QTL mapping of pre-harvest sprouting and stripe rust resistance in wheat cultivars Danby and Tiger

by

Mingqin Shao

B.S., Huazhong Agricultural University, 2005

M.S., Huazhong Agricultural University, 2008

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy

College of Agriculture

KANSAS STATE UNIVERSITY

Manhattan, Kansas

2017

Abstract

Wheat yield and quality is influenced by many abiotic and biotic environmental factors. Pre-harvest sprouting (PHS) occurs when physiologically matured spikes are exposed to wet field conditions before harvest, which results in seed germination and causes significant losses in yield and end-use quality. Wheat stripe rust is one of the most important biotic factors reducing grain yield and quality. To investigate the genetic basis of the resistance to PHS and stripe rust in hard white winter wheat cultivars Danby and Tiger and develop molecular markers for marker-assisted breeding, a double haploid (DH) population, derived from those two cultivars, was genotyped with simple sequence repeats (SSR) markers and simple nucleotide polymorphism (SNP) markers. This DH population was assessed for resistance to PHS and stripe rust in both greenhouse and field experiments. For PHS, one major resistant quantitative trait locus (QTL) was consistently detected on the short arm of chromosome 3A in all three experiments conducted and explained 21.6% to 41.0% of the phenotypic variation (PVE). This QTL is corresponding to a previously cloned gene, *TaPHS1*. A SNP in the promoter of *TaPHS1* co-segregated with PHS resistance in this mapping population. Meanwhile, two other QTLs, *Qphs.hwwg-3B.1* and *Qphs.hwwg-5A.1*, were consistently detected on the chromosome arms 3BS and 5AL in two experiments. These two QTLs showed significant additive effects with *TaPHS1* in improving PHS resistance. For stripe rust, three major QTLs were consistently detected in four out of six environments for infection type (IT) or disease severity (DS). Two of them, *QYr.hwwg-2AS1* and *QYr.hwwg-4BL1*, contributed by the Danby allele explained up to 28.4% of PVE for IT and 60.5% of PVE for DS. The third QTL, *QYr.hwwg-3BS1*, contributed by the Tiger allele, had PVE values up to 14.7% for IT and 22.9% for DS. *QYr.hwwg-2AS1* and *QYr.hwwg-4BL1* are likely the same resistance genes reported previously on chromosome arms 2AS and 4BL. However, *QYr.hwwg-3BS1* might be different from the reported gene cluster near the distal end of 3BS where *Yr57*, *Yr4*, *Yr30* and *Sr2* were located. Significant additive effects on reducing IT and DS were observed among these three major QTLs. In order to pyramid multiple QTLs in breeding, user-friendly Kompetitive allele specific PCR (KASP) markers were successfully developed for several QTLs identified in this study. The QTLs and their interactions found in this

study together with those novel flanking KASP markers developed will be useful not only for understanding genetic mechanisms of PHS and stripe rust resistance but also for marker-assisted breeding to improve wheat resistance to PHS and stripe rust by gene pyramiding.

QTL mapping of pre-harvest sprouting and stripe rust resistance in wheat cultivars Danby and Tiger

by

Mingqin Shao

B.S., Huazhong Agricultural University, 2005

M.S., Huazhong Agricultural University, 2008

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy

College of Agriculture

KANSAS STATE UNIVERSITY

Manhattan, Kansas

2017

Approved by:

Co-major Professor
Dr. Guorong Zhang

Approved by:

Co-major Professor
Dr. Guihua Bai

Copyright

Abstract

Wheat yield and quality is influenced by many abiotic and biotic environmental factors. Pre-harvest sprouting (PHS) occurs when physiologically matured spikes are exposed to wet field conditions before harvest, which results in seed germination and causes significant losses in yield and end-use quality. Wheat stripe rust is one of the most important biotic factors reducing grain yield and quality. To investigate the genetic basis of the resistance to PHS and stripe rust in hard white winter wheat cultivars Danby and Tiger and develop molecular markers for marker-assisted breeding, a double haploid (DH) population, derived from those two cultivars, was genotyped with simple sequence repeats (SSR) markers and simple nucleotide polymorphism (SNP) markers. This DH population was assessed for resistance to PHS and stripe rust in both greenhouse and field experiments. For PHS, one major resistant quantitative trait locus (QTL) was consistently detected on the short arm of chromosome 3A in all three experiments conducted and explained 21.6% to 41.0% of the phenotypic variation (PVE). This QTL is corresponding to a previously cloned gene, *TaPHS1*. A SNP in the promoter of *TaPHS1* co-segregated with PHS resistance in this mapping population. Meanwhile, two other QTLs, *Qphs.hwwg-3B.1* and *Qphs.hwwg-5A.1*, were consistently detected on the chromosome arms 3BS and 5AL in two experiments. These two QTLs showed significant additive effects with *TaPHS1* in improving PHS resistance. For stripe rust, three major QTLs were consistently detected in four out of six environments for infection type (IT) or disease severity (DS). Two of them, *QYr.hwwg-2AS1* and *QYr.hwwg-4BL1*, contributed by the Danby allele explained up to 28.4% of PVE for IT and 60.5% of PVE for DS. The third QTL, *QYr.hwwg-3BS1*, contributed by the Tiger allele, had PVE values up to 14.7% for IT and 22.9% for DS. *QYr.hwwg-2AS1* and *QYr.hwwg-4BL1* are likely the same resistance genes reported previously on chromosome arms 2AS and 4BL. However, *QYr.hwwg-3BS1* might be different from the reported gene cluster near the distal end of 3BS where *Yr57*, *Yr4*, *Yr30* and *Sr2* were located. Significant additive effects on reducing IT and DS were observed among these three major QTLs. In order to pyramid multiple QTLs in breeding, user-friendly Kompetitive allele specific PCR (KASP) markers were successfully developed for several QTLs identified in this study. The QTLs and their interactions found in this

study together with those novel flanking KASP markers developed will be useful not only for understanding genetic mechanisms of PHS and stripe rust resistance but also for marker-assisted breeding to improve wheat resistance to PHS and stripe rust by gene pyramiding.

Table of Contents

List of Figures

List of Tables

Acknowledgements

My thesis would not have been possible without the inspiration, support, help and directory from many wonderful individuals. I would like to express my thanks and appreciations to all of them for being part of the most import journey in my life and making this thesis possible. First of all, I owe my deepest gratitude to my supervisors, Dr. Guihua Bai and Dr. Guorong Zhang. Without their enthusiasm, encouragement, optimism, and continuous support, this thesis would not have been completed.

Co-major advisor Dr. Guorong Zhang has guided me and encouraged me to carry on through these years and has contributed to my research projects with a major impact, especially for experiment design, research material selection, and result explanations. He is always ready to proofread and mark up my manuscripts sentence by sentence. He taught me how to make my academic writing more logic and readable, always with big doses of patience through the whole process of my scientific writing. He also let me be involved into his breeding programs to learn practical breeding skills and gave me great chances to learn how to connect research with production.

As my co-major advisor, Dr. Bai was always there to talk about my ideas and also ask me many questions to help me think through my problems. Most importantly he was always patient to listen to me and give proper advice whenever I need. He taught me how to organize and emphasize critical components and key ideas in scientific reports, proposals and manuscripts. He also showed me different ways to approach a research problem by critic thinking and the need to be persistent to accomplish any goal. He is always the first person I turn to when I have any problem in laboratory or greenhouse work.

Besides my major advisors, I would like to thank the rest of my thesis committees. Dr. Harold Trick not only gave insightful comments and advises on my research projects but also introduced me with important common knowledge of intelligence protection and commercialization of biotechnology. He is also a great advisor for my academic writing by giving practical advises and introducing handful references. Dr. Allen Fritz is not only a productive

wheat breeder, but also a great coach and mentor for future breeders. He taught me a lot on wheat breeding by showing me how to do selection in nursery fields hand by hand and explaining different breeding strategies to me patiently. He is very generous to let me join his lab meetings to discuss all kinds of breeding or research topics from which I learned a lot on how to balance breeding and research as a breeder. I also would like to thank Dr. Mark Ungerer for his valuable time serving as the outside chairman of my final examination.

I want to express my deep gratitude to our most important collaborator, Dr. Bob Bowden, for his help on stripe rust experiment design, phenotyping and result analysis, Dr. Jesse Poland and Dr. Trevor W. Rife for their help on genotyping by sequencing of the mapping population. I am also very grateful to all the colleagues in Dr. Bai's lab and all the members in Dr. Zhang's breeding team for their help, assistance and discussions during the genotyping and phenotyping processes of my research.

A very special word of thanks goes for my family: my parents Shanxiu Nie and Changzi Shao for giving me life in the first place, for nursing me with all they have, for unconditional supports and encouragements to pursue my dream; and my wife Li Lei for her understanding, supporting, and advising in my whole Ph.D. study period.

Chapter 1 - Literature review

Wheat and wheat evolution

Importance of wheat

Wheat is grown on the largest land area among all commercialized food crops because of its wide adaptation, covering from 67° N in Russia to 45° S in Argentina and includes places not only in the temperate zones but also in the tropics and sub-tropics regions like Indian, Pakistan and other countries in southeastern Asia (Feldman, 1995).

Wheat is the second largest crop in term of production. In 2016, the whole world produced 751.1 million tonnes, which was second to maize (1,341.7 million tones) but more than rice (480 million tonnes) (http://usda.mannlib.cornell.edu/usda/current/wasde/wasde-03-09-2017.pdf). Wheat contributes more than 20% of total calories consumed by humans (Pfeifer et al. 2014). It also has about 13% protein in grain by weight with many essential amino acids, critical minerals, vitamins, beneficial phytochemicals, and fiber components for human diet (Shewry 2009). The relatively higher protein content in wheat comparing to other cereals crops makes wheat the major supplier of vegetable proteins in daily diet. That is why it is the staple food for more than 30% of the human population (International Wheat Genome Sequencing 2014). Good adaptability and rich nutrition together make bread wheat the most important cereal crop in the world.

Origin and evolution of wheat

Before hexaploid common wheat (genome AABBDD), there were other two kinds of cultivated wheat, diploid einkorn (genome AA) and tetraploid emmer (genome AABB), which originated from the southeastern Turkey based on their genetic relationships (Dubcovsky and Dvorak 2007).

Early farming practices first used wild diploid wheat species as major food resources and then advanced to domesticated diploid and polyploidy wheat species (Marcussen et al. 2014). The first cultivated common wheat was about 10,000 years ago and was critical for the transition

from hunting and gathering food to a settled agricultural food supply for human beings, which was the most important reason for the rapid increase of the human population (Shewry 2009).

The complicated genome (AABBDD) of common wheat was formed through multiple hybridization processes among its progenitors (International Wheat Genome Sequencing 2014). The predicted closest extant representatives of the ancestral diploid progenitor species are *Triticum* urartu (AA genome), *Aegilops* speltoides (SS genome related to the BB genome), and *Aegilops* tauschii (DD genome) (Choulet et al. 2014).

The first genome hybridization brought together the genomes of *Triticum urartu* (AA) and *Aegilops speltoides* (SS) several hundred thousand years ago (Petersen et al. 2006). This hybridization formed the allotetraploid *Triticum turgidum* (AABB), an ancestor of wild emmer wheat and the domesticated durum wheat grown for pasta. The second hybridization happened between *T. turgidum* (AABB) and *Aegilops tauschii* (DD) and produced the ancestral allohexaploid *T. aestivum* (AABBDD) (International Wheat Genome Sequencing 2014; Petersen et al. 2006). After this hybridization, the allopolyploid wheat was formed with 21 pairs of chromosomes consisting of three homeologous sets of seven chromosomes in each of the A, B, and D subgenomes.

Those two hybridization events resulted in a large and highly redundant genome with more than 80% of the genome consisting of highly repeated sequences (Eversole et al. 2014). After cultivation of more than 10,000 years, the bread wheat now accounts for over 95% of the wheat grown worldwide (International Wheat Genome Sequencing 2014).

Wheat pre-harvest sprouting

Pre-harvest sprouting (PHS) is the germination of wheat kernels within the spike before harvest because of prolonged rainfall and high humidity after the grain has ripened (Hilhorst, 2007). The major effect of PHS is the reduction in both yield and end-product quality (Cabrera et al. 1995; Flintham 2000; Shorinola et al. 2016a). PHS resistance is, therefore, a highly desirable trait for a good wheat cultivar. However, it is also a complex trait that is controlled by many genetic and environmental factors.

