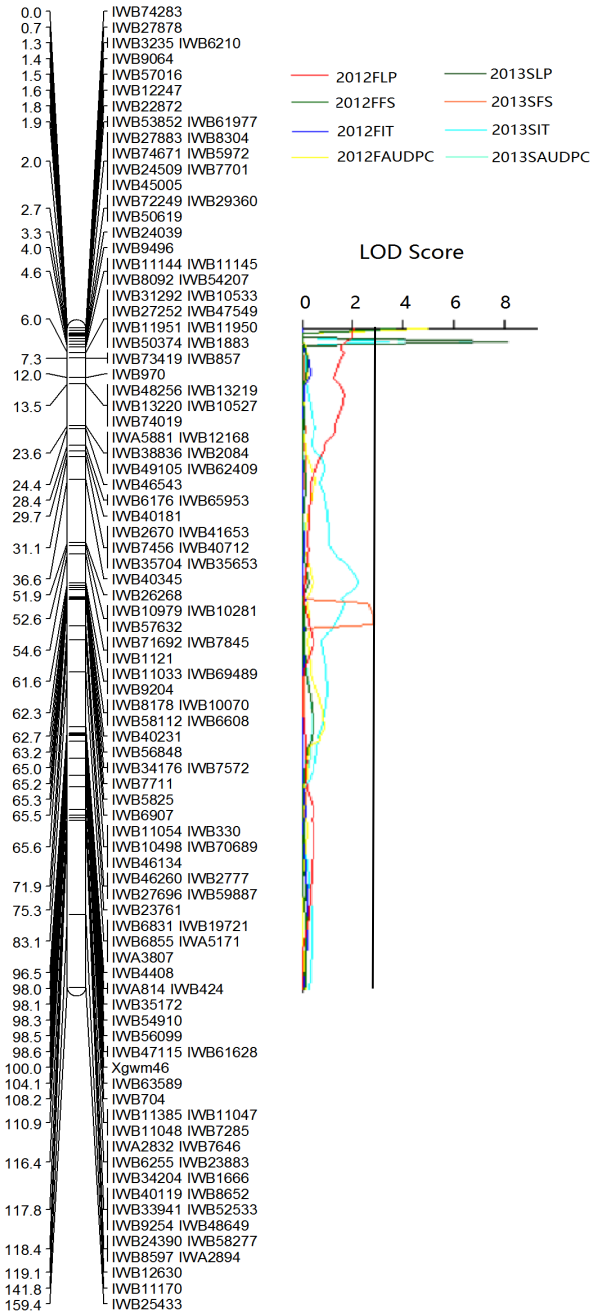


Figure 3.5 Part of the high-density linkage map for chromosome 7A constructed using wheat 90K-SNP arrays and SSR markers (left) and map location of *QLr.hwwgru-7A* for slow-rusting mapped in the doubled haploid (DH) population of CI13227 x Lakin (right).

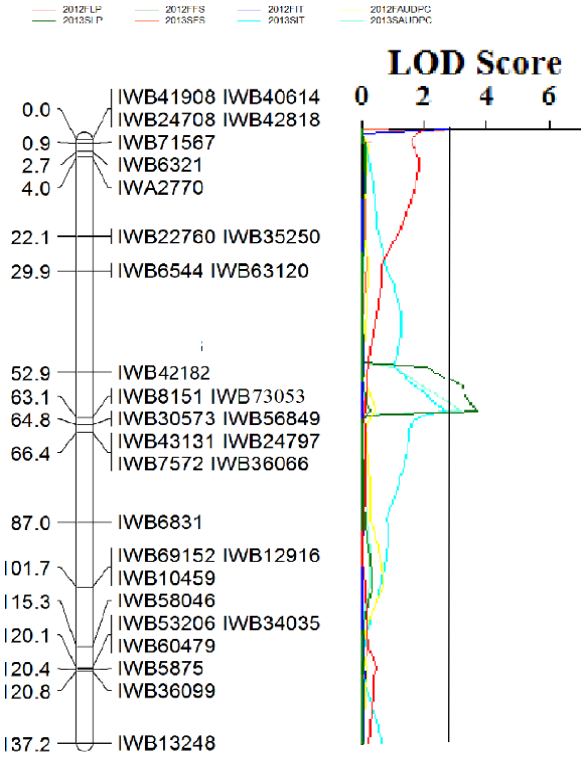


Figure 3.6 Part of the high-density linkage map for chromosome 3B constructed using wheat 90K-SNP arrays and SSR markers (left) and map location of *QLr.hwwgru-3B_1* for slow-rusting mapped in the doubled haploid (DH) population of CI13227 x Lakin (right)

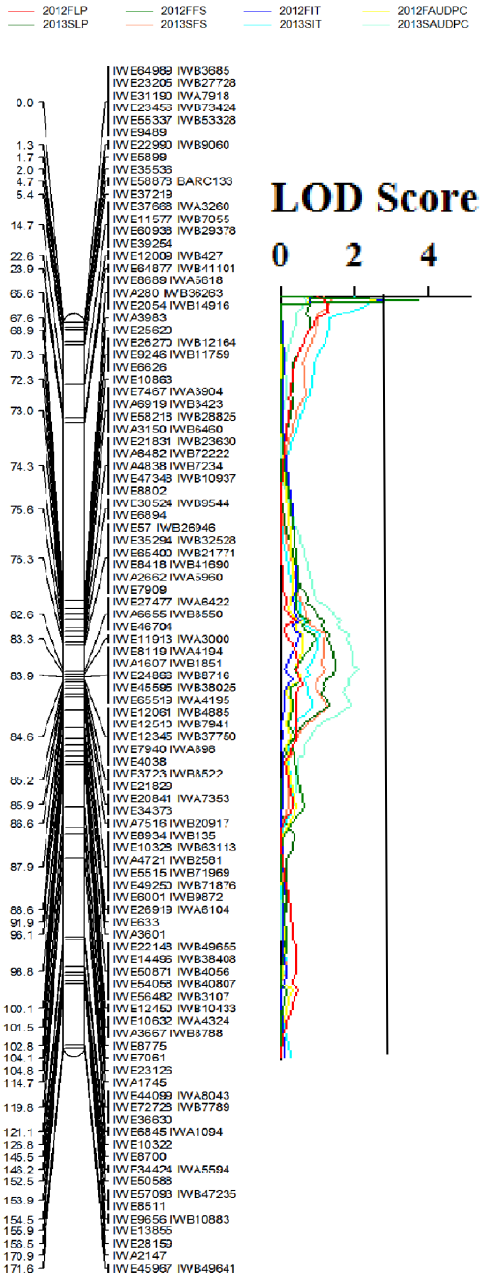
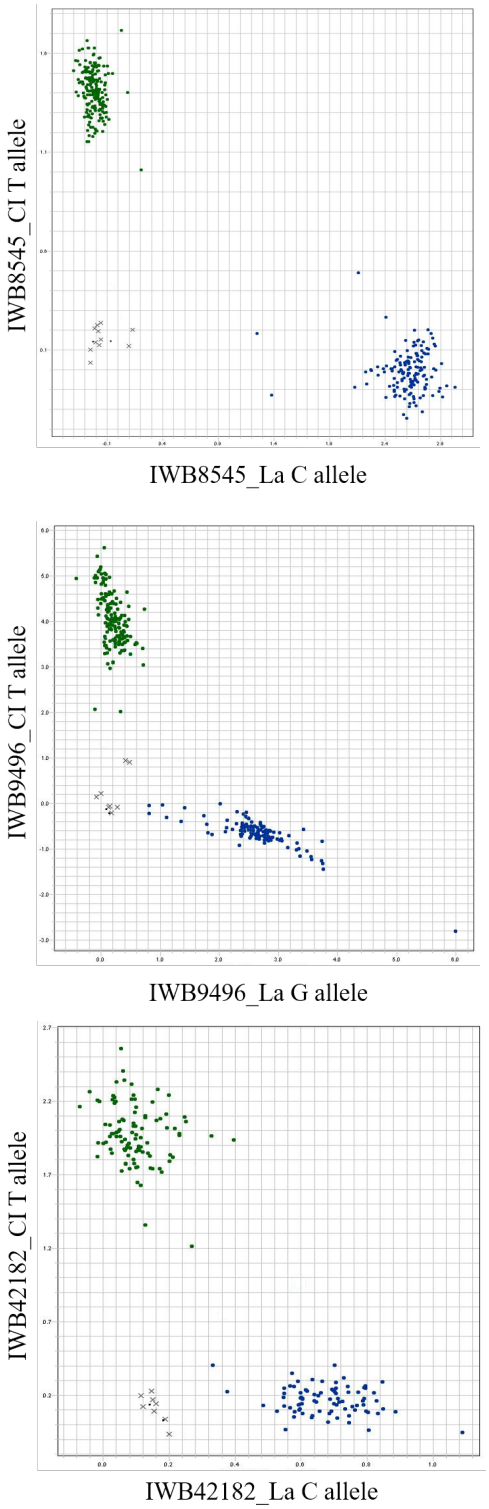
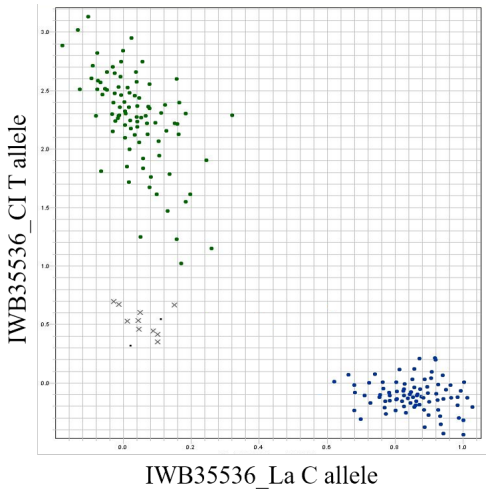


Figure 3.7 A KASPar assay profile to show allelic segregation of SNPs in the doubled haploid population of CI13227 x Lakin. The blue and green dots show different alleles, and the dark dots indicate the water control.




IWB35536_CI T allele
IWB35536_La C allele

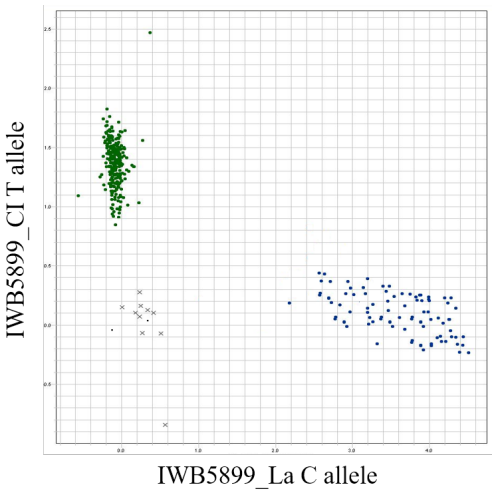

IWB5899_CI T allele
IWB5899_La C allele

Figure 3.8 Quantitative trait loci (QTL) effect for area under disease progress curve (AUDPC) for different allele combinations using Duncan multiple range test comparison at alpha 0.05 probability level: AA, BB, and CC represent the marker alleles from CI13227 linked to resistance at QTLs on 2DS, 7BL and 7A, respectively, and aa, bb and cc represent corresponding opposite alleles on the three QTLs from Lakin, respectively. Means with the same letter are not significantly different.