Economic losses caused by pre-harvest sprouting

PHS can cause significant economic losses from reduced yield and grain quality (Simsek et al. 2014). The lower yield mainly comes from the reduced test weight of PHS damaged grains due to the degradation of starch and protein in early germination processes, which is also the major cause of significant quality reduction(Cabrera et al. 1995; Flintham 2000; Shorinola et al. 2016a). The main cause of starch degradation is the high α-amylase activity in sprouted grains, which breaks down the starch quickly and efficiently during water absorption (Barbeau et al. 2006; De Laethauwer et al. 2013). Baked goods from this kind of flour will have a smaller volume and a compact, sticky crumb structure (Payannavar et al. 1993; Sorrells et al. 1989). Seriously damaged grains can only be used for animal feed and could have many potential negative health effects in this kind of feed (Farlin et al. 1971).

Pre-harvest sprouting is observed across all major wheat growing regions in the world and losses resulting from PHS are huge for wheat growers and grain processing industry. In the major wheat production region of western Canada, the average annual losses due to PHS are approximately $100 million (DePauw et al. 2012). In the United States, PHS caused low falling numbers resulting in the loss of millions of dollars for wheat farmers in Washington State in 2013 as wheat grain price was reduced by 25 cents per bushel for every 25 seconds below 300 seconds of the falling number. (http://public.wsu.edu/~csteber/publications.html#WheatLifeMagazine). PHS also occurs frequently and significantly reduces grain yield and quality in China, especially in the Yangtze River Valley and southern parts of the Yellow and Huai River Valleys, where less attention has

been paid to PHS resistance in wheat breeding programs comparing to yield potential and disease resistance (Xiao et al. 2002). On a worldwide basis estimated annual economic losses due to PHS exceed one billion US dollars (DePauw et al. 2012).

Evaluation methods for pre-harvest sprouting

Field visual inspection is only effective for very serious situations. In a field with severe PHS caused by enough precipitation and favorable temperature, visible indications including kernel swelling, germ discoloring, seed coat splitting, and the root and shoot emerging. However, in most cases, indications are not so obvious due to subtler PHS damage. Therefore, field inspection is neither suitable nor convenient for evaluation for grain grading. In most cases, PHS damage is investigated in the laboratory (Trethowan et al. 1996).

There are two kinds of laboratory evaluation methods in general usage. One is the alpha-amylase based methods for grain trading and milling industries, which are mostly concerned with the flour quality milled from the target grain samples. In this case, the wheat flour is the target for evaluation. Falling number test is the most popular tool used by grain trade and milling industries (Mares and Mrva 2008). Falling number can be evaluated in breeding programs but there is a need to assay PHS resistance among different cultivars or experimental materials (Trethowan et al. 1996). In this situation, we want to evaluate PHS resistance every season even in seasons without any rain before harvest, where it is impossible to investigate PHS directly in the field. To simulate the conditions that introduce PHS, wheat researchers and breeders employ proper combinations of simulated precipitation and temperature to evaluate the PHS resistance such as germination tests with threshed seeds and sprouting rate tests with intact spikes in a closed chamber (Gordon et al. 1979; Gordon et al. 1977).

Since PHS will reduce grain quality in the form of poor flour usage, a reliable and efficient method to evaluate the damage is critical not only for grain merchants but also for food processors. Flour quality will be greatly reduced by alpha-amylase which will activate starch digestion in germinated kernels. Alpha-amylase activity can be initiated by a small amount of water uptake by the seed, even without visible evidence of seed germination (Olaerts et al. 2016). Therefore, a sensitive test method is critical to detect the initial damage, especially for

grain commodity grading. FN test is based on the fact that a mixture of wheat flour with water will gelatinize upon boiling, but starch chains cleaved by alpha-amylase cannot gelatinize well (Mares and Mrva 2008). The more active alpha-amylase in the sample, the faster the stirrer falls, the lower the falling number. Flour made from sprout-damaged grain will result in much lower falling number because some of the starch will have already been broken down by alpha-amylase during sprouting. In general, falling number values less than 250 seconds indicate poor quality flour for milling and baking purposes. This kind of grain is unsuitable for milling and will be discounted or directly sold to the livestock feed market at a much lower price than the milling market (Simsek et al. 2014). The Hagberg-Perten falling number test, an industry standard since 1968, measures the damage to starch caused by amylase enzyme activity in the flour milled from sprouting damaged grains (Mares and Mrva 2008).

For the wheat research and breeding community, two phenotyping methods have been broadly accepted and used to evaluate the seed dormancy and pre-harvest sprouting under simulated conditions in the laboratory. The first method is the weighted germination index test with threshed seeds germinated in Petri dishes in a temperature controlled chamber (Chen et al. 2008; Chono et al. 2015; Hughes et al. 2010; Knox et al. 2012; Nonogaki 2014; Rasul et al. 2012; Wang et al. 2011). In the germination index test as described by Walker-Simmons (1988), spikes were harvested at physiological maturity stage and manually threshed after being naturally dried at room temperature. Fifty to sixty seeds were placed on two layers of wet paper towel in a petri dish and 10 ml of clean water were added every three days to supply enough moisture for seed germination. Petri dishes were kept in a controlled chamber at 20°C. Germinated seeds were counted daily from the 1st day to the 7th day and removed after each counting. Germination index (GI) was calculated with the maximum weight given to the grains that germinated earlier and less weight to those that germinated later by the following formula: GI = $[(7\times n1 + 6\times n2 + 5\times n3 + 4\times n4 + 3\times n5 + 2\times n6 + 1\times n7)/(7 \times \text{total number of grains})] \times 100$, where n1, n2, ..., n7 is the number of germinated kernels in different days .

The second phenotyping method in the laboratory is to evaluate the sprouting rate with un-threshed spikes in a misting chamber at a controlled temperature (Chen et al. 2008; Lin et al. 2015; Liu et al. 2011; Liu et al. 2008; Liu et al. 2013; Liu et al. 2015; Rasul et al. 2012). To

evaluate the sprouting rate, five to ten spikes are harvested at physiological maturity (when peduncles turn yellow) from the field or greenhouse. Harvested spikes are air dried for 5-6 days at room temperature and then stored in a freezer at -20°C to maintain their dormancy. After harvest of all materials, the samples are air-dried again at room temperature for 3-5 days. After the second drying period, spikes are incubated in a mist chamber for 7 d. Visibly germinated and non-germinated kernels in each tested spike are counted to calculate the percentage of sprouting for each genotype.

Several studies compared GI to sprouting rate and FN. GI and sprouting rate were compared for environmental sensitivity and reliability in a three-year study (Paterson et al. 1989). In this research, effects of non-genetic factors were minimal for both germination index and sprouting rate. Meanwhile, this study also validated that storage of spikes or seeds in -20°C was effective to retain seed dormancy for up to 3 months. Another examination of different methods for PHS evaluation in white-grained bread wheat included sprouting test, germination index, and falling number (Trethowan 1995). Sprouting test with spikes in a rain simulator was useful for more susceptible materials. However, it is not as accurate as germination index in assessment of individual genotypes because large interactions between genotypes and years were detected under simulated rainfall. GI was also compared with FN for accuracy and reliability (Singh et al. 2008a). Although FN is the industry standard method for grain grading, it was demonstrated that the germination test was more reliable than FN or alpha-amylase activity test for measuring PHS resistance for genetic studies or breeding selection relatively little seed is generally available. FN may also be influenced by too many other factors, which are difficult to control or simulate in a laboratory (Singh et al. 2008a). Another challenge for comparing PHS and FN is that, sometimes, high amylase activity or low FN may occur in cultivars without significant PHS because of the late maturity amylase, also known as prematurity amylase, which will reduce the FN(Mares and Mrva 2008; Mares and Mrva 2014; Singh et al. 2008a).

Factors affecting pre-harvest sprouting

PHS is affected by many factors such as grain dormancy, seed coat color, temperature during grain filling, water uptake and drying speed, wheat spike structure, and the remobilization of

nutrients to support germination (King and Richards 1984; Mares and Mrva 2014; Tuttle et al. 2015).

The most important factor responsible for the genetic variation of PHS is the degree of seed dormancy at harvest, which has been validated by many genetic and physiological studies (Benech-Arnold 2009; Bewley 1997; Gubler et al. 2005; Mapes et al. 1989; Shu et al. 2015; Walkersimmons 1987). Seed dormancy could be influenced by a combination of many factors associated with water uptake, drying rate of the ear and the mobilization of storage reserves to support germination (Garello and Le Page-Degivry 1999; Tuttle et al. 2015).

Seed dormancy is the most frequently investigated PHS-associated trait. This is not only true for the model plant, Arabidopsis, but also for many crops including wheat, rice, barley, and sorghum (Gao et al. 2013). Seed dormancy is thought to be one of the most important adaptation and domestication traits in plants (Doebley et al. 2006). The level of seed dormancy in domesticated or cultivated wheats is relatively lower than their progenitors and wild relatives (Liu et al. 2015; Torada et al. 2016). Seed dormancy is an important adaptation trait in nature as it provides a survival strategy under adverse environmental conditions. Dormancy has been significantly altered by selection in the domestication and breeding processes (Sugimoto et al. 2010).

The level of seed dormancy is determined not only by the amount of abscisic acid (ABA) and gibberellin (GA) but also by the seed sensitivity to these two hormones (Finkelstein et al. 2008; Koch et al. 1982). The two hormones play contrasting roles in regulating seed dormancy (Son et al. 2016). ABA (a germination suppressor) promotes and maintains dormancy, whereas GA (a germination promoter) releases and reduces seed dormancy (Gao et al. 2012). Many environmental factors like temperature, drought and heat could regulate these two hormones' levels or sensitivity of seed embryo to them through biosynthetic and catabolic enzymes (Suzuki et al. 2000; Walker-Simmons 1987). During seed development, dormancy increases with ABA sensitivity but the GA sensitivity is decreasing during this process. However, during the after-ripening process when seed dormancy is decreasing, dormant seed first become sensitivity to GA, a germination promoter, and then lose sensitivity to ABA, a germination suppressor (Gao et al. 2012; Tuttle et al. 2015).

There is also direct gene mutation evidence of the association between ABA sensitivity and seed dormancy in wheat. A wheat mutation *(ERA8)*, identified from a soft white wheat cultivar "Zak", after treated by chemical mutagenesis, demonstrated increased sensitivity to the plant hormone ABA resulting in increased seed dormancy (Schramm et al. 2013). It showed high PHS resistance because of elevated sensitivity to ABA, which induces seed dormancy during embryo maturation. No obvious yield decrease was observed compared to the un-mutated primary line (Schramm et al. 2013). This mutation line has been backcrossed twice to wild-type Zak and showed similar morphological and grain quality traits to the original Zak (Martinez et al. 2014). Based on the distinct function for PHS tolerance and rare negative effect on yield or grain quality, this mutation, with increased sensitivity to ABA, could be a good genetic resource to improve PHS tolerance in white wheat breeding programs.

Temperature is one of the most important environmental factors during grain filling associated with seed dormancy and PHS (Mares et al. 2009; Mares 1984). Grain tend to have more dormancy when growing under cool conditions than under warm conditions. Even within a single spike, grains located at different positions may have different dormancy levels because their maturity time is different and they might experience different temperature during grain filling (Auld and Paulsen 2003). After maturity, cool and moist conditions can break seed dormancy and lead to PHS. A previous study (Yamauchi et al., 2004) has shown that cold stratification can reduce dormancy because of decreased ABA and increased GA hormone levels in Arabidopsis, a similar effect was also observed in wheat (Tuttle et al. 2015).

There is also considerable evidence for the association between grain color and PHS resistance. Usually white wheat cultivars are more susceptible to PHS than red ones, which could be due to pleiotropic effects of red grain color genes (Groos et al. 2002; Lawson et al. 1997; Lin et al. 2016; Pellizzaro et al. 2016). The pleiotropic effect of grain color genes on PHS was validated by grain color mutated wheat lines, which were changed from red to white and became more susceptible to PHS (Himi et al. 2002; Warner et al. 2000). The other supporting evidence for the association between grain color and PHS resistance is that many PHS resistance QTLs were co-localized with grain color QTLs (dos Santos et al. 2010; Groos et al. 2002; Kumar et al. 2009; Lin et al. 2016; Pellizzaro et al. 2016). Since many PHS resistance QTL were linked with grain color,

they could hardly be used to improve the PHS resistance in white wheat, highlighting the importance of identifying color-independent PHS resistance genes. Fortunately, there are also many color-independent genes associated with seed dormancy and some of them have been used in breeding for many years (DePauw et al. 2012; Depauw et al. 1992; Depauw et al. 1985; Mccaig and Depauw 1992; Morris and Paulsen 1987, 1989).