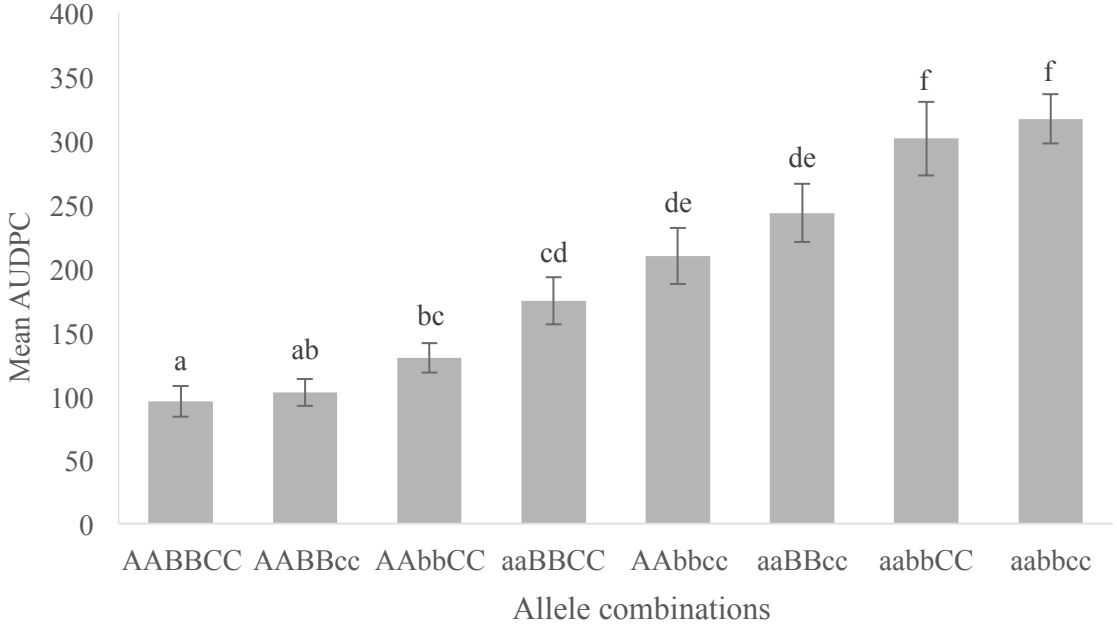


Figure 3.9 Comparison of the current *Qlr.hwwgru-2DS* QTL map with three previously published genetic maps of chromosome 2DS. A. A map with *Lr39/Lr41* from Sun et al. (2009); B. A map with *QLrlp.ous_2DS* from Xu et al. (2005); and C. The current QTL map with *Qlr.hwwgru-2DS* constructed by the DH population from the cross between CI13227 and Lakin; and D. A map with *Lr22a* from Hiebert et al. (2007).

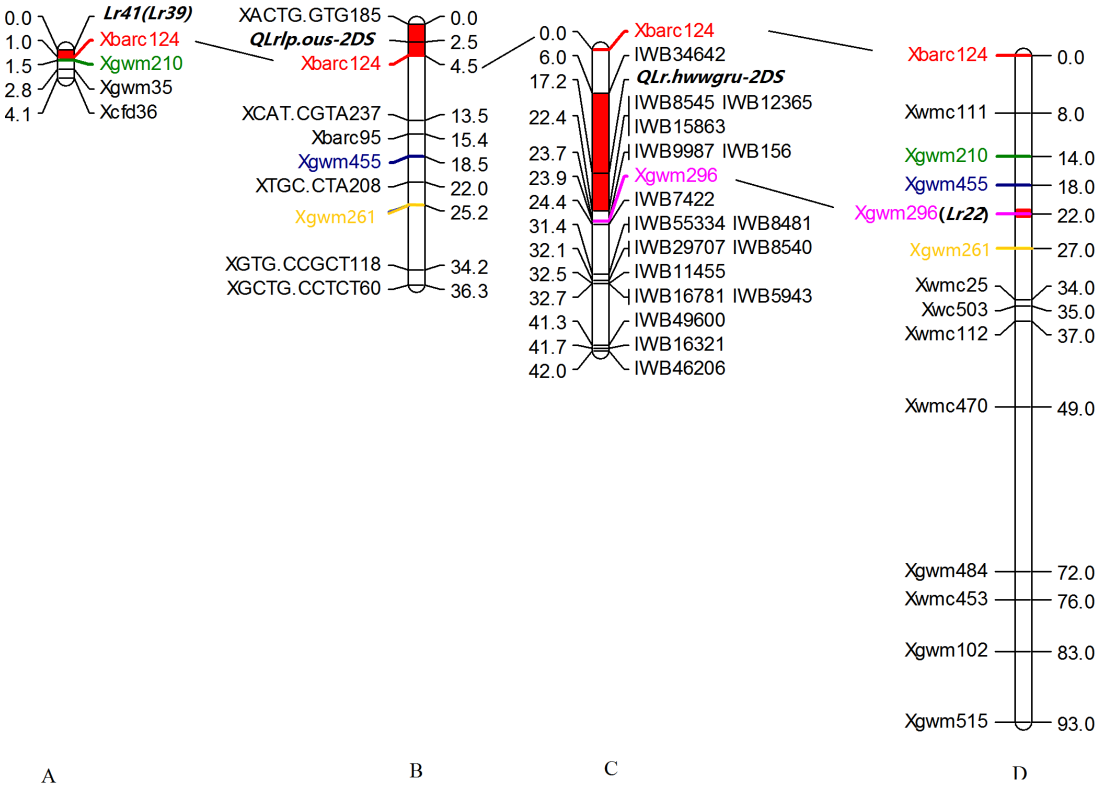


Figure 3.10 Comparison of the current *Qlr.hwwgru-7BL* map (left) with the map of Xu et al. (2005) (right)

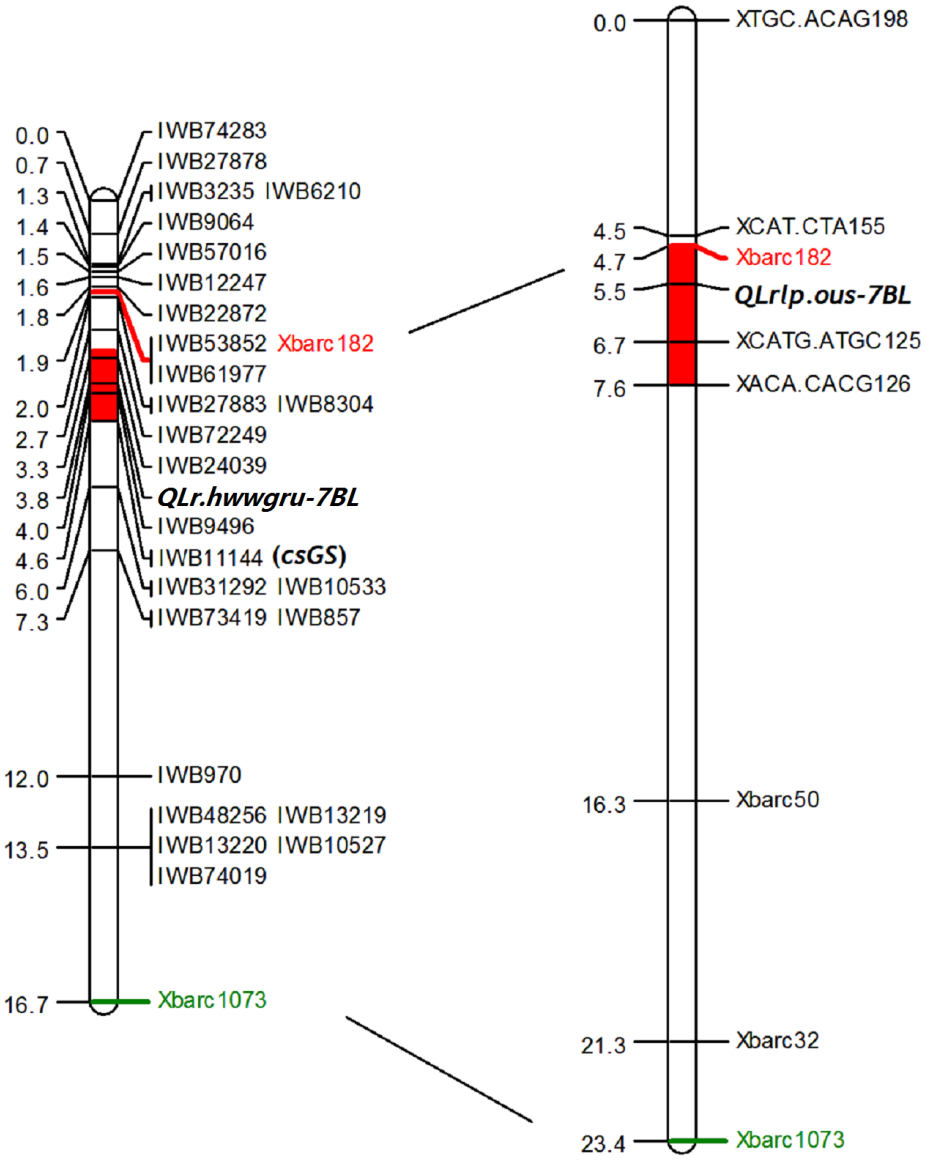


Table 3.1 Infection type (IT), area under disease progress curve (AUDPC), final severity (FS), and latent period (LP) of CI13227, Lakin, and their doubled haploid (DH) population (n=181) in 2012 fall and 2013 spring greenhouse experiments

	Year	IT	AUDPC	FS %	LP day
CI13227	2012F	2	96.7	28.8	11
	2013S	2	92.3	27	11
Lakin	2012F	8	277.4	65.3	7
	2013S	8	266.8	80.8	7
DH means	2012F	5	178.7	46.6	8
	2013S	5	205	47.7	9
DH ranges	2012F	1-9	5.67-557.1	5.0-98.0	7-13
	2013S	1-9	4.4-531.1	4.9-94.2	7-15

Table 3.2 Correlation coefficients among four slow-rusting traits, final severity (FS), latent period (LP), infection type (IT) and areas under disease progress curve (AUDPC)

	LP	FS	IT
FS	-0.91***		
IT	-0.63***	0.68***	
AUDPC	-0.89***	0.96***	0.71***

*** $p < 0.0001$

Table 3.3 Chromosomal locations, marker intervals, determination coefficients (r^2), additive effects and logarithm of the odds (LOD) values for significant quantitative trait loci (QTLs) identified in the CI13227 x Lakin doubled haploid (DH) population

Trait	Chromosome	QTL	Flanking Markers	Interval (cM)	LOD	r^2 (%)	Add
LP2012F	2D	*QLr.hwwgru-2DS*	IWB8545-IWB34642	16.4	5.8	11.2	-0.58
FS2012F	2D	*QLr.hwwgru-2DS*	IWB8545-IWB34642	16.4	9.9	20.5	16.12
IT2012F	2D	*QLr.hwwgru-2DS*	IWB8545-IWB34642	16.4	11.5	24.1	1.49
AUDPC2012F	2D	*QLr.hwwgru-2DS*	IWB8545-IWB34642	16.4	12.5	19.5	73.17
LP2013S	2D	*QLr.hwwgru-2DS*	IWB8545-IWB34642	16.4	10.5	13.3	-1.06
FS2013S	2D	*QLr.hwwgru-2DS*	IWB8545-IWB34642	16.4	10.4	17.5	12.92
IT2013S	2D	*QLr.hwwgru-2DS*	IWB8545-IWB34642	16.4	13	25.6	1.53
AUDPC2013S	2D	*QLr.hwwgru-2DS*	IWB8545-IWB34642	16.4	12.2	19.3	81.44
FS2012F	7B	*QLr.hwwgru-7BL*	IWB9496-IWB24039	1.8	3.6	6.3	6.78
AUDPC2012F	7B	*QLr.hwwgru-7BL*	IWB9496-IWB24039	1.8	5	8.1	35.83
LP2013S	7B	*QLr.hwwgru-7BL*	IWB9496-IWB24039	1.8	8.2	13.9	-0.83
IT2013S	7B	*QLr.hwwgru-7BL*	IWB9496-IWB24039	1.8	3.5	6.1	0.57
AUDPC2013S	7B	*QLr.hwwgru-7BL*	IWB9496-IWB24039	1.8	7.5	12.6	50.29
LP2013S	7A	*QLr.hwwgru-7A*	IWB42182-IWB73053	10.2	3.2	5	-0.53
FS2013S	7A	*QLr.hwwgru-7A*	IWB42182-IWB73053	10.2	3.8	7.6	7.21
FS2012F	3B	*QLr.hwwgru-3B_1*	IWB35536-IWB5899	0.4	3.1	5.4	-6.26
IT2012F	3B	*QLr.hwwgru-3B_1*	IWB35536-IWB5899	0.4	5	8.1	-0.62
AUDPC2012F	3B	*QLr.hwwgru-3B_1*	IWB35536-IWB5899	0.4	2.7	4.2	-25.95

Table 3.4 List of KASP primers designed based on sequences from 90K-SNP arrays

Primer name[a]	Position	Primer sequence (5'-3')
IWB8545_CI	*QLr.hwwgru-2DS*	AGCGGTTTCTTTTAACCATTCTTGT
IWB8545_La	*QLr.hwwgru-2DS*	AGCGGTTTCTTTTAACCATTCTTGC
IWB8545_R	*QLr.hwwgru-2DS*	CTGGCGTTGTATATTGGACAAGGT
IWB34642_CI	*QLr.hwwgru-2DS*	GGAATCGCCTAACCAATGTTGT
IWB34642_La	*QLr.hwwgru-2DS*	GGAATCGCCTAACCAATGTTGC
IWB34642_R	*QLr.hwwgru-2DS*	GCCATGAACATCCTGCAACA
IWB9496_CI	*QLr.hwwgru-7BL*	TGTGATCTGATCCAACAAAACTCT
IWB9496_La	*QLr.hwwgru-7BL*	TGTGATCTGATCCAACAAAACTCG
IWB9496_R	*QLr.hwwgru-7BL*	ACAGGCAATTCCACCTTTACTT
IWB24039_CI	*QLr.hwwgru-7BL*	GCTTTGGTCCACTCCACTAGT
IWB24039_La	*QLr.hwwgru-7BL*	GCTTTGGTCCACTCCACTAGC
IWB24039_R	*QLr.hwwgru-7BL*	AAGGAGGCGCTCATGACG
IWB42182_CI	*QLr.hwwgru-7A*	CAGATGTGCAAGATCATTGATTCT
IWB42182_La	*QLr.hwwgru-7A*	CAGATGTGCAAGATCATTGATTCC
IWB42182_R	*QLr.hwwgru-7A*	ACCCTCTACCTGGAAGCAT
IWB73053_CI	*QLr.hwwgru-7A*	GAGGATGCCCTGCCGACA
IWB73053_La	*QLr.hwwgru-7A*	GAGGATGCCCTGCCGACG
IWB73053_R	*QLr.hwwgru-7A*	CCCAACTCCACGCTCCTCTT
IWB35536_CI	*QLr.hwwgru-3B_1*	TGAGTACTTGGGAATATCTTTGCAT
IWB35536_La	*QLr.hwwgru-3B_1*	TGAGTACTTGGGAATATCTTTGCAC
IWB35536_R	*QLr.hwwgru-3B_1*	GGGATAGACCAACAAAAGAAACATC
IWB5899_CI	*QLr.hwwgru-3B_1*	CTTGTCCTCACAACAATACATGTT
IWB5899_La	*QLr.hwwgru-3B_1*	CTTGTCCTCACAACAATACATGTC
IWB5899_R	*QLr.hwwgru-3B_1*	CGGCGACACAGCGAGTATAT

[a]CI forward primer with CI13227 alleles, La forward primer with Lakin allele, R reverse primer

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Chapter 4 - Mapping and Validation of Quantitative trait loci for Slow-rusting Traits in Wheat using KASP and GBS Markers

Abstract

In this study, slow-rusting traits (LP, FS, AUDPC and IT) were evaluated in a RIL population derived from the cross between CI13227 and Everest for two seasons in 2015. Genotyping-by-sequencing (GBS) was used to genotype the RIL population using 2037 markers. Five QTL regions on 2DS, 7BL, 7AL, 3B and 1D that were significantly associated with slow-rusting traits were detected and explained 6.82 to 28.45% of the phenotypic variance. Seven Kompetitive allele specific polymorphism (KASP) markers closely linked to QTLs on 2DS, 7BL and 7AL that were developed from the previous studies (chapter 2 and 3) were remapped to the QTL regions and validated in the new RIL population. Analysis of different combinations of QTLs using validated KASP markers showed that the RILs containing all three or two of the QTLs had the longest average LP. KASP markers validated in this study can be applied to transfer these QTLs into adapted wheat cultivars.

Introduction

Molecular markers have experienced an evolution from the low and medium throughput systems including Restriction fragment length polymorphisms (RFLPs), Radom amplified polymorphic DNAs (RAPDs), Single sequence repeats (SSRs) and Amplified fragment length polymorphisms (AFLPs) to the high and ultra-high throughput systems such as Single nucleotide polymorphisms (SNPs), Kompetitie allele-specific PCR (KASP), and Genotyping by sequencing (GBS) (Lateef 2015). The development and application of these marker systems facilitate mapping of disease resistance genes including leaf rust resistance genes in wheat. Currently, SNP markers have attracted the attention of breeders and researchers, and applied to various aspects of genetic studies such as association mapping, QTL mapping, and genomic prediction (Bajgain et al. 2016). KASP, as a new SNP genotyping platform, has evolved to be a global benchmark technology for marker-assisted selection in breeding due to its priority of analyzing a small number of SNPs with large sample size (Semagn et al. 2014). However, there are very few publications relating to its application in wheat improvement. The main objectives of this study were to 1) detect new QTLs associated with slow-rusting traits in the population of CI13227 x Everest, 2) convert newly detected GBS-SNPs closely linked to the QTLs into KASP markers and 3) validate the effect of previously detected QTLs using KASP markers.

Material and Methods

Leaf rust evaluation

A mapping population of 184 F_7 RILs derived from the cross CI13227 x Everest was developed by single-seed decent. CI13227 is the same resistant parent used in

Chapter 2, whereas Everest is a hard red winter wheat with the pedigree of HBK1064-3/Betty 'S'//VBF0589-1/ IL89-6483 (Pioneer 9021L//Roland/IL77-2656) and was developed cooperatively by K-State Research and Extension and the Agricultural Research Service, United States Department of Agriculture, for growing in eastern Kansas (Fritz et al. 2011).

Both parents and the 184 RILs were evaluated for latent period (LP), final severity (FS), area under disease progress curve (AUDPC), and infection type (IT) in two greenhouse experiments conducted in Kansas State University, Manhattan, KS in the spring and summer of 2015. About 5 plants per replication were inoculated with a uredinospore suspension of *P. triticina* isolate MFPSC (PRTUS 54) after heading and LP, FS and AUDPC were evaluated as described in Chapter 2. Additionally, at 12 d after inoculation, the infected plants were evaluated for IT using a 0 to 9 scale (Roelfs 1985).

GBS library construction and SNPs identification

The GBS library was constructed as described in Chapter 2 (Poland et al. 2012). The size-selected library was sequenced on an Ion Proton system (Life TechnologiesInc.) Barcodes allowed assignment of raw data to individual samples. SNP calling was conducted using the same method as Chapter 2.

KASP markers

Ten KASP markers developed from the previous study (Table 4.1) were evaluated for polymorphisms between the two parents, CI13227 and Everest. Polymorphic markers were used to genotype the mapping population. KASP assays were performed as described in Chapter 2.

Genetic map construction and QTL analysis

A linkage map was constructed using GBS-SNPs and polymorphic KASP markers developed form the previous studies (Chapters 2 and 3) using the method described in Chapter 2.

Results

Slow-rusting traits of parents and RILs

CI13227 showed resistance to leaf rust with low IT, FS and AUDPC, and long LP, in contrast, Everest had a relatively higher IT, FS and AUDPC, and shorter LP in the adult plant stage (Table 4.2). The RILs showed continuous distributions for all four traits measured (Figure. 4.1), indicating the quantitative nature of leaf rust resistance. Transgressive segregation was observed for all traits in the both experiments (Figure. 4.1), suggesting both parents contributed resistance QTLs.

Correlation coefficients for IT (r = 0.43), AUDPC (r = 0.42), FS (r = 0.51), and LP (r = 0.62) were significant ($p < 0.0001$) between the two experiments. Among those traits, correlation coefficients between AUDPC and FS (r = 0.95, $p < 0.0001$) were the highest (Table 4.4), indicating the two traits are most likely under the same genetic control. LP was negatively correlated ($p < 0.0001$) with AUDPC, FS, and IT with correlation coefficients of -0.74, -0.72 and -0.58, respectively, suggesting that a slow-rusting cultivar usually has lower AUDPC, FS and IT, but a longer LP than these in a susceptible cultivar. The correlations between IT and AUDPC, FS and LP were relatively lower (0.56, 0.67 and -0.58, respectively) than among AUDPC, FS and LP, suggesting that some gene(s) controlling IT may be different from these controlling other three traits.

Linkage map construction

Among 2073 polymorphic SNPs analyzed in the RIL population, 2050 were mapped in a linkage map. The map consists of 51 linkage groups with at least five makers per group, and represents all 21 chromosomes at a total length of 2630.2 cM with an average interval of 1.2 cM between markers. The B genome had the most mapped markers (42.3%), followed by the A genome (36.7%), and the D genome (21%). Chromosome 2B had the most markers (158) at a density of 0.85 cM per marker, and chromosome 5D had the least at a density of 5.7 cM per marker.