QTL mapping and gene cloning for PHS resistance

To investigate the genetic mechanisms and discover useful molecular markers for wheat breeding, significant efforts have been made to map quantitative trait loci controlling seed dormancy and pre-harvest sprouting in wheat. So far, many PHS resistance QTLs or genes have been reported in wheat, and they were mapped on almost all wheat chromosomes (Anderson et al. 1993; Flintham et al. 2002; Groos et al. 2002; Kato et al. 2001; Li et al. 2004; Mares and Mrva 2001; Roy et al. 1999; Zanetti et al. 2000). Among those QTLs, the QTLs on chromosomes 3AS and 4AL were investigated intensively since they contributed large phenotypic variations and were consistently detected in different genetic backgrounds and environments (Albrecht et al. 2015; Bi et al. 2014; Cao et al. 2016; Fakthongphan et al. 2016; Lei et al. 2013; Lin et al. 2015; Liu et al. 2011; Liu et al. 2008; Lohwasser et al. 2013; Miao et al. 2013; Miura et al. 2002; Mori et al. 2005; Rasul et al. 2009; Xiao et al. 2012). They were considered as the most important targets for fine mapping and gene cloning and they were successfully cloned recently (Liu et al. 2011; Liu et al. 2008; Liu et al. 2013; Mares et al. 2005; Mori et al. 2005; Nakamura et al. 2011; Torada et al. 2008; Torada et al. 2016).

The QTL on 3AS chromosome arm was first successfully cloned by two different research groups with different strategies using different genetic resources (Liu et al. 2013; Nakamura et al. 2011). Nakamura, S. et al. (2011) cloned this gene through a gene differential expression study with microarray. They identified a wheat homolog gene of *MOTHER OF FT AND TFL1* (*TaMFT*), which was up regulated in dormant seeds grown and maturated at a low temperature. The differential gene expression was correlated with a single nucleotide polymorphism (SNP) in the promoter region of the identified gene. Through mapping-based cloning, Liu et al. also identified a PHS resistance gene, at the same position as *TaMFT*, and it was designated as

TaPHS1. In this study, two SNP sites in the gene coding region were identified as the causal mutations and were further validated by RNA interference-mediated knockdown and association analysis. Apparently, these two research groups identified different casual variations in the same gene responsible for regulating seed dormancy and PHS, which might be due to the different evaluation methods used. In the first study, the differential gene expression and phenotypic effect of the promoter variation was investigated with seeds developed under 13°C, which is much lower than normal temperature (20°C to 25°C) for grain development in the field (Nakamura et al. 2011). In contrast, the second study was conducted with seeds grown under normal greenhouse and field temperatures. The functional difference between the promoter and coding region is still unclear.

On chromosome arm 4AL, a major QTL (*Phs1*) was also recently cloned by different research groups. It was first cloned in 2015 through RNA-sequencing of multiple near-isogenic lines segregating for this QTL (Barrero et al. 2015). In this study, two adjacent candidate genes, *PM19-A1* and *PM19-A2*, were identified in the QTL region. They are gene members of ABA-induced wheat plasma membrane 19 family. Their expression levels were associated with dormancy variations, which was validated in a diverse panel of bread and durum wheat. One year later, another gene *TaMKK3-A*, under the *Phs1* QTL region and only 0.5 cM away from PM19-A1, was cloned by a map-based approach (Torada et al. 2016). This mitogen-activated protein kinase gene is critical in phosphorylation of proteins involved in signal transduction including ABA signaling. Another study reported that *PM19-A1* and *PM19-A2* are 0.3 cM away from *Phs-A1* gene by comparative analysis of *Phs-A1* intervals in wheat and *Brachypodium* using high-resolution fine-mapping with two bi-parental mapping populations (Shorinola et al. 2016b). Above all, there could be more than one responsible candidate genes under the major PHS resistance QTL on chromosome 4AL, but further study is in need to uncover whether both PM19 genes and MKK3 genes are causal genes of seed dormancy in bread wheat.

PHS improvement in wheat breeding

There are many limitations for genetic improvement of PHS, especially the difficulties during phenotypic screening in breeding programs (DePauw et al. 2012). A favorable field environment

for PHS is not always available for direct phenotypic selection in breeding. Although greenhouse screening is used as an alternative selection method in some breeding programs (Humphreys and Noll 2002), it is not only time consuming but also difficult to control the moisture and temperature for a large number of segregating populations involved in breeding programs (Li et al. 2004). Field conditions cannot be totally simulated in controlled environments because PHS is affected by many environmental factors and their interactions, such as temperature during grain filling and drying, harvest time, grain storage environment and so on (Benech-Arnold 2009; Finch-Savage and Leubner-Metzger 2006; Gao et al. 2013; Humphreys et al. 2012; Linkies et al. 2010; Mares and Mrva 2014; Nonogaki 2014; Nyachiro 2012; Willis et al. 2014). This complicated field environment is far different from simulated conditions in laboratory. To overcome the difficulties of phenotypic selection in breeding, validated molecular markers could provide a useful tool in PHS resistance breeding (Graybosch et al. 2013; Kumar et al. 2010). Although there are already many molecular markers available, most of them were developed from QTL mapping results (Gao et al. 2013; Mares and Mrva 2014).

Wheat stripe rust and its genetic resistance

Wheat stripe rust, pathogen, host, and infection condition

Wheat stripe rust or yellow rust, caused by *Puccinia striiformis* f.sp. tritici (PST), is one of the most damaging diseases in bread wheat, especially for those growing under cool conditions (Brown and Hovmoller 2002; Thach et al. 2016; Wellings 2011).

Infected wheat plants show yellow-colored stripes parallel along each leaf blade, leaf sheath and even on spikes at the later growth stage under favorable conditions (Line 2002). Following the stripe rust infection, plants are stunted and weakened and have fewer grains, shriveled grains, low test weight, and reduced dry matter production (Sorensen et al. 2016).

Puccinia striiformis has a hemiform life cycle with only uredinial and telial stages growing in the intercellular space between the mesophyll cells. In favorite environments, six to seven days after infection, chlorotic symptoms can be observed on the infected leaf. Then, narrow and yellow or orange pustules will be observed on leaves and on the inner surfaces of glumes and lemmas of spikelets after one more week (Cartwright and Russell 1981; Sorensen et al. 2016).

Common wheat, durum wheat, barley, triticale and rye are the primary hosts of wheat stripe rust. However, wheat stripe rust cannot infect other grass species and stripe rust identified in other grasses doesn't have the ability to infect common wheat (Wellings 2011). For wheat stripe rust, autumn-sown and volunteer wheat was the only known overwintering host until 2010 when *Berberis* spp. were identified as the alternate host of wheat stripe rust for overwinter survival and confirmed by artificial inoculation and DNA analysis (Jin et al. 2010). Compared to the relatively few overwinter hosts, there are more oversummer wheat plants existing in various environments like susceptible volunteer wheat plants in fields, grain storage sites, transporting facilities and roadsides (McIntosh and Brown 1997).

Cool temperature and high humidity are the basic conditions for stripe rust infection and spread. Although the optimum temperature for infection is between 15 and 22 °C, infection can occur when the field temperature is between 2 and 15 °C (Akin et al. 2016; Sorensen et al. 2016). More recently, races active at higher temperatures were also reported and they caused

severe yield losses under temperatures between 23 and 35 °C (Akin et al. 2016). In high elevation regions, wheat stripe rust is often a threat because the temperature is low at night during flowering stage (Brown and Hovmoller 2002).

Wheat stripe rust distribution, diversity, viability, migration, and epidemics

Wheat stripe rust is present in all temperate wheat growing areas of the world and disease epidemics have been associated with large yield losses without adequate disease control (Chen 2005; Wellings 2011). In other areas near the equator, the life cycles of stripe rust change with altitude just as the flowering and maturity time of wheat changes with altitude (Saari and Prescott, 1985).

Large-scale population structure analyses were used to investigate the migration patterns and diversity for stripe rust pathogens. Population genetic analysis showed an obvious regional heterogeneity resulting from significant sexual recombination in the Himalayan and near-Himalayan regions. Therefore, this region could be the possible center of origin for PST because of the high genetic diversity, clear recombinant population structure and highly sexual reproduction ability (Ali et al. 2014). Recently, through a worldwide population structure analysis with 212 single isolates collected from 1958 to 1991, Thach et al. (2016) had a similar finding. Meanwhile, by comparison analysis between historic strains and newly emerging ones, Thach et al. (2016) also identified the sources of the recently emerged strains for the major geographical regions across the world (Thach et al. 2016). Based on this study, Middle East Africa is the origin of the high temperature tolerant races which have recently spread widely around the world. Europe is the primary source of the current PST populations in North America and Australia. Mediterranean-Central Asian is the primary origin of major South African PST races. These migration patterns of the newly spreading strains will be helpful to effectively predict and control future epidemics.

By comparing the population structure of two collections, one isolated from the past (1958–1991) and the other from 1992 to 2009, Thach et al (2016) found significant changes in Nepalese, the Mediterranean and Pakistani populations, which have greater diversity than the Chinese and the Middle East populations. The Northwestern European population has the least

diversity. The higher divergence in the Mediterranean may be partially explained by the population substitution with a new aggressive strain, PstS2, which has been present in the region since 2003 (Ali et al. 2014; Bahri et al. 2009). The Northwestern European population is the most stable due to its clonal reproduction model. In this population the mutation and subsequent selection by host resistance gene could result in less genetic diversity (de Vallavieille-Pope et al. 2012; Hovmoller and Justesen 2007). However, the diversity increased dramatically since 2011 when a new exotic strain largely replaced the Northwestern European population (Hovmoller et al. 2016).

The stripe rust pathogen is evolving and migrating quicker than before and has developed new characteristic that influence epidemiology in the last two decades. Firstly, wheat stripe rust has spread to broader areas and caused more yield losses (Hovmoller et al. 2008; Wellings et al. 2003). For instance, stripe rust was reported in western Australia for the first time in 2002 (Wellings et al. 2003). However, the stripe rust race found there is of foreign origin and likely derived from races that were either introduced or migrated from east Africa. Secondly, the evolution of the stripe rust races is becoming faster than before and allowing them to break resistant cultivars more easily and more frequently (Hovmoller and Justesen 2007). Thirdly, stripe rust epidemics are becoming more and more severe in the major wheat growing areas including China, Europe, North American and Canada since 2000 (Chen 2005; Hovmoller et al. 2010; Wellings 2011).

Several significant epidemics were recorded in China. The most destructive ones recorded in 1950, 1964, 1990 and 2002 with 6.0, 3.2, 1.8 and 1.3 million tons of wheat losses, respectively. The lack of resistance in the dominant cultivars is the most important casual factor of the earlier epidemics (Wan et al. 2004; Zeng and Luo 2006, 2008). During the latest two epidemics, the fungicides were used timely and properly and it caused much less yield loss.

In Europe, wheat stripe didn't cause significant losses until 2010, which is mainly due to a few long-term effective resistance genes deployed in most cultivars (Hovmoller et al. 2016). However, a new virulent race, termed 'Warrior', was found on both wheat and triticale in 2011 across several European countries and rapidly spread over most of the European continent. This virulent race defeated most of the resistance mediated by former race-specific resistance genes

(Hovmoller et al. 2016). Only a few resistance genes (*Yr5, Yr8, Yr10, Yr15*, and *Yr24*) remained effective in Germany during the epidemics (Losert et al. 2016). After molecular analysis and comparison with other races, scientists found that 'Warrior' did not evolve through mutation or recombination within the European population, but is of exotic origin from sexually recombining populations in the near-Himalayan region of Asia, the diversity center of stripe rust pathogen (Hovmoller et al. 2016). The "Warrior' races has had a huge impact on wheat production in Europe. The dominance of the 'Warrior' race is a good example of how the invasion of a single exotic pathogen race can initiate a serious disease epidemic by wiping out most of the resistances established through many years of breeding (Losert et al. 2016).

In the United States, the disease can cause significant damage in the western states almost every year and has become increasingly important in the Great Plains and eastern states (Chen 2005, 2007; Chen et al. 2002, 2010; Line 2002; Wan and Chen 2014). In the United States, yearly losses exceeding 1 million metric tons since 2000 occurred in 2001 (1,081,903 t), 2003 (2,418,950 t), 2005 (2,004,234 t), and 2010 (2,606,401 t) (http://striperust.wsu.edu). Following the widespread epidemics throughout the United States in 2010, stripe rust also caused significant damage in 2011 and 2012 (Wan and Chen 2014). In 2011, stripe rust was reported in 20 states, especially in the Pacific Northwest (Wan et al. 2016). In total, national yield losses due to stripe rust were 1.76% or 959,416 metric t of grain, which is worth more than $250 million. In 2012, stripe rust was wider spread, affecting more than 25 states including the Great Plains from Texas to North Dakota. Nationally, the yield loss was estimated as 2.41% (1,491,252 t). In 2013 and 2014, the distribution and the severity of wheat stripe rust was not greater than 2012 or 2011 (https://www.ars.usda.gov/midwest-area/st-paul-mn/cereal-disease-lab/docs/cereal-rust-bulletins/). In 2015 and 2016, wheat stripe rust was more severe than any previous year in the Great Plains. The severe epidemics in 2015 resulted in an estimated yield loss of 12.7% on winter wheat and 7.2% on spring wheat. Stripe rust was found all the way from Texas to the Canadian border in North Dakota by early June of 2015 and the worst stripe rust occurred in Colorado and Nebraska (Kolmer et al. 2016). In 2016, wheat stripe rust was broadly widespread across 31 states and 4 Canadian provinces. The estimated yield loss was 7.1% on winter wheat and 1.8% on spring wheat. This was the widest distribution since 2010

(https://www.ars.usda.gov/ARSUserFiles/50620500/Cerealrustbulletins/16CRBFIN.pdf). In conclusion, wheat stripe rust has been increasing in area affected, frequency of epidemics and loss of yield in the last 15 years.