Analysis of KASP markers

Seven out of 10 KASP markers previously developed from the RIL population CI13227 x Lakin showed polymorphisms between CI13227 and Everest and mapped to the GBS-SNPs map. Three of them, IWB34642, IWB8545 and GBS_snpj2228, were closely linked to *QLr.hwwgru-2DS,* two, GBS_snp1637 and IWB24039, were closely linked to *QLr.hwwgru-7BL,* and another two, IWB73053 and IWB42182, were linked to *QLr.hwwgru-7AL.*

QTLs for slow-rusting traits

CIM analysis identified five QTLs associated with slow-rusting traits on chromosomes 2DS, 7BL, 7AL, 3B and 1D across the two greenhouse experiments (Table 4.4). The resistance parent, CI13227, contributed resistance alleles of three QTLs on 2DS, 7BL and 7AL and the susceptible parent, Everest, contributed another two resistance loci.

The most stable locus with the largest effect across environments was *QLr.hwwgru-2DS* located between KASP markers IWB8545 and GBS_snpE2044 (Fig

4.2). The other two KASP markers GBS_snpj2228 and IWB34642 were also mapped at 0.8 and 1.1 cM away from GBS_snpE2044 (Figure. 4.3), respectively. This QTL explained 28.45, 12.73, and 12.96% of the phenotypic variance for LP, FS and IT in 2015 spring experiment, respectively, and explained 9.61, 9.32, and 9.16 of the phenotypic variance for LP, FS and AUDPC in 2015 summer experiment, respectively.

Another QTL with a large effect was *QLr.hwwgru-7BL* located between GBS markers GBS_snpE1588 and GBS_snp1637 (Figure. 4.4). The third KASP marker IWB24039 was located at 1.0 cM away from GBS_snp1637 (Figure. 4.5). This QTL explained 13.60 and 6.82% of the phenotypic variance of IT and LP in the 2015 summer experiment and explained 9.87 to 10.36% of the phenotypic variance for all slow-rusting traits except IT in the 2015 spring experiment.

The third QTL was *QLr.hwwgru-7AL* located between GBS_snpE0495 and GBS_snpE1632 (Fig 4.6). The two KASP markers IWB42182 and IWB73053 were located at 5.1 and 6.4 cM away from GBS_snpE1632 (Figure. 4.7). This QTL explained 7.36 and 8.89% of the phenotypic variance for LP in 2015 spring and summer experiments, respectively.

The fourth QTL, *QLr.hwwgru-3B_2,* was flanked by GBS_snpE1131 and GBS_snpE0820 (Figure. 4.8), and explained 8.71 and 9.89% of the phenotypic variance of AUDPC and IT in 2015 spring.

The fifth QTL was identified in the marker interval GBS_snpE1845 and GBS_snpE1357 on chromosome 1D (Figure.4.9) and explained 9.2 % of the phenotypic variance of LP in 2015 summer.

Effects of QTLs on leaf rust resistance

To verify the effect of individual QTLs on leaf rust resistance, 184 RILs were grouped based on their allele combinations at the three QTLs (2D, 7B, and 7A) from CI13227. Their allele substitution effects were compared using KASP markers. The eight possible allelic combinations of the three QTLs are AABBCC, AABBcc, AAbbCC, AAbbcc, aaBBCC, aaBBcc, aabbCC, and aabbcc, where AA, BB, and CC represent the marker alleles linked to the QTLs on 2DS, 7BL and 7A of CI13227, respectively, and aa, bb and cc represent corresponding alleles on the three QTLs from Everest, respectively. The closest SNP markers to the three QTLs (IWB8545 on 2DS, GBS_snp1637 on 7BL, and GBS_snpE1632 on 7AL) were selected to represent these QTLs (Figure. 4.10). Two contrasting alleles at each of the three SNPs exhibited a 1:1 segregation ratio in the RIL population. All eight genotype combinations could be found in the DH population. Mean LP for the eight genotypic groups of 184 RILs ranged from 7.8 to 11.6 (Figure. 4.11). The mean LP for the genotypic group with one of the resistance QTLs, *QLr.hwwgru-2DS* or *QLr.hwwgru-7BL,* was significantly longer than the genotypic group with none of QTLs. The genetic groups with two of the three resistance QTLs (*QLr.hwwgru-2DS, QLr.hwwgru-7BL,* and *QLr.hwwgru-7AL*) had significantly longer LP than the genotypic groups with only one of the resistance QTLs. The genetic group with all the three resistance QTLs had a significantly longer LP than the genetic groups with the two resistance QTLs (*QLr.hwwgru-7BL* and *QLr.hwwgru-7AL*). Thus *QLr.hwwgru-2DS* and *QLr.hwwgru-7BL* had larger effect on leaf rust resistance than *QLr.hwwgru-7AL*. When *QLr.hwwgru-2DS* combined with one of the other QTL *QLr.hwwgru-7BL* or *QLr.hwwgru-7AL*, LP will be prolonged in this population.

Discussion

After analysis of phenotypic data from two experiments and sequencing-based SNP markers, we found five genomic regions that were significantly associated with slow-rusting resistance to leaf rust. Both parents, CI13227 and Everest, contributed positive alleles towards leaf rust resistance, suggesting using transgressive segregation in breeding may enhance slow-rusting resistance.

Among four components of slow-rusting measured in this study, a strong correlation were observed between FS and AUDPC ($r = 0.95$, $p < 0.0001$) (Table 4.4), which agrees with previous reports (Das et al. 1993). Autocorrelations may exist between the two parameters because AUDPC was calculated based on the rust severities collected over time during disease development including FS. Both the correlation and QTL data suggested that the AUDPC, FS and LP were under the same genetic control and reflected different aspects of the slow-rusting. However, the correlations between IT and three other traits were relatively low, since IT is also the major character of race specific resistance. Compared with other slow rust resistance traits, IT may be less reliable to reflect slow-rusting resistance although it can partially reflect the resistance level of APR. Hence among the four slow-rusting traits, LP, FS, and AUDPC are more reliable traits for slow-rusting.

The application of GBS facilitates generation of a large number of SNPs for QTL detection. Conversion of GBS-SNPs to KSAP markers facilities marker-assisted selection in breeding program (Mammadov et al. 2012). Seven KASP markers derived from the previous QTL mapping studies of a RIL population and a DH population (Chapters 2 and 3) (3 KASP markers for *QLr.hwwgru-2DS*, 2 for *QLr.hwwgru-7BL*, and 2 for

QLr.hwwgru-7AL) were analyzed in CI13227 x Everest population to validate their relationship with the QTLs in a new genetic background. *QLr.hwwgru-2DS* and *QLr.hwwgru-7BL* were successfully validated by the KASP markers, IWB8545 and GBS_snp1637, respectively, as one of the flanking markers for the major QTL contributed by CI13227 in our study (Figure 4.11). The KASP markers linked to QTL on chromosome 7A in previous study, IWB42182 and IWB73053, were located 4.1 and 3.4 cM away from one of the flanking marker GBS_snpE1632 in this study, thus, the QTL region shifted 3.4 cM away. This difference could be attributed to the increased number of meiotic events allowing the opportunity for additional recombinations in the RIL population compared to the DH population (Somers et al. 2004). It is also possible due to phenotyping variation between experiments. However, that all these KASP markers were within 5cM of the QTL region in this study, indicates the three QTLs (*QLr.hwwgru-2DS, QLr.hwwgru-7BL*, and *QLr.hwwgru-7AL*) were the same QTLs as detected in Chapters 2 and 3. Those QTLs were stable in different experiments and KASP markers closely linked to the QTLs are effective for marker-assisted selection in breeding.

Additionally, a QTL on chromosome 3B was detected in a single environment of 2015 summer associated with AUDPC and IT that was contributed by the susceptible parent Everest in this study. The most important QTL previously reported by Mago et al. (2011) is a multiple resistance locus on chromosome arm 3BS in wheat confers resistance to stem rust (*Sr2*), leaf rust (*Lr27*) and powdery mildew, and a marker Xgwm493 was the closely linked marker to *Sr2/Lr27*. In the current study, Xgwm493 was closely linked to the 3BS QTL and explained about 20% of the phenotypic variation, suggesting that the QTL on 3B was most likely the same as *Sr2/Lr27*. This QTL did not show up in the 2015

summer in greenhouse. Another QTL with a minor effect was detected in the single environment of 2015 summer and located on chromosome 1D. This most likely could be due to the difference in environment, especially the temperature for rust development. In the summer experiment, the higher temperature made the faster disease growth and this might be the major cause of failure to detect the QTL.

The joint effects of the three verified QTLs, *QLr.hwwgru-2DS*, *QLr.hwwgru-7BL* and *QLr.hwwgru-7AL* were clearly observable because most RILs had a longer LP (average of 12 d) if all three QTLs were combined, whereas RILs had none of the QTLs had a shortest LP (average of 7.8 d). Mean LP of RILs with one of the QTLs on 2DS or 7B was significantly longer when compared with RILs with no QTL (Figure.4.7). However, 7AL QTL only became significant in the presence of 2DS or 7BL QTLs, the joint effects of three QTLs was not significantly larger than 2DS plus one of the other QTLs, but significantly larger than a combination of 7BL and 7AL QTLs (Figure. 4.7). The enhanced effects of *Lr34* was demonstrated in the previous studies by German and Kolmer (1992). Their results showed in Thatcher line with both *Lr16* and *Lr34* had higher resistance level than the Thatcher isogenic lines with only one of the genes. Vanzetti et al. (2011) also found that by combining some seedling resistance genes with APR gene *Lr34*, *Sr2*, or *Lr46*, it can provide durable resistance to leaf rust. Analysis of different combinations of QTLs in our study showed that the RILs containing all three or at least two QTLs (*QLr.hwwgru-2DS*, *QLr.hwwgru-7BL*) had a longer LP. Deployment of these QTLs in combination with other effective genes will lead to successful control of leaf rust.

Conclusion

Five QTL regions for slow-rusting on 2DS, 7BL, 7AL, 3B and 1D were identified using GBS-SNP markers in this study. Three of them *QLr.hwwgru-2DS*, *QLr.hwwgru-7BL*, *QLr.hwwgru-7AL* were the QTL identified in previous studies and validated in this study using KASP markers. A combination of the three QTLs (*QLr.hwwgru-2DS*, *QLr.hwwgru-7BL* and *QLr.hwwgru-7AL*) can significantly prolong LP. Validated KASP markers in this study can be applied in breeding to improve wheat durable rust resistance.

Figures and Tables

Figure 4.1 Frequency distribution of latent period (LP), final severity (FS), area under disease progress curve (AUDPC), and infection type (IT) for parents, CI13227 and Everest and their 184 recombinant inbred lines (RILs) measured in the two greenhouse experiments

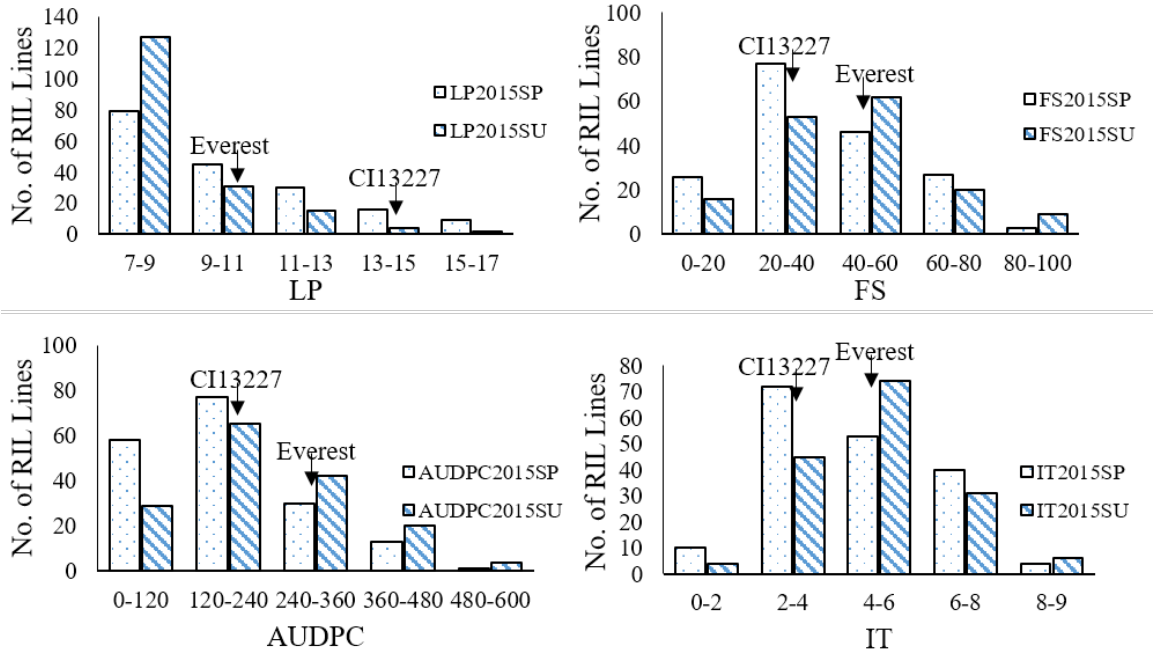


Figure 4.2 Part of the high-density linkage map for chromosome 2D constructed using GBS-SNP markers (left) and map location of *QLr.hwwgru-2DS* for slow-rusting mapped in the recombinant inbred line (RIL) population of CI13227 x Everest

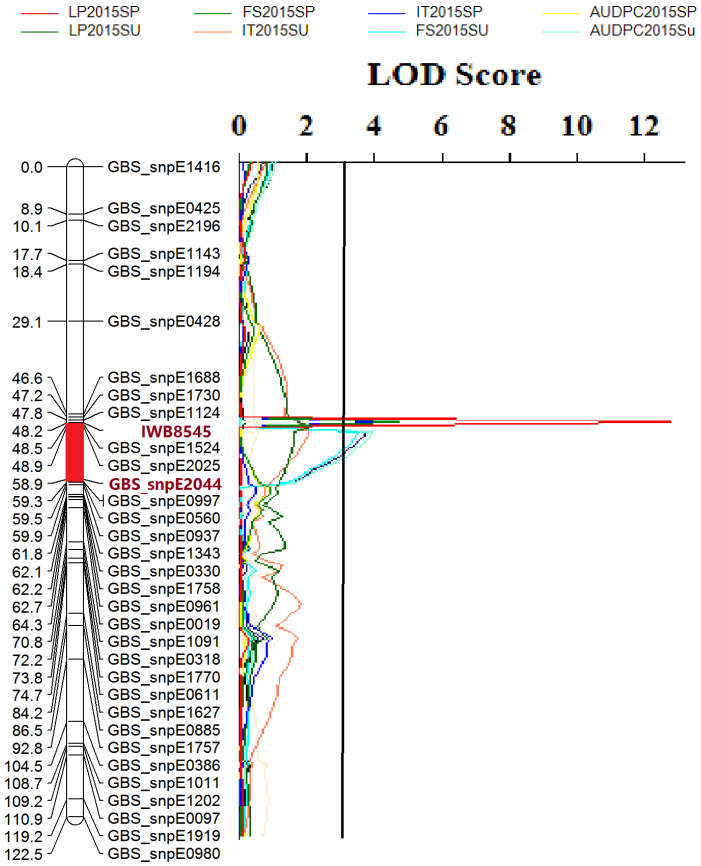


Figure 4.3 Comparison of *QLr.hwwgru-2DS* QTL map in three populations. A. QTL map constructed using CI13227 x Suwon RIL population. B. QTL map constructed using CI13227 x Everest RIL population. C. QTL map constructed using CI13227 x Lakin DH population. Common KASP markers were marked in the same color in different populations.

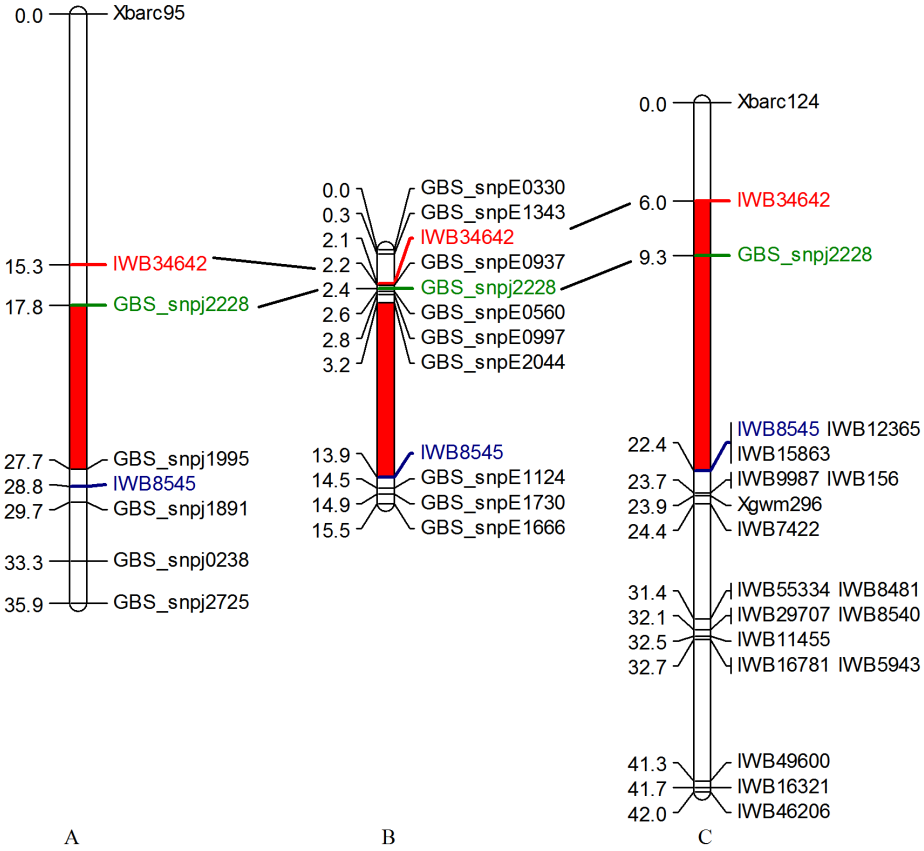


Figure 4.4 Part of the high-density linkage map for chromosome 7B constructed using GBS-SNP markers (left) and map location of *QLr.hwwgru-7BL* for slow-rusting mapped in the recombinant inbred line (RIL) population of CI13227 x Everest

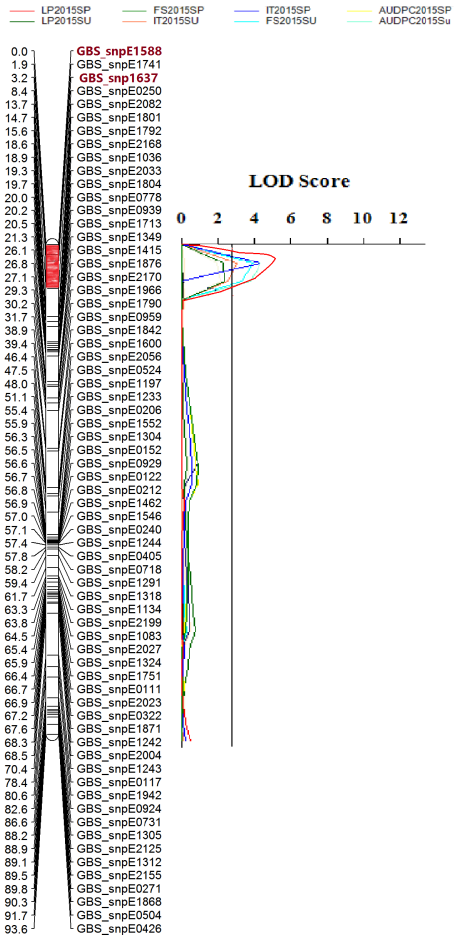


Figure 4.5 Comparison of *QLr.hwwgru-7BL* QTL map in three populations. A. QTL map constructed using CI13227 x Suwon RIL population. B. QTL map constructed using CI13227 x Everest RIL population. C. QTL map constructed using CI13227 x Lakin DH population. Common KASP markers were marked in the same color in different populations.

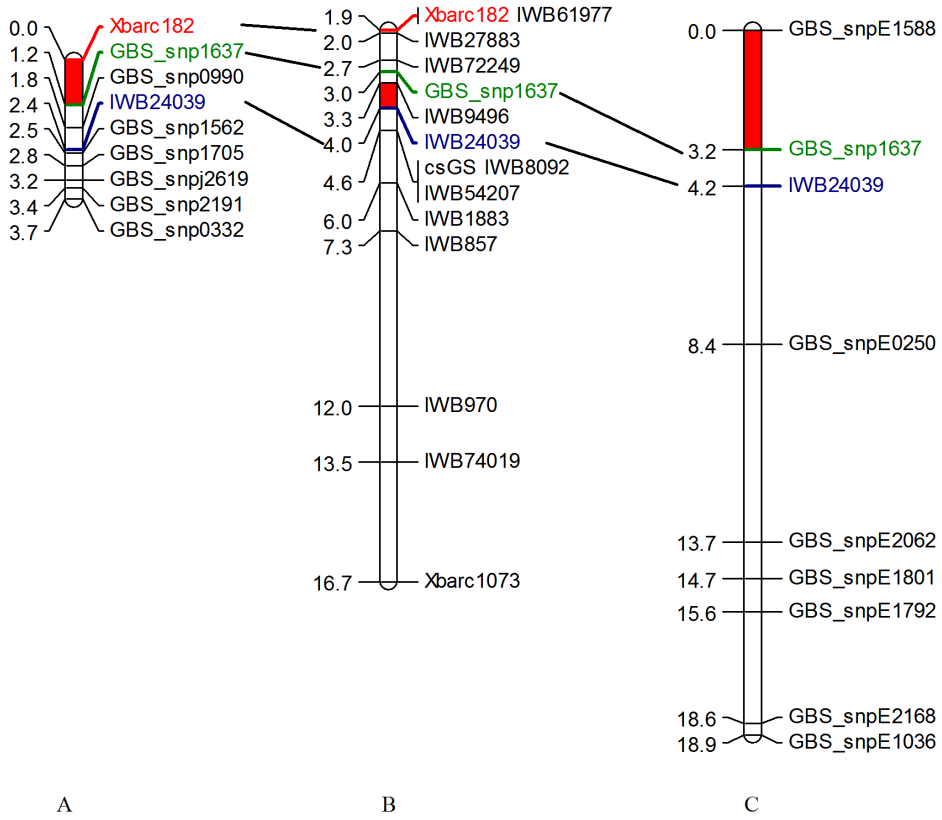


Figure 4.6 Part of the high-density linkage map on chromosome 7A constructed using GBS-SNP markers (left) and map location of *QLr.hwwgru-7A* for slow-rusting mapped in the recombinant inbred line (RIL) population of CI13227 x Everest