Disease control

Some traditional agricultural practices are proven effective methods to control wheat stripe rust. These cultural practices can be a great complement to other control methods such as fungicides and genetic resistance and were used broadly and effectively for a long time before fungicides were invented. Early maturing cultivars or planting of normal maturity cultivars earlier are good ways to avoid wheat stripe rust epidemics (McIntosh1976). Removing volunteer plants before planting with tillage is also an effective practice to reduce epidemics. Stripe rust epidemics also can be controlled by proper timing, frequency and amount of irrigation and fertilization (Zadoks and Bouwman 1985). A proper special arrangement of different cultivars based on maturity and wind direction might help to control stripe rust spreading within a certain area, such as planting early maturing cultivars downwind of late maturing cultivars(Singh and Rajaram 1993). There is not a single practice effective for all conditions, but using a series of cultural practices after proper pre-testing could greatly reduce the epidemics (Hovmoller and Henriksen 2008).

Chemical control has been successfully applied in Europe for a long time (Buchenauer, 1982; Stubbs and de Bruin, 1970). In the United States, chemicals work well and are accepted by farmers in high yield growing areas. In lower yielding areas, chemicals will be used to control stripe rust only when the potential benefit from increased yield can cover the cost of the chemical control. The first large-scale, successful fungicide controlled epidemics in North America occurred in 1981 and prevented multi-million dollar losses (Line 2002). In the 1990s, Cu and Line developed an expert system called MoreCrop, which combined information on agronomic practices, fungicides and resistant cultivars into an integrated disease management program and it had a managerial option for reasonable and economical control of rusts and other pathogens (Chen et al. 2003a). MoreCrop is still in use today and it has successfully reduced yield losses due to stripe rust. In 2002, fungicide application saved wheat growers of

Washington State about 30 million US dollars when stripe rust was widespread on susceptible cultivars (Chen et al. 2003a).

Growing resistant cultivars has been the principle method of controlling wheat stripe rust for many years (Chen 2005). Most of the resistant cultivars can keep effective resistance to the predominant races for about five years. It is long enough cover most of the lifespan of a adapted cultivar. Although fungicides are effective in the control of stripe rust, there are many other disadvantages that arise from their application. The use of fungicides adds an additional cost to wheat production, which is a burden for many growers in developing countries. To avoid these problems, growing cultivars with adequate level of durable resistance is the best strategy to control stripe rust. Consequently, breeding of resistant cultivars is the most critical step to sustainably control wheat stripe rust.

Types of stripe rust resistance

Genetics of stripe rust resistance has been studied for over a century. The first research was reported in 1905, which demonstrated the Mendelian inheritance model for stripe rust resistance. Up to now, more than seventy resistance genes have been reported (see reviews by Ayliffe et al. 2008; Chen et al. 2014; Chen 2005; Ellis et al. 2014; Losert et al. 2016; Rehman et al. 2013; Rosewarne et al. 2013; Singh et al. 2008b). (Ayliffe et al. 2008; Chen et al. 2014; Chen 2005; Ellis et al. 2014; Losert et al. 2016; Rehman et al. 2013; Rosewarne et al. 2013; Singh et al. 2008b). Generally there are two types stripe rust resistance, race-specific (all-stage) resistance and non-race-specific resistance (Chen et al. 2014; Chen 2005; Ellis et al. 2014).

Race-specific resistance, also known as all-stage resistance, mostly functions from seedling to adult growth stages and is most often controlled by a single gene (Chen 2005; Chen and Line 1992). One drawback of these single gene controlled resistances is their nondurable nature as they could be quickly overcome by the rapidly evolving pathogen populations (Losert et al. 2016). Cultivars with all-stage resistance usually lost their resistance in only a few years after release (Chen 2005).

In contrast, non-race-specific resistance genes are expressed at late stages of plant development, provide a broader range of resistance to pathogens, and tend to be more durable

than race-specific resistance genes. In contrast to most race-specific resistance genes, the resistance conferred by single non-race-specific resistance gene is only partial, permitting considerable disease development on infected host plants (Lupton et al. 1971; Priestley and Dodson 1976; McIntosh et al. 1995). While a single non-race-specific resistance gene only provides a low level of resistance, combining many non-race-specific genes is necessary to obtain a significant resistance level (Miedaner and Korzun 2012). This none-race-specific resistance has received much attention and has been successfully incorporated into quite a few cultivars that conferred resistance for more years than race-specific resistance (Chen et al. 2014).

One specific class of APR is referred to as high-temperature adult-plant (HTAP) resistance. It is effective after stem elongation and when average night temperatures remain above 10°C and day temperatures are between 25°C and 30°C (Qayoum and Line 1985; Milus and Line 1986a, b; Line and Chen 1995). Cultivars with only HTAP demonstrate resistance to all races only in adult plant stage, but not the seedling stage. HTAP resistance reduces epidemics in the late growth stage by reducing both the infection type and severity to get lighter and slower development of the disease. As early as 1980s, HTAP resistance has been reported in the winter wheat cultivars 'Gaines', 'Nugaines', and 'Luke' (Milus and Line (1986a, 1986b). Chen and Line (1995a, 1995b) also studied the genetics of HTAP resistance and the relationships with race-specific all stage resistance in wheat cultivars 'Druchamp' and 'Stephens'. Their results suggested that it is possible to combine HTAP with other all-stage resistance sources to improve the resistance level and durability (Chen and Line 1995b).

Resistance QTL mapping, gene cloning and marker development

Resistant cultivars are the most important component of a sustainable disease management system in wheat. Characterization of resistance genes and understanding their underlying genetic architecture is of utmost importance for breeding resistant cultivars. There are 76 designated and more than 40 temporarily designated genes or quantitative trait loci (*Yr1–Yr76*) described for stripe rust resistance in bread wheat (McIntosh et al. 2013; Rosewarne et al. 2013). Those genes covered 47 chromosomal regions across all wheat chromosomes except

chromosome 5D (Rosewarne et al. 2013).

Only a few of the mapped resistance genes/QTLs have been successfully cloned and characterized with relatively clear gene structures and functions. Early successes in wheat disease resistance gene cloning focused on race-specific resistance genes, such as *Yr10* (Spielmeyer and Lagudah, 2003). The cloned gene sequence from *Yr10* showed common molecular motifs for race-specific resistance genes such as nucleotide-binding sites and leucine repeat regions (NBS-LRR). Different from the race-specific resistance genes, a functional study of *Yr39* by Coram et al. (2008a) showed evidence for broad, non-race-specific defense responses, including the induction of several resistance protein homologues and wider induction of the defense transcripts. These mechanisms are different from those of the NBS-LRR resistance genes, and hence *Yr39* was predicted and proven to be a durable source of resistance to stripe rust (Lin and Chen, 2007; Coram et al., 2008a).

Another cloned non-race-specific resistance gene is *Yr18* (*Lr34*and *Pm38*) that has been providing durable resistance to stripe rust, leaf rust and powdery mildew for more than 50 years in wheat cultivars around the world (Lagudah et al. 2009; Lagudah et al. 2006; Lillemo et al. 2008; Suenaga et al. 2003). The proteins translated from *Yr18* resemble adenosine triphosphate–binding cassette transporters that belong to the pleiotropic drug resistance subfamily (Krattinger et al. 2009). More interestingly, the necrotic leaf tip can serve as a useful morphological marker for phenotypic based selection in breeding (Dyck 1991; Lagudah et al. 2006; McIntosh 1992; Singh 1992; Sivasamy et al. 2014). The observation of multiple pathogen resistance of this gene demonstrates the existence of single gene mediated durable resistance to multiple diseases.

Additionally, *Yr36* was also characterized as a non-race-specific resistance gene conferring resistance to a broad range of pathogen races under relatively high temperatures (25° to 35°C). This gene encodes a kinase and a putative START lipid-binding domain. *Yr36* has supplied effective resistance for many cultivars in California for many years (Fu et al., 2009).

During QTL mapping and gene cloning, hundreds of molecular markers have been identified for different resistance genes or QTL (as reviewed by Ayliffe et al. 2008; Chen et al. 2014; Chen

2005; Ellis et al. 2014; Losert et al. 2016; Rehman et al. 2013; Rosewarne et al. 2013; Singh et al. 2008b). Most of those markers were amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs) and simple sequence repeats (SSR). There are only a few kompetitive allele specific PCR (KASP) markers developed based on single-nucleotide polymorphism (SNP). Since molecular markers are critical for pyramiding resistance genes or QTLs to achieve stronger and more durable resistance in breeding, marker development for marker-assisted selection has been among the most studied areas on stripe rust.

Breeding strategies for resistance improving

Multiline cultivar is a proven strategy for stripe rust management and has been used in club wheat for a long time (Chen 2005). Multiline cultivar utilizes a mixture of lines having different resistance genes in a common genetic background to provide durable resistance to stripe rust. One example of multiline cultivar is 'Rely' released in 1993 (Allan et al. 1993). 'Rely' has been widely grown in the pacific north-western states since its release, and its overall resistance remains adequate and durable (Finckh and Mundt 1992). Other than club wheat, about one third of the common wheat acreage in Washington is also planted with multiline cultivars for stripe rust (Chen 2005).

Combining several resistance genes in one cultivar through gene pyramiding is another important strategy to improve wheat stripe rust resistance. While a single non-race-specific gene induces only a low level of resistance, combining several minor, durable resistance genes can notably improve resistance levels, especially to a new pathogen or in complex disease environments (Miedaner and Korzun 2012). Meanwhile combining HTAP resistance with effective all-stage resistance is also an effective approach to breed new cultivars with high level and durable resistance. With this combinational effect in the resistant cultivars, the all-stage resistance can provide complete control as long as it is effective, and the HTAP resistance can reduce damage when the all-stage resistance is overcome by new evolved pathogen races (Chen 2005).

In addition, anticipatory resistance breeding is an effective pre-breeding strategy of potential

deployment of resistance genes for future possible epidemics. Anticipatory resistance breeding is to develop resistance to virulent pathotypes before they become prevalent and cause significant losses (McIntosh and Brown 1997). Anticipatory resistance breeding involves predicting future pathotypes and producing corresponding resistant germplasm to accelerate new resistance gene deployment.

References

Akin B, Chen XM, Morgunov A, Zencirci N, Wan AM, Wang MN (2016) High-temperature adult-plant resistance to stripe rust in facultative winter wheat. Crop Pasture Sci 67:1064-1074

Albrecht T, Oberforster M, Kempf H, Ramgraber L, Schacht J, Kazman E, Zechner E, Neumayer A, Hartl L, Mohler V (2015) Genome-wide association mapping of preharvest sprouting resistance in a diversity panel of European winter wheats. J Appl Genet 56:277-285

Ali S, Gladieux P, Leconte M, Gautier A, Justesen AF, Hovmoller MS, Enjalbert J, de Vallavieille-Pope C (2014) Origin, Migration Routes and Worldwide Population Genetic Structure of the Wheat Yellow Rust Puccinia striiformis f. sp tritici. PLoS pathogens 10

Anderson JA, Sorrells ME, Tanksley SD (1993) Rflp Analysis of Genomic Regions Associated with Resistance to Preharvest Sprouting in Wheat. Crop Sci 33:453-459

Auld AS, Paulsen GM (2003) Effects of drought and high temperature during maturation on preharvest sprouting of hard white winter wheat. Cereal Res Commun 31:169-176

Ayliffe M, Singh R, Lagudah E (2008) Durable resistance to wheat stem rust needed. Current opinion in plant biology 11:187-192

Bahri B, Leconte M, Ouffroukh A, De Vallavieille-Pope C, Enjalbert J (2009) Geographic limits of a clonal population of wheat yellow rust in the Mediterranean region. Mol Ecol 18:4165-4179

Barbeau WE, Griffey CA, Yan ZH (2006) Evidence that minor sprout damage can lead to significant reductions in gluten strength of winter wheats. Cereal Chem 83:306-310

Barrero JM, Cavanagh C, Verbyla KL, Tibbits JFG, Verbyla AP, Huang BE, Rosewarne GM, Stephen S, Wang PH, Whan A, Rigault P, Hayden MJ, Gubler F (2015) Transcriptomic analysis of wheat near-isogenic lines identifies PM19-A1 and A2 as candidates for a major dormancy QTL. Genome Biol 16

Benech-Arnold R (2009) Pre-harvest sprouting in cereals. Euphytica 168:289-289

Bewley JD (1997) Seed germination and dormancy. Plant Cell 9:1055-1066

Bi HH, Sun YW, Xiao YG, Xia LQ (2014) Characterization of DFR allelic variations and their associations with pre-harvest sprouting resistance in a set of red-grained Chinese wheat germplasm. Euphytica 195:197-207

Brown JKM, Hovmoller MS (2002) Epidemiology - Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 297:537-541

Cabrera G, Grossmann MVE, Beleia AP (1995) Laboratory Sprouting of Wheat - Effect on Grain and Flour Quality. Arq Biol Tecnol 38:1-13

Cao LZ, Hayashi K, Tokui M, Mori M, Miura H, Onishi K (2016) Detection of QTLs for traits associated with pre-harvest sprouting resistance in bread wheat (Triticum aestivum L.). Breeding science 662:260-270

Cartwright DW, Russell GE (1981) Development of Puccinia-Striiformis in a Susceptible Winter-Wheat Cultivar. T Brit Mycol Soc 76:197-204

Chen CX, Cai SB, Bai GH (2008) A major QTL controlling seed dormancy and pre-harvest sprouting resistance on chromosome 4A in a Chinese wheat landrace. Mol Breeding 21:351-358

Chen W, Wellings C, Chen X, Kang Z, Liu T (2014) Wheat stripe (yellow) rust caused by Puccinia striiformis f. sp.tritici. Mol Plant Pathol 15:433-446

Chen X. and Wan A, Stripe rust epidemics of wheat and barley and races of *Puccinia striiformis* identified in the United States in 2015 https://www.apsnet.org/meetings/Documents/2016_meeting_abstracts/aps2016_520.htm

Chen XM (2005) Epidemiology and control of stripe rust [Puccinia striiformis f. sp tritici] on wheat. Can J Plant Pathol 27:314-337

Chen XM, Line RF (1992) Inheritance of Stripe Rust Resistance in Wheat Cultivars Used to Differentiate Races of Puccinia-Striiformis in North-America. Phytopathology 82:633-637

Chono M, Matsunaka H, Seki M, Fujita M, Kiribuchi-Otobe C, Oda S, Kojima H, Nakamura S (2015) Molecular and genealogical analysis of grain dormancy in Japanese wheat cultivars, with specific focus on MOTHER OF FT AND TFL1 on chromosome 3A. Breeding science 65:103-109

Choulet F, Alberti A, Theil S, Glover N, Barbe V, Daron J, Pingault L, Sourdille P, Couloux A, Paux E, Leroy P, Mangenot S, Guilhot N, Le Gouis J, Balfourier F, Alaux M, Jamilloux V, Poulain J, Durand C, Bellec A, Gaspin C, Safar J, Dolezel J, Rogers J, Vandepoele K, Aury JM, Mayer K, Berges H, Quesneville H, Wincker P, Feuillet C (2014) Structural and functional partitioning of bread wheat chromosome 3B. Science 345:1249721

De Laethauwer S, De Riek J, Stals I, Reheul D, Haesaert G (2013) alpha-Amylase gene expression during kernel development in relation to pre-harvest sprouting in wheat and triticale. Acta Physiol Plant 35:2927-2938

de Vallavieille-Pope C, Ali S, Leconte M, Enjalbert J, Delos M, Rouzet J (2012) Virulence Dynamics and Regional Structuring of Puccinia striiformis f. sp tritici in France Between 1984 and 2009. Plant Dis 96:131-140

DePauw RM, Knox RE, Singh AK, Fox SL, Humphreys DG, Hucl P (2012) Developing standardized methods for breeding preharvest sprouting resistant wheat, challenges and successes in Canadian wheat. Euphytica 188:7-14

Depauw RM, Mccaig TN, Clarke JM, Mcleod JG, Knox RE, Fernandez MR (1992) Registration of Sprouting-Tolerant White-Kerneled Wheat Germplasms Sc8019r1 and Sc8021v2. Crop Sci 32:838-838

Depauw RM, Mccaig TN, Townleysmith TF (1985) Registration of a Sprouting Resistant White-Seeded Spring Wheat Germplasm Line. Crop Sci 25:577-578

Doebley JF, Gaut BS, Smith BD (2006) The molecular genetics of crop domestication. Cell 127:1309-1321

dos Santos LT, Pinto RJB, Franco FD, Schuster I (2010) Inheritance and potential use of grain color in the identification of genotypes resistant to pre-harvest sprouting in wheat. Crop Breed Appl Biot 10:218-224

Dubcovsky J, Dvorak J (2007) Genome plasticity a key factor in the success of polyploid wheat under domestication. Science 316:1862-1866

Dyck PL (1991) Genetics of Adult-Plant Leaf Rust Resistance in Chinese Spring and Sturdy Wheats. Crop Sci 31:309-311

Ellis JG, Lagudah ES, Spielmeyer W, Dodds PN (2014) The past, present and future of breeding rust resistant wheat. Front Plant Sci 5:641

Eversole K, Feuillet C, Mayer KFX, Rogers J (2014) Slicing the Wheat Genome. Science 345:285-285

Fakthongphan J, Bai GH, St Amand P, Graybosch RA, Baenziger PS (2016) Identification of markers linked to genes for sprouting tolerance (independent of grain color) in hard white winter wheat (HWWW). Theor Appl Genet 129:419-430

Farlin SD, Dahmen JJ, Bell TD (1971) Effect of Sprouting on Nutritional Value of Wheat in Cattle Diets. Can J Anim Sci 51:147-&

Feldman M. 1995. Wheats. In: Smartt J, Simmonds NW, eds. Evolution of crop plants. Harlow, UK: Longman Scientific and Technical, 185–192.

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol 171:501-523

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. Annual

Review of Plant Biology 59:387-415

Flintham J, Adlam R, Bassoi M, Holdsworth M, Gale M (2002) Mapping genes for resistance to sprouting damage in wheat. Euphytica 126:39-45

Flintham JE (2000) Different genetic components control coat-imposed and embryo-imposed dormancy in wheat. Seed Sci Res 10:43-50

Flor, H. H. (1971) Annu. Rev. Phytopathol. 9, 275-296.

Gao F, Jordan MC, Ayele BT (2012) Transcriptional programs regulating seed dormancy and its release by after-ripening in common wheat (Triticum aestivum L.). Plant Biotechnol J 10:465-476

Gao X, Hu CH, Li HZ, Yao YJ, Meng M, Dong J, Zhao WC, Chen QJ, Li XY (2013) Factors Affecting Pre-Harvest Sprouting Resistance in Wheat (Triticum Aestivum L.): A Review. J Anim Plant Sci 23:556-565

Garello G, Le Page-Degivry MT (1999) Evidence for the role of abscisic acid in the genetic and environmental control of dormancy in wheat (Triticum aestivum L.). Seed Sci Res 9:219-226

Gordon IL, Balaam LN, Derera NF (1979) Selection against Sprouting Damage in Wheat .2. Harvest Ripeness, Grain Maturity and Germinability. Aust J Agr Res 30:1-17

Gordon IL, Derera NF, Balaam LN (1977) Selection against Sprouting Damage in Wheat .1. Germination of Unthreshed Grain, with a Standard Wetting Procedure. Aust J Agr Res 28:583-596

Graybosch RA, St Amand P, Bai GH (2013) Evaluation of genetic markers for prediction of preharvest sprouting tolerance in hard white winter wheats. Plant Breeding 132:359-366

Groos C, Gay G, Perretant MR, Gervais L, Bernard M, Dedryver F, Charmet D (2002) Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a whitexred grain bread-wheat cross. Theor Appl Genet 104:39-47

Gubler F, Millar AA, Jacobsen JV (2005) Dormancy release, ABA and pre-harvest sprouting. Current opinion in plant biology 8:183-187

Hilhorst H. (2007). Definitions and hypotheses of seed dormancy. In Annual Plant Reviews Volume 27: Seed Development, Dormancy and Germination, K.J. Bradford, and H. Nonogaki, eds. (Blackwell Publishing), pp. 50–71.

Himi E, Mares DJ, Yanagisawa A, Noda K (2002) Effect of grain colour gene (R) on grain dormancy and sensitivity of the embryo to abscisic acid (ABA) in wheat. J Exp Bot 53:1569-1574

Hovmoller MS, Henriksen KE (2008) Application of pathogen surveys, disease nurseries and varietal resistance characteristics in an IPM approach for the control of wheat yellow rust. Eur J Plant Pathol 121:377-385

Hovmoller MS, Justesen AF (2007) Rates of evolution of avirulence phenotypes and DNA markers in a northwest European population of Puccinia striiformis f. sp tritici. Mol Ecol 16:4637-4647

Hovmoller MS, Walter S, Bayles RA, Hubbard A, Flath K, Sommerfeldt N, Leconte M, Czembor P, Rodriguez-Algaba J, Thach T, Hansen JG, Lassen P, Justesen AF, Ali S, de Vallavieille-Pope C (2016) Replacement of the European wheat yellow rust population by new races from the centre of diversity in the near-Himalayan region. Plant Pathol 65:402-411

Hovmoller MS, Walter S, Justesen AF (2010) Escalating Threat of Wheat Rusts. Science 329:369-369

Hovmoller MS, Yahyaoui AH, Milus EA, Justesen AF (2008) Rapid global spread of two aggressive strains of a wheat rust fungus. Mol Ecol 17:3818-3826

Hughes KR, Griffey CA, Parrish DJ, Barbeau WE, Souza E, Thomason WE (2010) Preharvest Sprouting Tolerance in Current Soft Red Winter Wheat Cultivars. Crop Sci 50:1449-1457

Humphreys DG, DePauw RM, Knox RE, Singh AK, Cuthbert R, Fox SL, Randhawa HS (2012) Weathering and pre-harvest sprouting resistance. Can J Plant Sci 92:599-600

Humphreys DG, Noll J (2002) Methods for characterization of preharvest sprouting resistance in a wheat breeding program. Euphytica 126:61-65

International Wheat Genome Sequencing C (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345:1251788

Jin Y, Szabo LJ, Carson M (2010) Century-Old Mystery of Puccinia striiformis Life History Solved with the Identification of Berberis as an Alternate Host. Phytopathology 100:432-435

Kato K, Nakamura W, Tabiki T, Miura H, Sawada S (2001) Detection of loci controlling seed dormancy on group 4 chromosomes of wheat and comparative mapping with rice and barley genomes. Theor Appl Genet 102:980-985

King RW, Richards RA (1984) Water-Uptake in Relation to Pre-Harvest Sprouting Damage in Wheat - Ear Characteristics. Aust J Agr Res 35:327-336

Knox RE, Clarke FR, Clarke JM, Fox SL, DePauw RM, Singh AK (2012) Enhancing the identification of genetic loci and transgressive segregants for preharvest sprouting resistance in a durum wheat population. Euphytica 186:193-206

Koch JL, Tamas IA, Sorrells ME (1982) The Role of Abscisic-Acid and Gibberellic-Acid in the Control of Preharvest Sprouting of Wheat. Hortscience 17:478-478

Kolmer JA, Jin Y, Hughes ME, Gale SW, AnnulWheatNewsletter Vol.62, 2016 https://wheat.pw.usda.gov/ggpages/awn/62/AWN_VOL_62.pdf.