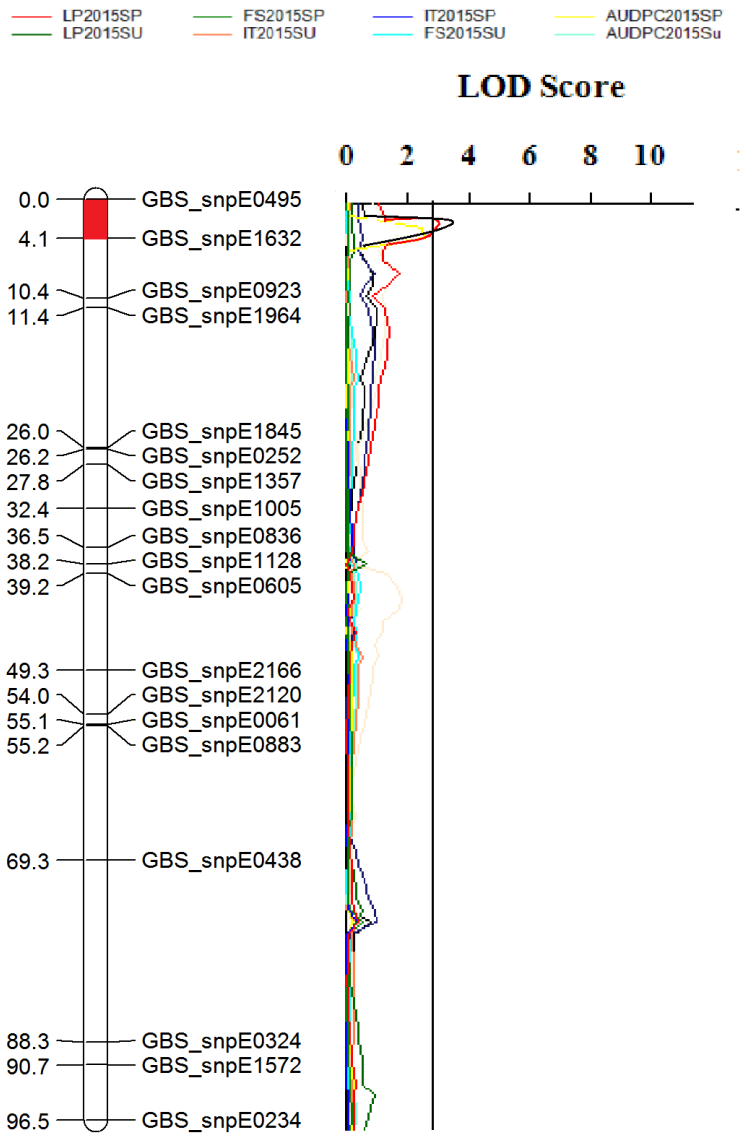


Figure 4.7 Comparison of *QLr.hwwgru-7AL* QTL map in three populations. A. QTL map constructed using CI13227 x Suwon RIL population. B. QTL map constructed using CI13227 x Everest RIL population. C. QTL map constructed using CI13227 x Lakin DH population. Common KASP markers were marked in the same color in different populations.

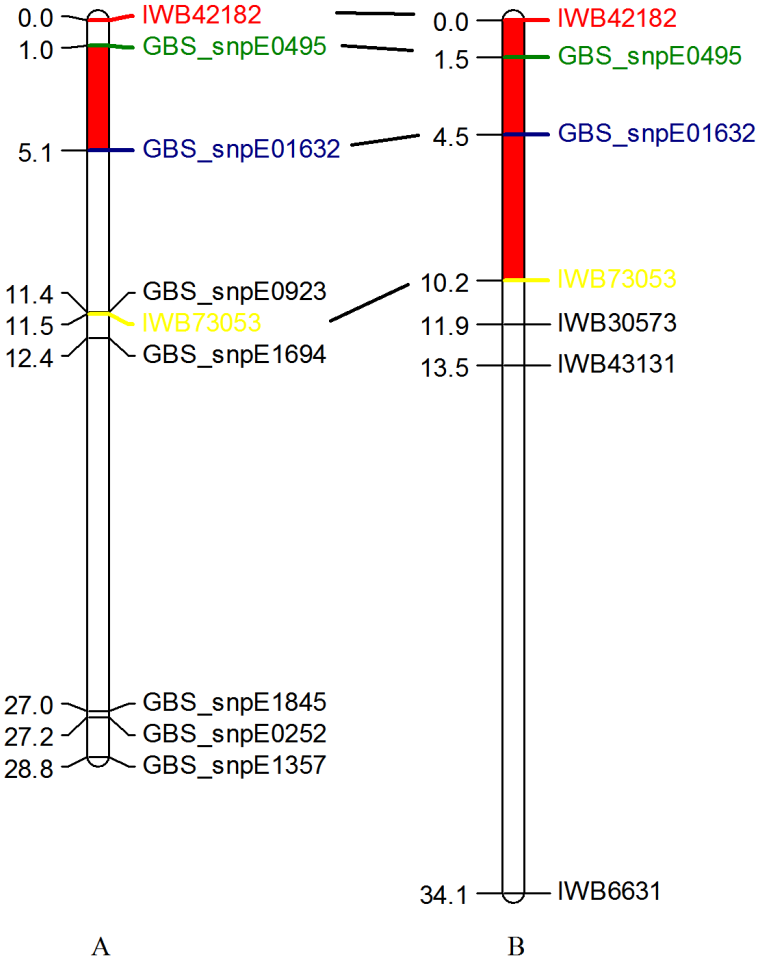


Figure 4.8 Part of the high-density linkage map for chromosome 3B constructed using GBS-SNP markers (left) and map location of *QLr.hwwgru-3B_2* for slow-rusting mapped in the recombinant inbred line (RIL) population of CI13227 x Everest

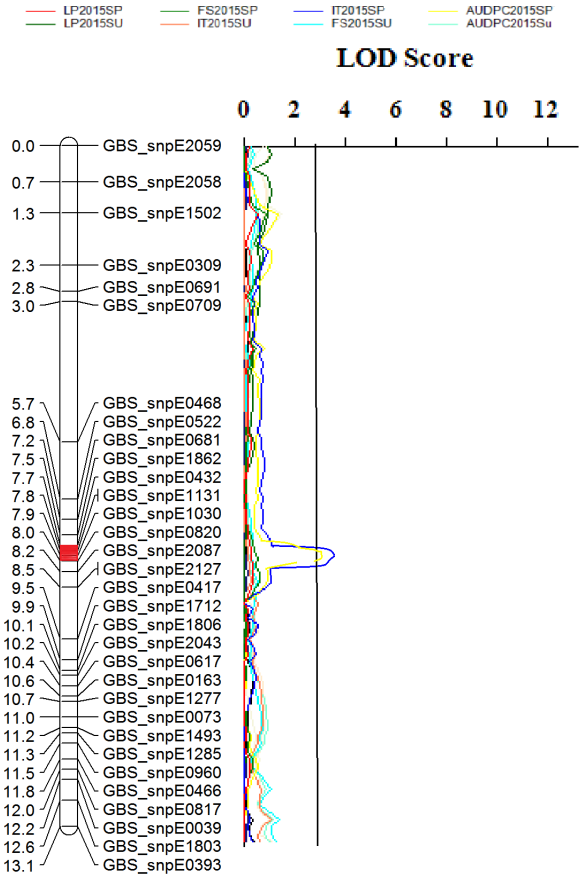


Figure 4.9 Part of the high-density linkage map for 1D constructed using GBS-SNP markers (left) and map location of *QLr.hwwgru-1D* for slow-rusting mapped in the recombinant inbred line (RIL) population of CI13227 x Everest