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A Putative ABC Transporter Confers Durable Resistance to Multiple Fungal Pathogens in Wheat. Science 323:1360-1363

Kumar A, Kumar J, Singh R, Garg T, Chhuneja P, Balyan HS, Gupta PK (2009) QTL analysis for grain colour and pre-harvest sprouting in bread wheat. Plant Sci 177:114-122

Kumar J, Mir RR, Kumar N, Kumar A, Mohan A, Prabhu KV, Balyan HS, Gupta PK (2010) Marker-assisted selection for pre-harvest sprouting tolerance and leaf rust resistance in bread wheat. Plant Breeding 129:617-621

Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B (2009) Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. Theor Appl Genet 119:889-898

Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, Spielmeyer W (2006) Molecular genetic characterization of the Lr34/Yr18 slow rusting resistance gene region in wheat. Theor Appl Genet 114:21-30

Lawson WR, Godwin ID, Cooper M, Brennan PS (1997) Genetic analysis of preharvest sprouting tolerance in three wheat crosses. Aust J Agr Res 48:215-221

Lei L, Zhu XK, Wang SW, Zhu MR, Carver BF, Yan LL (2013) TaMFT-A1 Is Associated with Seed Germination Sensitive to Temperature in Winter Wheat. Plos One 8

Li C, Ni P, Francki M, Hunter A, Zhang Y, Schibeci D, Li H, Tarr A, Wang J, Cakir M, Yu J, Bellgard M, Lance R, Appels R (2004) Genes controlling seed dormancy and pre-harvest sprouting in a rice-wheat-barley comparison. Funct Integr Genomics 4:84-93

Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, Bjornstad A (2008) The adult plant rust resistance loci Lr34/Yr18 and Lr46/Yr29 are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor Appl Genet 116:1155-1166

Lin M, Cai SB, Wang S, Liu SB, Zhang GR, Bai GH (2015) Genotyping-by-sequencing (GBS) identified SNP tightly linked to QTL for pre-harvest sprouting resistance. Theor Appl Genet

128:1385-1395

Lin M, Zhang DD, Liu SB, Zhang GR, Yu JM, Fritz AK, Bai GH (2016) Genome-wide association analysis on pre-harvest sprouting resistance and grain color in US winter wheat. Bmc Genomics 17

Line RF (2002) Stripe rust of wheat and barley in North America: A retrospective historical review. Annual review of phytopathology 40:75-118

Linkies A, Graeber K, Knight C, Leubner-Metzger G (2010) The evolution of seeds. New Phytol 186:817-831

Liu SB, Bai GH, Cai SB, Chen CX (2011) Dissection of genetic components of preharvest sprouting resistance in white wheat. Mol Breeding 27:511-523

Liu SB, Cai SB, Graybosch R, Chen CX, Bai GH (2008) Quantitative trait loci for resistance to pre-harvest sprouting in US hard white winter wheat Rio Blanco. Theor Appl Genet 117:691-699

Liu SB, Sehgal SK, Li JR, Lin M, Trick HN, Yu JM, Gill BS, Bai GH (2013) Cloning and Characterization of a Critical Regulator for Preharvest Sprouting in Wheat. Genetics 195:263-+

Liu SB, Sehgal SK, Lin M, Li JR, Trick HN, Gill BS, Bai GH (2015) Independent mis-splicing mutations in TaPHS1 causing loss of preharvest sprouting (PHS) resistance during wheat domestication. New Phytol 208:928-935

Lohwasser U, Arif MAR, Borner A (2013) Discovery of loci determining pre-harvest sprouting and dormancy in wheat and barley applying segregation and association mapping. Biol Plantarum 57:663-674

Losert D, Maurer HP, Leiser WL, Wurschum T (2016) Defeating the Warrior: genetic architecture of triticale resistance against a novel aggressive yellow rust race. Theor Appl Genet

McIntosh, R.A. 1976. Genetics of wheat and wheat rusts since Farrer: Farrer memorial oration 1976. J. Aust. Inst. Agric. Sci., 42: 203-216.

McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Appels R, Xia XC (2013) Catalogue of gene symbols for wheat. http://wheat.pw.usda.gov/GG2/Triticum/wgc/2013/

Mapes G, Rothwell GW, Haworth MT (1989) Evolution of Seed Dormancy. Nature 337:645-646

Marcussen T, Sandve SR, Heier L, Spannagl M, Pfeifer M, International Wheat Genome Sequencing C, Jakobsen KS, Wulff BB, Steuernagel B, Mayer KF, Olsen OA (2014) Ancient hybridizations among the ancestral genomes of bread wheat. Science 345:1250092

Mares D, Mrva K (2008) Late-maturity alpha-amylase: Low falling number in wheat in the absence of preharvest sprouting. J Cereal Sci 47:6-17

Mares D, Mrva K, Cheong J, Williams K, Watson B, Storlie E, Sutherland M, Zou Y (2005) A QTL located on chromosome 4A associated with dormancy in white- and red-grained wheats of diverse origin. Theor Appl Genet 111:1357-1364

Mares D, Rathjen J, Mrva K, Cheong J (2009) Genetic and environmental control of dormancy in white-grained wheat (Triticum aestivum L.). Euphytica 168:311-318

Mares DJ (1984) Temperature-Dependence of Germinability of Wheat (Triticum-Aestivum L) Grain in Relation to Pre-Harvest Sprouting. Aust J Agr Res 35:115-128

Mares DJ, Mrva K (2001) Mapping quantitative trait loci associated with variation in grain dormancy in Australian wheat. Aust J Agr Res 52:1257-1265

Mares DJ, Mrva K (2014) Wheat grain preharvest sprouting and late maturity alpha-amylase. Planta 240:1167-1178

Martinez SA, Schramm EC, Harris TJ, Kidwell KK, Garland-Campbell K, Steber CM (2014) Registration of Zak ERA8 Soft White Spring Wheat Germplasm with Enhanced Response to ABA and Increased Seed Dormancy. J Plant Regist 8:217-220

Mccaig TN, Depauw RM (1992) Breeding for Preharvest Sprouting Tolerance in White-Seed-Coat Spring Wheat. Crop Sci 32:19-23

McIntosh, R.A. 1976. Genetics of wheat and wheat rusts since Farrer: Farrer memorial oration 1976. J. Aust. Inst. Agric. Sci., 42: 203-216.

McIntosh RA (1992) CLOSE GENETIC-LINKAGE OF GENES CONFERRING ADULT-PLANT RESISTANCE TO LEAF RUST AND STRIPE RUST IN WHEAT. Plant Pathol 41:523-527

McIntosh RA, Brown GN (1997) Anticipatory breeding for resistance to rust diseases in wheat. Annual review of phytopathology 35:311-326

Miao XL, Zhang YJ, Xia XC, He ZH, Zhang Y, Yan J, Chen XM (2013) Mapping quantitative trait loci for pre-harvest sprouting resistance in white-grained winter wheat line CA 0431. Crop Pasture Sci 64:573-579

Miedaner T, Korzun V (2012) Marker-Assisted Selection for Disease Resistance in Wheat and Barley Breeding. Phytopathology 102:560-566

Miura H, Sato N, Kato K, Amano Y (2002) Detection of chromosomes carrying genes for seed dormancy of wheat using the backcross reciprocal monosomic method. Plant Breeding 121:394-

399

Mori M, Uchino N, Chono M, Kato K, Miura H (2005) Mapping QTLs for grain dormancy on wheat chromosome 3A and the group 4 chromosomes, and their combined effect. Theor Appl Genet 110:1315-1323

Morris CF, Paulsen GM (1987) Development of Preharvest Sprouting-Resistant Germplasm from Clarks Cream Hard White Winter-Wheat. Cereal Res Commun 15:229-235

Morris CF, Paulsen GM (1989) Registration of 5 Preharvest Sprouting-Resistant Hard White Winter-Wheat Germplasm Lines. Crop Sci 29:246-247

Nakamura S, Abe F, Kawahigashi H, Nakazono K, Tagiri A, Matsumoto T, Utsugi S, Ogawa T, Handa H, Ishida H, Mori M, Kawaura K, Ogihara Y, Miura H (2011) A Wheat Homolog of MOTHER OF FT AND TFL1 Acts in the Regulation of Germination. Plant Cell 23:3215-3229

Nonogaki H (2014) Seed dormancy and germination-emerging mechanisms and new hypotheses. Front Plant Sci 5:233

Nyachiro JM (2012) Pre-harvest sprouting in cereals PREFACE. Euphytica 188:1-3

Olaerts H, Roye C, Derde LJ, Sinnaeve G, Meza WR, Bodson B, Courtin CM (2016) Impact of Preharvest Sprouting of Wheat (Triticum aestivum) in the Field on Starch, Protein, and Arabinoxylan Properties. J Agr Food Chem 64:8324-8332

Paterson AH, Sorrells ME, Obendorf RL (1989) Methods of Evaluation for Preharvest Sprouting Resistance in Wheat Breeding Programs. Can J Plant Sci 69:681-689

Payannavar CS, Singhal RS, Kulkarni PR (1993) Studies on Bread-Making from Sprouted Wheat. Res Ind 38:138-143

Pellizzaro K, Braccini AL, Vieira ESN, Franco FD, Marchioro V, Schuster I (2016) Molecular Marker Linked to the Rb1 Gene and Association with Pre-Harvest Sprouting Tolerance in Wheat. Biosci J 32:908-914

Petersen G, Seberg O, Yde M, Berthelsen K (2006) Phylogenetic relationships of Triticum and Aegilops and evidence for the origin of the A, B, and D genomes of common wheat (Triticum aestivum). Mol Phylogenet Evol 39:70-82

Pfeifer M, Kugler KG, Sandve SR, Zhan B, Rudi H, Hvidsten TR, International Wheat Genome Sequencing C, Mayer KF, Olsen OA (2014) Genome interplay in the grain transcriptome of hexaploid bread wheat. Science 345:1250091

Rasul G, Humphreys DG, Brule-Babel A, McCartney CA, Knox RE, DePauw RM, Somers DJ (2009)

Mapping QTLs for pre-harvest sprouting traits in the spring wheat cross 'RL4452/AC Domain'. Euphytica 168:363-378

Rasul G, Humphreys GD, Wu JX, Brule-Babel A, Fofana B, Glover KD (2012) Evaluation of preharvest sprouting traits in a collection of spring wheat germplasm using genotype and genotype environment interaction model. Plant Breeding 131:244-251

Rehman AU, Sajjad M, Khan SH, Ahmad N (2013) Prospects of Wheat Breeding for Durable Resistance against Brown, Yellow and Black Rust Fungi. Int J Agric Biol 15:1209-1220

Rosewarne GM, Herrera-Foessel SA, Singh RP, Huerta-Espino J, Lan CX, He ZH (2013) Quantitative trait loci of stripe rust resistance in wheat. Theor Appl Genet 126:2427-2449

Roy JK, Prasad M, Varshney RK, Balyan HS, Blake TK, Dhaliwal HS, Singh H, Edwards KJ, Gupta PK (1999) Identification of a microsatellite on chromosomes 6B and a STS on 7D of bread wheat showing an association with preharvest sprouting tolerance. Theor Appl Genet 99:336-340

Schramm EC, Nelson SK, Kidwell KK, Steber CM (2013) Increased ABA sensitivity results in higher seed dormancy in soft white spring wheat cultivar 'Zak'. Theor Appl Genet 126:791-803

Shewry PR (2009) Wheat. J Exp Bot 60:1537-1553

Shorinola O, Bird N, Simmonds J, Berry S, Henriksson T, Jack P, Werner P, Gerjets T, Scholefield D, Balcarkova B, Valarik M, Holdsworth MJ, Flintham J, Uauy C (2016a) The wheat Phs-A1 pre-harvest sprouting resistance locus delays the rate of seed dormancy loss and maps 0.3 cM distal to the PM19 genes in UK germplasm. Journal of experimental botany

Shorinola O, Bird N, Simmonds J, Berry S, Henriksson T, Jack P, Werner P, Gerjets T, Scholefield D, Balcarkova B, Valarik M, Holdsworth MJ, Flintham J, Uauy C (2016b) The wheat Phs-A1 pre-harvest sprouting resistance locus delays the rate of seed dormancy loss and maps 0.3 cM distal to the PM19 genes in UK germplasm. J Exp Bot 67:4169-4178

Shu K, Meng YJ, Shuai HW, Liu WG, Du JB, Liu J, Yang WY (2015) Dormancy and germination: How does the crop seed decide? Plant biology 17:1104-1112

Simsek S, Ohm JB, Lu H, Rugg M, Berzonsky W, Alamri MS, Mergoum M (2014) Effect of Pre-Harvest Sprouting on Physicochemical Properties of Starch in Wheat. Foods 3:194-207

Singh R, Matus-Cadiz M, Baga M, Hucl P, Chibbar RN (2008a) Comparison of different methods for phenotyping preharvest sprouting in white-grained wheat. Cereal Chem 85:238-242

Singh RP (1992) Genetic Association of Leaf Rust Resistance Gene Lr34 with Adult-Plant Resistance to Stripe Rust in Bread Wheat. Phytopathology 82:835-838

Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R, Herrera-Foessel SA, Ward RW (2008b) Will stem rust destroy the world's wheat crop? In: Sparks DL (ed) Advances in Agronomy, Vol 98, pp 271-309

Singh RP, Rajaram S (1993) Genetics of Adult-Plant Resistance to Stripe Rust in 10 Spring Bread Wheats. Euphytica 72:1-7

Sivasamy M, Aparna M, Kumar J, Jayaprakash P, Vikas VK, John P, Satyaprakash RN, Sivan K, Punniakotti E (2014) Phenotypic and Molecular Confirmation of Durable Adult Plant Leaf Rust Resistance (APR) Genes Lr34+, Lr46+ and Lr67+ Linked to Leaf Tip Necrosis (LTN) in Select Registered Indian Wheat (T. aestivum) Genetic Stocks. Cereal Res Commun 42:262-273

Son S, Chitnis VR, Liu AH, Gao F, Nguyen TN, Ayele BT (2016) Abscisic acid metabolic genes of wheat (Triticum aestivum L.): identification and insights into their functionality in seed dormancy and dehydration tolerance. Planta 244:429-447

Sorensen CK, Thach T, Hovmoller MS (2016) Evaluation of Spray and Point Inoculation Methods for the Phenotyping of Puccinia striiformis on Wheat. Plant Dis 100:1064-1070

Sorrells ME, Paterson AH, Finney PL (1989) Milling and Baking Quality of Soft White Wheat Genotypes Subjected to Preharvest Sprouting. Cereal Chem 66:407-410

Suenaga K, Singh RP, Huerta-Espino J, William HM (2003) Microsatellite markers for genes Lr34/Yr18 and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93:881-890

Sugimoto K, Takeuchi Y, Ebana K, Miyao A, Hirochika H, Hara N, Ishiyama K, Kobayashi M, Ban Y, Hattori T, Yano M (2010) Molecular cloning of Sdr4, a regulator involved in seed dormancy and domestication of rice. P Natl Acad Sci USA 107:5792-5797

Suzuki T, Matsuura T, Kawakami N, Noda K (2000) Accumulation and leakage of abscisic acid during embryo development and seed dormancy in wheat. Plant Growth Regul 30:253-260

Thach T, Ali S, de Vallavieille-Pope C, Justesen AF, Hovmoller MS (2016) Worldwide population structure of the wheat rust fungus Puccinia striiformis in the past. Fungal Genetics and Biology 87:1-8

Torada A, Koike M, Ikeguchi S, Tsutsui I (2008) Mapping of a major locus controlling seed dormancy using backcrossed progenies in wheat (Triticum aestivum L.). Genome 51:426-432

Torada A, Koike M, Ogawa T, Takenouchi Y, Tadamura K, Wu JZ, Matsumoto T, Kawaura K, Ogihara Y (2016) A Causal Gene for Seed Dormancy on Wheat Chromosome 4A Encodes a MAP Kinase Kinase. Curr Biol 26:782-787

Trethowan RM (1995) Evaluation and Selection of Bread Wheat (Triticum-Aestivum L) for Preharvest Sprouting Tolerance. Aust J Agr Res 46:463-474

Trethowan RM, Rajaram S, Ellison FW (1996) Pre-harvest sprouting tolerance of wheat in the field and under rain simulation. Aust J Agr Res 47:705-716

Tuttle KM, Martinez SA, Schramm EC, Takebayashi Y, Seo M, Steber CM (2015) Grain dormancy loss is associated with changes in ABA and GA sensitivity and hormone accumulation in bread wheat, Triticum aestivum (L.). Seed Sci Res 25:179-193

Van Ooijen JW (2006) JoinMap® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen

Walkersimmons M (1987) Aba Levels and Sensitivity in Developing Wheat Embryos of Sprouting Resistant and Susceptible Cultivars. Plant Physiol 84:61-66

Wan AM, Chen XM, Yuen J (2016) Races of Puccinia striiformis f. sp tritici in the United States in 2011 and 2012 and Comparison with Races in 2010. Plant Dis 100:966-975

Wan AM, Zhao ZH, Chen XM, He ZH, Jin SL, Jia QZ, Yao G, Yang JX, Wang BT, Li GB, Bi YQ, Yuan ZY (2004) Wheat stripe rust epidemic and virulence of Puccinia striiformis f. sp tritici in China in 2002. Plant Dis 88:896-904

Wang JR, Liu YX, Wang Y, Chen ZH, Dai S, Cao WG, Fedak G, Lan XJ, Wei YM, Liu DC, Zheng YL (2011) Genetic variation of Vp1 in Sichuan wheat accessions and its association with pre-harvest sprouting response. Genes Genom 33:139-146

Warner RL, Kudrna DA, Spaeth SC, Jones SS (2000) Dormancy in white-grain mutants of Chinese Spring wheat (Triticum aestivum L.). Seed Sci Res 10:51-60

Wellings CR (2011) Global status of stripe rust: a review of historical and current threats. Euphytica 179:129-141

Wellings CR, Wright DG, Keiper F, Loughman R (2003) First detection of wheat stripe rust in Western Australia: evidence for a foreign incursion. Australas Plant Path 32:321-322

Willis CG, Baskin CC, Baskin JM, Auld JR, Venable DL, Cavender-Bares J, Donohue K, Rubio de Casas R, Group NEGW (2014) The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. New Phytol 203:300-309

Xiao SH, Zhang HP, You GX, Zhang XY, Yan CS, Chen X (2012) Integration of marker-assisted selection for resistance to pre-harvest sprouting with selection for grain-filling rate in breeding of white-kernelled wheat for the Chinese environment. Euphytica 188:85-88

Xiao SH, Zhang XY, Yan CS, Lin H (2002) Germplasm improvement for preharvest sprouting resistance in Chinese white-grained wheat: An overview of the current strategy. Euphytica 126:35-38

Zadoks, J.C. & Bouwman, J.J. 1985. Epidemiology in Europe. In A.P. Roelfs & W.R. Bushnell, eds. The cereal rusts, vol. 2, Diseases, distribution, epidemiology, and control, p. 329-369. Orlando, FL, USA, Academic Press.

Zanetti S, Winzeler M, Keller M, Keller B, Messmer M (2000) Genetic analysis of pre-harvest sprouting resistance in a wheat x spelt cross. Crop Sci 40:1406-1417

Zeng SM, Luo Y (2006) Long-distance spread and interregional epidemics of wheat stripe rust in China. Plant Dis 90:980-988

Zeng SM, Luo Y (2008) Systems analysis of wheat stripe rust epidemics in China. Eur J Plant Pathol 121:425-438

Chapter 2 - QTL mapping of pre-harvest sprouting resistance in white wheat cultivar Danby

Abstract

Pre-harvest sprouting (PHS) causes significant losses in grain yield and end-use quality in wheat. Although white wheat is preferred for Asian noodle and steamed bread, it is usually more susceptible to PHS than red wheat. Therefore, use of none grain color-related PHS resistance quantitative trait loci (QTLs) is essential for improvement of PHS resistance in white wheat. To identify PHS resistance QTLs in the white wheat cultivar Danby, and determine their effects, a doubled haploid (DH) population derived from a cross of Danby × Tiger was genotyped using genotyping-by-sequencing (GBS) markers and phenotyped for PHS resistance in two greenhouse and one field experiments. One major QTL was consistently detected on the short arm of chromosome 3A in all three experiments and explained 21.6% to 41.0% of the phenotypic variation. This QTL corresponds to a previously cloned gene, *TaPHS1*. A SNP in the promoter of *TaPHS1* co-segregated with PHS resistance in this population. In addition, two minor QTLs were detected on chromosome arms 3BS and 5AL in two experiments and one on 2AS in one experiment. The two minor QTLs on chromosome arms 3BS and 5AL together with *TaPHS1* showed significant additive effects. The results demonstrated that pyramiding those three QTLs with new, breeder-friendly KASP markers developed and validated in this study could greatly improve PHS resistance in white wheat

Introduction

Pre-harvest sprouting (PHS) in wheat (*Triticum aestivum* L.) occurs when physiologically matured spikes are exposed to a long period of wet field conditions before harvest (Cabral et al. 2014; Li et al. 2004; Mares and Mrva 2014). PHS may cause significant losses of grain yield and quality due to degraded starch and protein in germinated kernels (Flintham 2000; Shorinola et al. 2016). In the United States, PHS caused millions of dollars of losses to wheat growers in Washington State in 2013 alone (Steber et al. 2014). The average annual losses due to PHS are approximately $100 million in Canada, and more than $1 billion worldwide (DePauw et al. 2012).

Compared with red wheat, white wheat usually has higher flour yield and lighter color in its end-use products, making it more attractive for production of Asian noodle, steamed bread and many other white wheat flour based products (Fakthongphan et al. 2016). Although hard red winter wheat predominates wheat production in the U.S. Great Plains, hard white winter wheat production acreage has been increasing since the late 1980s due to strong international market demands (Fakthongphan et al. 2016). However, PHS in white wheat has been a major barrier for expansion of the production area. Breeding cultivars more tolerant to pre-harvest sprouting is an effective method to reduce the losses from sprouted grains in wheat production (Barrero et al. 2015; Gao and Ayele 2014). Therefore, improvement of PHS resistance in white wheat is critical for increasing its production in the U.S. Great Plains to meet the growing international market demands.

Seed dormancy (SD) refers to the temporary resistance to germination for a viable seed under favorable environmental conditions. Lack of adequate SD is the major risk factor for PHS (Li et al. 2004). Besides SD, grain color and wheat spike structure, such as presence of awns, ear nodding angle and glume tenacity, also affect PHS resistance (King and Richards 1984; Mares and Mrva 2014). Red wheat cultivars are typically more resistant than white wheat cultivars (Morris and Paulsen 1992; Groos et al. 2002). The association between PHS resistance and grain color might be due to either tight genetic linkage between genes for PHS resistance and grain color or pleiotropic effects of the grain color genes (Flintham 2000; Lin et al. 2016). Although

red wheat is more resistant to PHS than white wheat, several white wheat cultivars released for the Great Plains are PHS-resistant including “Rio Blanco”, “Trego”, and “Danby.” These cultivars share a similar source of PHS resistance (http://wheatpedigree.net). Other genetic sources of PHS resistance might be available in white wheat and continuously combining those different resistance sources may enhance PHS resistance in new cultivars (Fakthongphan et al. 2016; Graybosch et al. 2013).

PHS can be evaluated in both the field and controlled environments. Field evaluation of PHS resistance depends on the presence of weather conditions conducive for seed sprouting after maturity but, in most environments, cannot be tested reliably due to variable environmental conditions across locations and years (Graybosch et al. 2013; Kato et al. 2001). Therefore, PHS is usually evaluated under controlled environments. Evaluating sprouting rate of whole spikes in a misting chamber is one of the most common methods, but seed germination tests in petri dishes (Clarke et al. 2005) and indirect assays using flour falling number tests (Barnard and Bona 2004; Hareland 2003) are also efficient methods to evaluate PHS. However, all methods of PHS phenotyping are both time consuming and labor intensive. Marker-assisted selection (MAS) is a desirable alternative that can reduce phenotyping cost, improve accuracy and shorten breeding cycles.

Markers tightly linked to PHS resistance QTLs are essential for MAS (Gao et al. 2013; Kulwal et al. 2012; Mares and Mrva 2014). Many PHS resistance QTLs have been reported and are located on almost all wheat chromosomes (Anderson et al. 1993; Flintham et al. 2002; Groos et al. 2002; Kato et al. 2001; Li et al. 2004; Mares and Mrva 2001; Roy et al. 1999; Zanetti et al. 2000). Among them, the QTLs on chromosome arms 3AS and 4AL show major effects on PHS and have been investigated intensively (Albrecht et al. 2015; Bi et al. 2014; Cao et al. 2016; Fakthongphan et al. 2016; Lei et al. 2013; Lin et al. 2015; Liu et al. 2011; Liu et al. 2008; Lohwasser et al. 2013; Miao et al. 2013; Miura et al. 2002; Mori et al. 2005; Rasul et al. 2009; Xiao et al. 2012). Recently, the underlying genes for those two PHS resistance QTLs have been cloned and their causal mutations have been reported (Barrero et al. 2015; Liu et al. 2013; Nakamura et al. 2011; Torada et al. 2016). Two independent studies reported cloning of the QTL on chromosome arm 3AS designated as *TaPHS1* (Liu et al. 2013), and identified three different causal SNPs

(Nakamura et al. 2011, Liu et al. 2013). A SNP in the promoter region was associated with SD of a red wheat that was grown at a low temperature (13°C) (Nakamura et al. 2011), whereas two SNPs in the gene-coding region were reported to be responsible for PHS resistance in a white wheat cultivar, Rio Blanco, grown under normal temperatures (Liu et al. 2013).

Danby has been the most popular white wheat cultivar in the Great Plains for about ten years and it has a high level of PHS resistance. However, the PHS resistance in Danby is not yet well understood. The objectives of this study were to identify PHS resistance QTLs in Danby, determine their effects, and develop user-friendly DNA markers for MAS.

Materials and methods

Plant materials

A population of 211 doubled haploid (DH) lines was developed from a cross between Danby and "Tiger" (PI 661995, Martin et al. 2013), a PHS-susceptible white wheat cultivar. This population and its parents were evaluated for PHS in three environments, two in the greenhouse and one in the field. In addition, an association mapping population of 167 U.S. winter wheat cultivars and elite breeding lines (Lin et al. 2016) was used for marker validation.