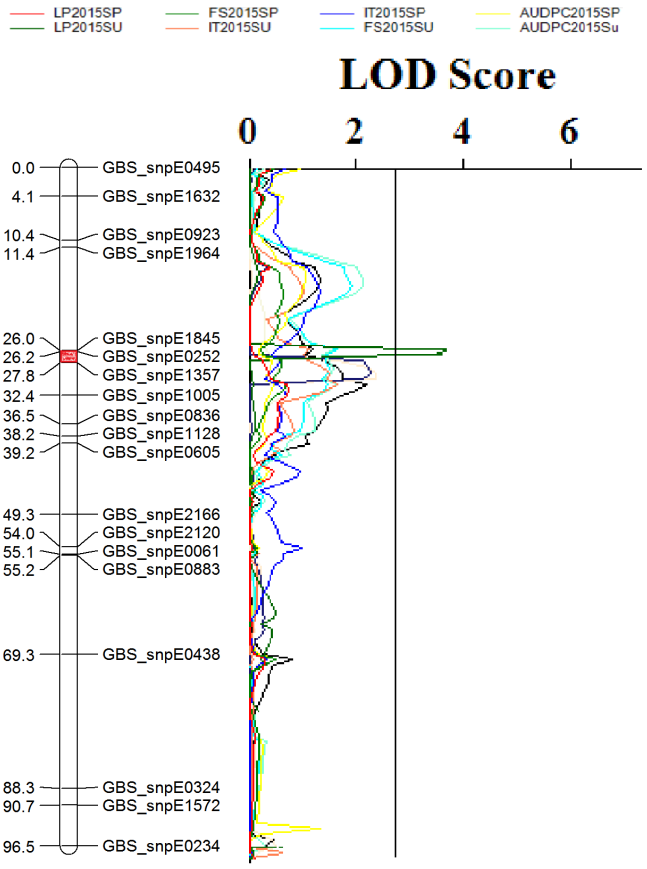
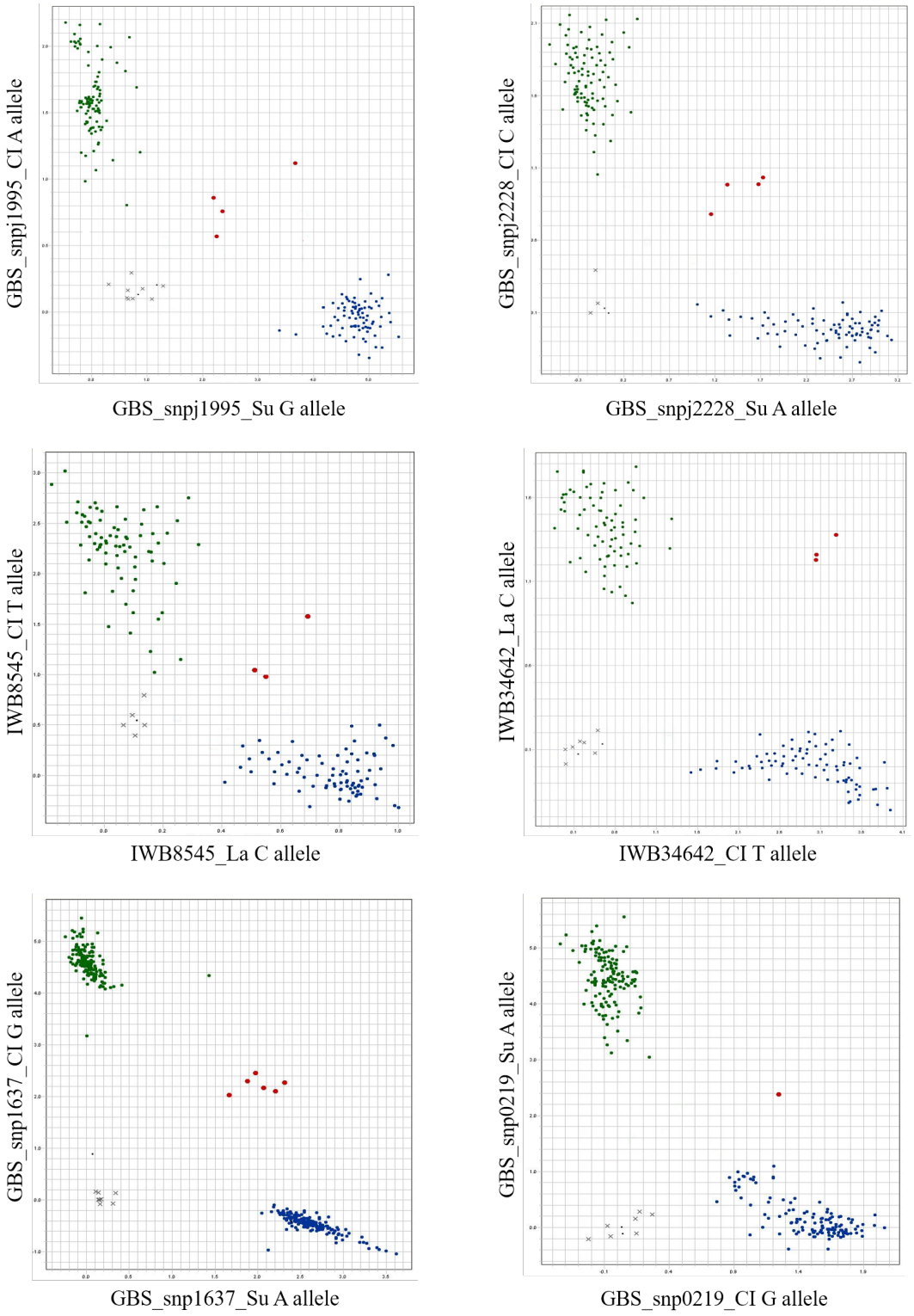
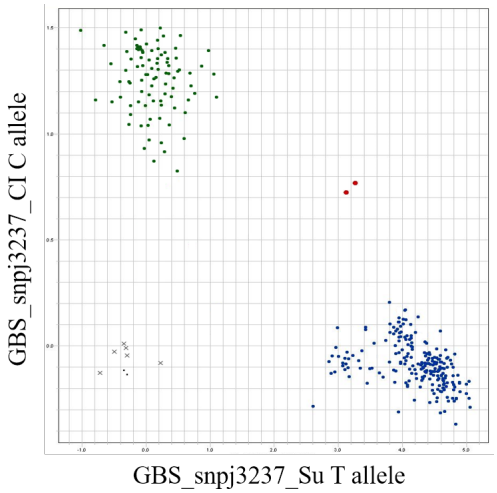
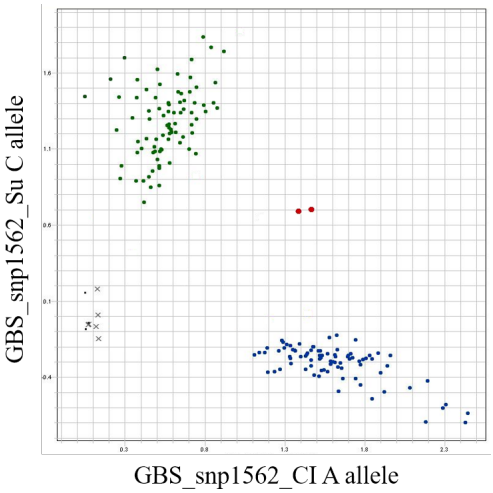
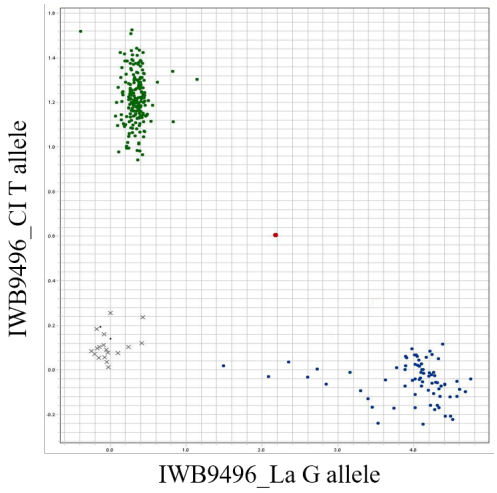


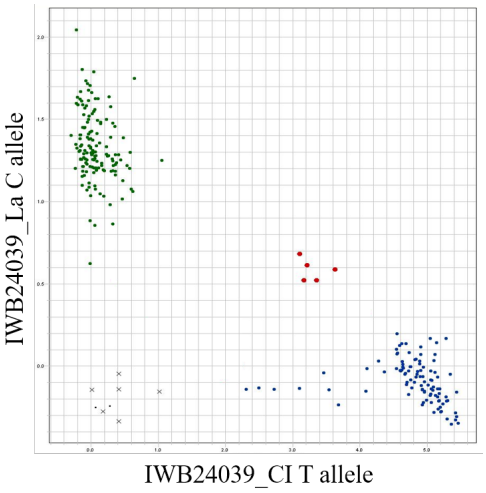
Figure 4.10 A KASPar assay profile to show allelic segregation of SNPs in the RIL population of CI13227 x Everest. The blue and green dots show different alleles, and the dark dots indicate the water control.

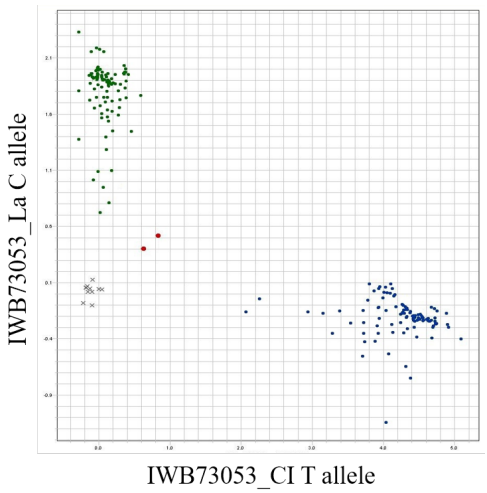



GBS_snpj3237_CI C allele
GBS_snpj3237_Su T allele


GBS_snp1562_Su C allele
GBS_snp1562_CI A allele


IWB9496_CI T allele
IWB9496_La G allele


IWB24039_La C allele
IWB24039_CI T allele


IWB73053_La C allele
IWB73053_CI T allele

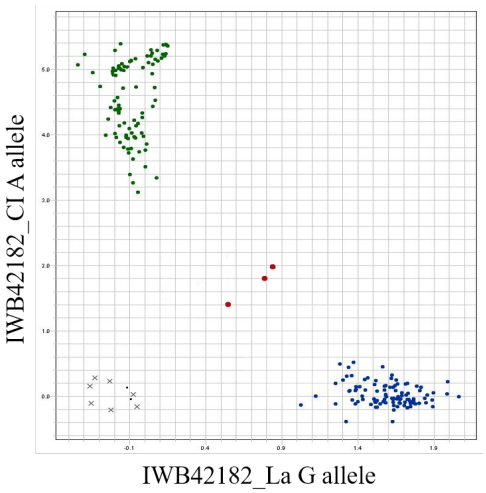

IWB42182_CI A allele
IWB42182_La G allele

Figure 4.11 Quantitative trait loci (QTL) effect latent period (LP) for area under disease progress curve (AUDPC) for different allele combinations using Duncan multiple range test comparison at alpha 0.05 probability level: AA, BB, and CC represent the marker alleles from CI13227 linked to resistance at QTLs on 2DS, 7BL and 7AL, respectively, and aa, bb and cc represent corresponding opposite alleles on the three QTLs from Lakin, respectively. Means with the same letter are not significantly different.

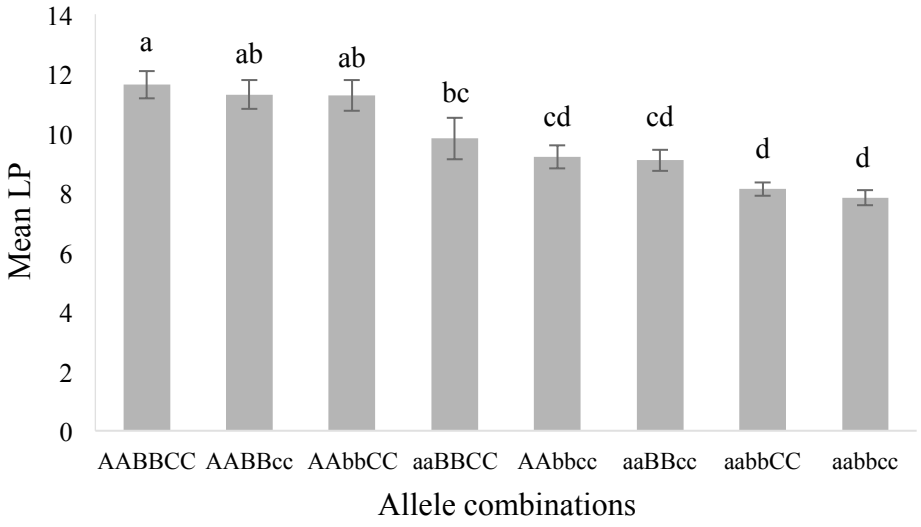
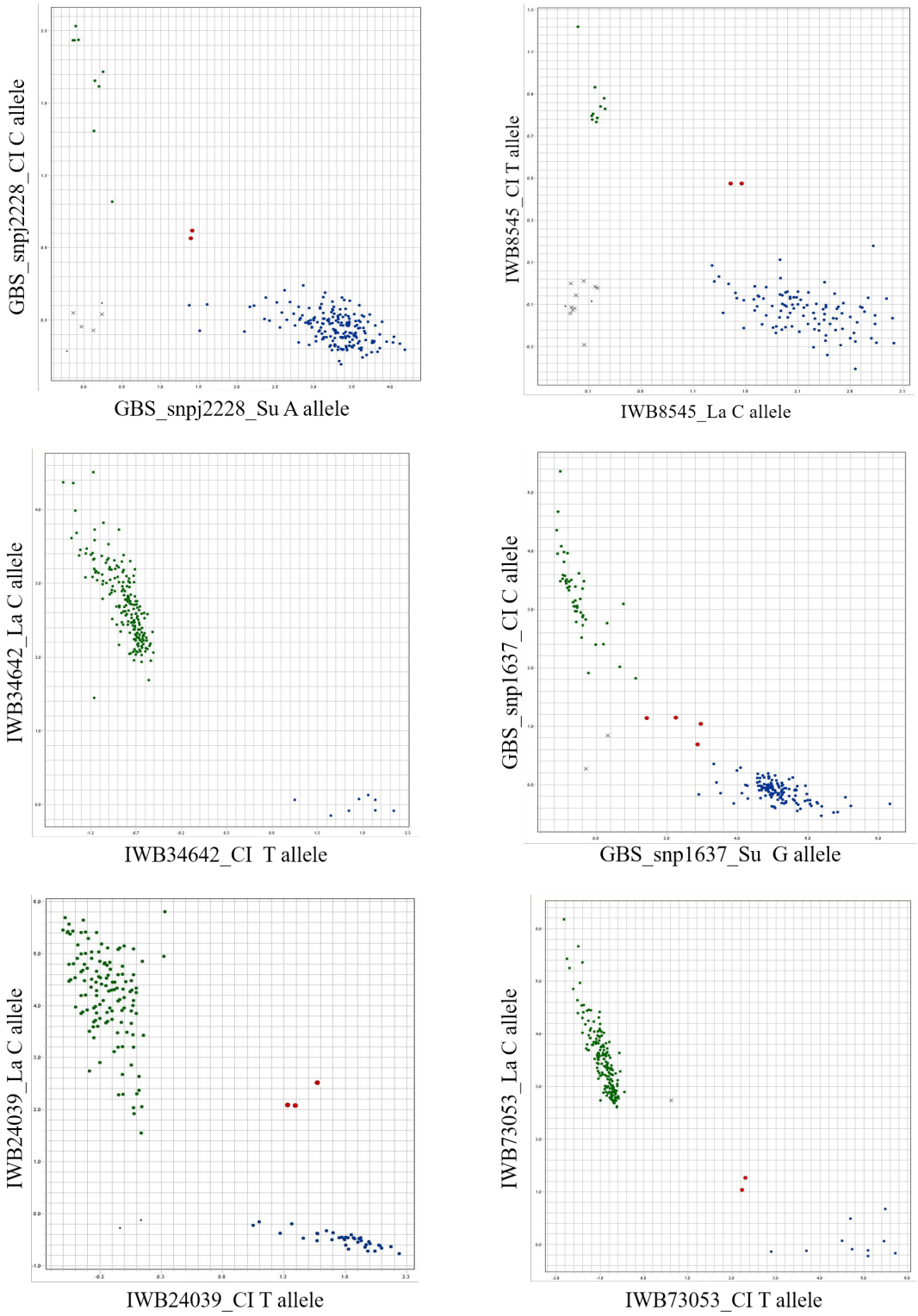


Figure 4.12 A KASPar assay profile to show allelic segregation of SNPs in 192 elite U.S lines. The blue and green dots show different alleles, and the dark dots indicate the water control.



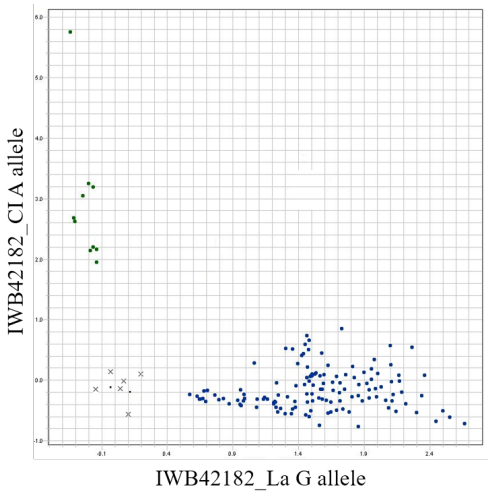

IWB42182_CIA allele
IWB42182_La G allele

Table 4.1 List of KASP primers designed based on sequences from GBS-SNPs and 90K-SNPs

Primer name[a]	Chromosome	Primer sequence (5'-3')
GBS_snpj1995_CI	2DS	TGCAGCCTCATAGAAA
GBS_snpj1995_Su	2DS	TGCAGCCTCATAGAAG
GBS_snpj1995_R	2DS	CCTTCCTTGGTGTCGT
GBS_snpj2228_CI	2DS	GCAGTGAACTTGGC
GBS_snpj2228_Su	2DS	GCAGTGAACTTGGA
GBS_snpj2228_R	2DS	AGCGTGCTAAGGAGT
IWB8545_CI	2DS	AGCGGTTTCTTTTAACCATTCTTGT
IWB8545_La	2DS	AGCGGTTTCTTTTAACCATTCTTGC
IWB8545_R	2DS	CTGGCGTTGTATATTGGACAAGGT
IWB34642_CI	2DS	GGAATCGCCTAACCAATGTTGT
IWB34642_La	2DS	GGAATCGCCTAACCAATGTTGC
IWB34642_R	2DS	GCCATGAACATCCTGCAACA
GBS_snp1637_CI	7BL	ACTCTCGCAGCAGGCCAG
GBS_snp1637_Su	7BL	CTCTCGCAGCAGGCCAA
GBS_snp1637_R	7BL	ACCGATGGCCACGAGAGT
GBS_snp0219_CI	7BL	TAAACGGCAGCCATCTCG
GBS_snp0219_Su	7BL	TAAACGGCAGCCATCTCA
GBS_snp0219_R	7BL	GCGGTAGGGTTGTACATGCT
GBS_snpj3237_CI	7BL	CTGCAGTTTCAGC
GBS_snpj3237_Su	7BL	CTGCAGTTTCAGT
GBS_snpj3237_R	7BL	TGCATACGGTGATACGGAT
GBS_snp1562_CI	7BL	AGCAGTACATGCTTCTGTCA
GBS_snp1562_Su	7BL	AGCAGTACATGCTTCTGTCC
GBS_snp1562_R	7BL	CGCGCGCCCATTAG
IWB9496_CI	7BL	TGTGATCTGATCCAACAAAACTCT
IWB9496_La	7BL	TGTGATCTGATCCAACAAAACTCG
IWB9496_R	7BL	ACAGGCAATTCCACCTTTACTT
IWB24039_CI	7BL	GCTTTGGTCCACTCCACTAGT
IWB24039_La	7BL	GCTTTGGTCCACTCCACTAGC
IWB24039_R	7BL	AAGGAGGCGCTCATGACG
IWB73053_CI	7AL	CAGATGTGCAAGATCATTGATTCT
IWB73053_La	7AL	CAGATGTGCAAGATCATTGATTCC
IWB73053_R	7AL	ACCCTCTACCTGGAAGCAT
IWB42182_CI	7AL	GAGGATGCCCTGCCGACA
IWB42182_La	7AL	GAGGATGCCCTGCCGACG
IWB42182_R	7AL	CCCAACTCCACGCTCCTCTT

[a]CI forward primer with CI13227 alleles, La forward primer with Lakin allele, Su forward primer with Suwon92 allele, R reverse primer

Table 4.2 Latent period (LP), final severity (FS), and area under disease progress curve (AUDPC), of CI13227, Everest, and their recombinant inbred line (RIL) population (n=184) in 2015 spring and 2015 summer greenhouse experiments

	Year[a]	LP day	FS %	AUDPC	IT
CI13227	2015SP	14	33.2	100.5	3
	2015SU	13	29.2	96.9	2
Everest	2015SP	10	55.7	200.5	5
	2015SU	10	58.2	253.1	6
RILs means	2015SP	9	39.02	180.1	4.7
	2015SU	8	43.00	234.4	4.2
RILs ranges	2015SP	7-17	5.0-88.3	4.5-547.0	1-9
	2015SU	7-17	5.0-89.2	24.0-567.0	2-9

[a] 2015SP = 2015 spring, 2015SU = 2015 summer

Table 4.3 Correlation coefficients among four slow-rusting traits, latent period (LP), final severity (FS), and area under disease progress curve (AUDPC) and infection type (IT)

	LP	FS	AUDPC
FS	-0.74***		
AUDPC	-0.72***	0.95***	
IT	-0.58***	0.67***	0.56***

*** $p < 0.0001$

Table 4.4 Chromosomal locations, flanking markers, determination coefficients (r^2), additive effects (Add) and logarithm of the odds (LOD) values for significant quantitative trait loci (QTLs) identified in the CI13227 x Everest recombinant inbred line (RIL) population

TraitName	Chromosome	Position	Left Marker	Right Marker	LOD	r^2 (%)	Add
LP2015SP	2D	51.2	IWB8545	GBS_snpE2044	12.9	28.45	-1.34
FS2015SP	2D	51.2	IWB8545	GBS_snpE2044	5.0	12.73	6.17
IT2015SP	2D	51.2	IWB8545	GBS_snpE2044	5.1	12.96	0.53
LP2015SU	2D	51.2	IWB8545	GBS_snpE2044	4.2	9.61	-0.52
FS2015SU	2D	51.2	IWB8545	GBS_snpE2044	4.1	9.32	5.92
AUDPC2015SU	2D	51.2	IWB8545	GBS_snpE2044	4.1	9.16	31.29
IT2015SP	7B	2.7	GBS_snpE1588	GBS_snp1673	5.3	13.60	0.67
LP2015SP	7B	2.7	GBS_snpE1588	GBS_snp1673	3.0	6.82	-0.35
FS2015SU	7B	2.7	GBS_snpE1588	GBS_snp1673	4.2	10.10	5.74
AUDPC2015SU	7B	2.7	GBS_snpE1588	GBS_snp1673	4.0	9.87	30.27
IT2015SU	7B	2.7	GBS_snpE1588	GBS_snp1673	4.2	10.36	0.55
LP2015SP	7A	3.2	GBS_snpE0495	GBS_snpE1632	3.2	7.36	-0.38
LP2015SU	7A	3.2	GBS_snpE0495	GBS_snpE1632	3.8	8.89	-0.48
AUDPC2015SP	3B	8.0	GBS_snpE1131	GBS_snpE0820	3.8	9.89	2.80
IT2015SP	3B	8.0	GBS_snpE1131	GBS_snpE0820	2.9	8.71	37.75
LP2015SU	1D	26.8	GBS_snpE1845	GBS_snpE1357	3.9	9.20	0.54

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