PHS phenotyping in field and greenhouse experiments

The DH population and its parents were grown for PHS evaluation in the greenhouse at Kansas State University, Manhattan, KS in 2014 and 2015, and in the field at Hays, KS in 2015. The association mapping population was evaluated for PHS in both greenhouse (2012 and 2013) and field (2013 and 2014) experiments at Manhattan, KS and in field (2013 and 2014) experiments at Hays, KS. In the greenhouse experiments, five plants per line were grown in a 13 x 13 cm plastic pot, and the pots were arranged in a randomized complete block design with two replications. The greenhouse was set at 22°C day / 17°C night with 12 h supplemental light. In the field experiments, all the lines were planted in 90-cm long single-row plots with two replications using a randomized complete block design.

Evaluation of pre-harvest sprouting

To evaluate PHS resistance, five spikes were harvested from each line at their physiological maturity when both the peduncle and spike turned yellow. Harvested spikes were air-dried for 5 d at room temperature and then stored in a freezer at -20°C to maintain their dormancy. After all were harvested, they were air-dried again for 6 d at room temperature, and then incubated in a misting chamber for 7 d (Liu et al. 2008). Visibly germinated and non-germinated kernels in each tested spike were counted and the mean percentage of geminated kernels was calculated for each line for subsequent analysis.

Analysis of variance and heritability

Analysis of variance was conducted by SAS program (SAS 9.4, SAS Institute, 2012) using a general linear model (GLM) $y_{ijkl} = \mu + G_i + E_j + B_{k(j)} + GE_{ij} + e_{ijkl}$, where G_i is the effect of the ith genotype, E_j is the effect of the jth environment, $B_{k(j)}$ is the blocking effect, GE_{ij} is the interaction effect between genotype and environment, and e_{ijkl} is the random error in individual plots (Imtiaz et al. 2008). To account for missing data, type III sum of squares were used. The broad sense heritability across three experiments was estimated using the following equation (Toojinda et al. 1998): $H^2 = V_g / (V_g + V_{ge} / e + V_e / re)$, where V_g is the genotypic variance, V_{ge} is the variance of genotype by environment, V_e is the error variance, *r* is the number of replications, and *e* is the number of environments.

Assays of genotyping-by-sequencing and simple sequence repeat markers

Genomic DNA was isolated from leaf tissues collected at three-leaf stage using a BioSprint 96 DNA Plant Kit (Qiagen, Hilden, Germany) for genotyping-by-sequencing (GBS) and simple sequence repeats (SSR) markers. For GBS, each DNA sample was normalized to 20 ng/µl for library construction following the protocol described by Poland et al. (Poland et al. 2012). Briefly, DNA samples from both DH lines and parents were digested using *PstI* and *MspI* restriction enzymes and ligated to forward and reverse adapters. The ligated DNA samples from the parents and all DH lines were pooled into a single tube for PCR amplification. The PCR products were cleaned up and sequenced using Illumina HiSeq2000 (Illumina, Inc., CA, USA). SNPs were called using a population-based custom Java script and TASSEL (Bradbury et al. 2007). Raw sequence reads were parsed and assigned to samples using barcodes and trimmed to 64 bps in length. To identify SNPs in the DH population, all pairs of tags were evaluated first for one or two base-pair differences. Bi-allelic SNPs were determined by querying the filtered tags for pairs of sequences (Poland et al. 2012) if they differed in only one or two SNPs, were detected in at least 20% genotypes of the population, and could pass a Fisher Exact independence test. SNPs were discarded if 10% or more DH lines were heterozygotes in the population. Only SNPs with less than 20% missing data were used for further map construction.

Thirteen polymorphic SSR markers were randomly selected from a wheat consensus map

(Somers et al. 2004) for validating chromosome locations that were assigned based on GBS-SNPs. A 10 µl PCR mix for a SSR marker contained 20-40 ng DNA, 0.4 mM each of reverse and M13-tailed forward primers, 0.4 mM fluorescence-labeled M13 primer, 0.08 mM of each dNTP, 1.2 µl 10X PCR buffer, 1 mM $MgCl_2$, and 0.6 units of *Taq* polymerase. PCR was performed using a touch-down program (Liu et al. 2008) in a DNA Engine® Peltier Thermal Cycler (Bio-Rad Lab, Hercules, CA, USA). Four different plates of PCR products labeled with one of the four dyes (FAM, VIC, NED and PET) were pooled into one plate using a Biomek NX^P liquid handling system (Beckman Coulter Inc., CA, USA). The pooled PCR products were analyzed in an ABI Prism 3730 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) and their fragment lengths and polymorphisms of amplicons were scored using GeneMarker (SoftGenetics LLC, State College, PA, USA).

Linkage map construction and QTL analysis

A linkage map was constructed with GBS-SNP and SSR markers using JoinMap 4.1 (Van Ooijen, 2006) and the Kosambi mapping function (Kosambi, 1944). A minimum logarithm of odds (LOD) score of 5 and a maximum recombination frequency of 0.35 were set to identify linkage groups. Chromosome names and genetic locations of QTL on the wheat reference genome were assigned by blasting the GBS tags of mapped SNPs to the flow-sorted Chinese Spring survey sequences (Mayer et al. 2014) and the PopSeq sequence data (Chapman et al. 2015) using a web-based blasting tool (http://129.130.90.211/wpdb/gbsloc, Akhunov, et al. 2016). WinQTL Cart 2.5 (Wang et al. 2007) was used for QTL analysis using composite interval mapping function (Silva Lda et al. 2012). Significant QTLs were claimed if the LOD scores were above the threshold that was derived from 1000 permutations (Doerge and Churchill 1996).

To investigate the combined effects of the identified QTLs, all DH lines were grouped based on different allele combinations of the QTLs. The closest marker to each QTL was selected to represent that QTL. The mean sprouting rates of each allelic group were compared using Tukey's multiple comparison (Altman 1991).

KASP marker development and screening

Kompetitive allele specific PCR (KASP) was assayed following manufacturer's instruction (http://www.lgcgroup.com/LGCGroup/media/PDFs/Products/Genotyping/KASP-genotyping-chemistry-User-guide.pdf). A new gene-specific KASP marker was designed based on the reported sequence that harbors the causal SNP at position -222 (SNP-222) in the promoter of *TaPHS1* (Nakamura et al. 2011). Forward and reverse primers were designed using Primer3 web version 4.0.0 (http://primer3plus.com/primer3web/primer3web_input.htm).

For the GBS-SNP markers tightly linked to the QTLs, primers were designed using the web-based primer design pipeline (http://polymarker.tgac.ac.uk/), which was developed specifically to design homologue-specific KASP assays for the polyploidy wheat genome (Ramirez-Gonzalez et al. 2015). The newly-designed KASP primers were then tested for parental polymorphisms and the polymorphic SNPs were genotyped in the mapping population. The KASP-SNP data were used to replace their corresponding GBS-SNPs and the map was re-constructed for QTL validation.

The KASP assay was performed in a 6 µl PCR mix that consisted of 2.9 µl of reaction mix (LGC Genomics, Beverly, MA, USA), 0.1 µl of primer assay mix, and 3 µl of DNA at a concentration of 15 ng/µl. PCR was assayed following manufacturer's instruction (LGC, 2007) using an ABI 7900HT Real-Time PCR System (Life Technology, Grand Island, NY, USA).

Results

Phenotypic analysis

The DH population and its parents were evaluated for PHS resistance in three experiments. Danby displayed a significantly lower ($P < 0.01$) PHS rate (14.3%) than Tiger (64.9%) on average across three experiments (Fig. 2.1). The population had the highest PHS rate in 2014 greenhouse experiment with a mean of 49.1% and a range from 0 to 98.6%, and the lowest PHS rate for the 2015 field experiment with a mean of 26.9% and a range from 0 to 87.8% (Fig. 2.1).

The analysis of variance for PHS rates across three experiments showed highly significant variations ($P < 0.0001$) not only for genotypes but also for environments (Table 2.1). Significant genotype × environment interactions were also detected. The broad sense heritability for PHS resistance was moderately high (0.72) across three experiments.

Genetic linkage map

A total of 5578 and 2580 GBS-SNPs was identified with less than 50% and 20% missing data, respectively. Those GBS-SNPs with less than 20% missing data were combined with 13 SSR markers to construct a linkage map. A total of 1811 GBS-SNPs and all 13 SSR markers were mapped on 31 linkage groups of 1476 cM covering all the 21 chromosomes of common wheat with an average marker interval of 1.2 cM. The average length of linkage groups was 47.6 cM. The marker distribution was uneven across three genomes, with 721 markers (39.5%) on A genome, 648 markers (35.5%) on B genome, and 455 markers (24.9%) on D genome (Fig. 2.2). The A Genome had the highest marker density (1.5 markers/cM), followed by the B genome (1.2 markers/cM) and D genome (0.8 markers/cM). Within each genome, the numbers of markers varied greatly among chromosomes. In the A genome, chromosome 2A and 5A had the most markers (165 markers each), while chromosome 1A had the fewest (16 markers). In the B genome, chromosome 3B was the largest with 163 markers while 1B was the smallest with only nine markers. In the D genome, chromosome 6D was the largest with 194 markers while 4D was the smallest one with only two markers.

QTLs for PHS resistance

Composite interval mapping identified four QTLs for PHS resistance on chromosome arms 2AS (*Qphs.hwwg-2A.1*), 3AS (*Qphs.hwwg-3A.1*), 3BS (*Qphs.hwwg-3B.1*) and 5AL (*Qphs.hwwg-5A.1*) (Fig. 2.3, Table 2.2), and they were all consistently detected in at least two experiments. Three of them, *Qphs.hwwg-3A.1*, *Qphs.hwwg-3B.1* and *Qphs.hwwg-5A.1*, were contributed by the resistant parent Danby, while *Qphs.hwwg-2A.1* showed contradictory allelic effects between two experiments. *Qphs.hwwg-3A.1* was identified in all the three experiments and explained 21.6% to 41.0% of the phenotypic variation (PVE). *Qphs.hwwg-3B.1* explained 4.7% of the phenotypic variation in the 2014 greenhouse and 2015 field experiments. *Qphs.hwwg-5A.1* explained 6.4% and 5.4% of the phenotypic variation in the 2014 and 2015 greenhouse experiments, respectively. *Qphs.hwwg-2A.1* was identified in the 2014 greenhouse and 2015 field experiments with PVE of 4.7% and 15.3%, respectively, however, Danby contributed the resistant allele in the 2014 greenhouse experiment and the susceptible allele in the 2015 field experiment.

Additiveeffects among the QTLs

Three resistance QTLs, *Qphs.hwwg-3A.1* (A), *Qphs.hwwg-3B.1* (B) and *Qphs.hwwg-5A.1* (C) for which Danby contributed resistance alleles, were selected to investigate their combined effects. The DH lines were grouped into eight groups (abc, aBc, abC, aBC, Abc, AbC, ABc, ABC) based on allele combinations of those three QTLs, and the sprouting rates were compared among the groups (Fig. 2.4).

When *Qphs.hwwg-3A.1* was absent, *Qphs.hwwg-3B.1* (aBc) or *Qphs.hwwg-5A.1* (abC) only significantly reduced the sprouting rate ($P < 0.05$) in the 2015 field experiment and the 2014 greenhouse experiment, respectively (Fig. 2.4). However, when those two minor QTLs were combined (aBC), the sprouting rates were significantly lower ($P < 0.05$) than those with neither QTL (abc) in all three experiments, demonstrating a significant additiveeffect of the two minor QTLs on reducing PHS. The two minor QTLs together reduced the sprouting rate from 78.8% (abc) to 51.8% (aBC) in the 2014 greenhouse experiment, from 53.0% to 34.4% in the 2015 greenhouse experiment, and from 45.1% to 28.9% in the 2015 field experiment.

When the major resistance QTL, *Qphs.hwwg-3A.1*, was present, adding either *Qphs.hwwg-3B.1* or *Qphs.hwwg-5A.1* did not significantly ($P > 0.05$) reduce PHS rate (Fig. 2.4). However, when both minor QTLs were present with *Qphs.hwwg-3A.1* (ABC), PHS rates were significantly reduced ($P < 0.05$) compared to the genotypes with only the *Qphs.hwwg-3A.1* resistance allele (Abc) in all three experiments (Fig. 2.4). In the 2014 greenhouse experiment, the sprouting rate of the ABC group (20.3%) was half that for the Abc group (40.1%). Similar trends were observed in the 2015 greenhouse (12.1% vs 23.9%) and 2015 field experiment (5.4% vs 20.7%). Therefore, the addition of the two minor QTLs could also greatly enhance the effect of *Qphs.hwwg-3A.1.*

Development of KASP markers

Qphs.hwwg-3A.1 was mapped to a similar position as *TaPHS1* based on the common linked SSR marker *Xbarc321* (Liu and Bai 2010; Liu et al. 2008; Mori et al. 2005; Nakamura et al. 2011). Two KASP markers, SNP646 and SNP666, in the coding region of *TaPHS1* (Liu et al. 2013) were monomorphic between the two parents in this study. Therefore, a new KASP marker (SNP-222) was developed based on the SNP information in the promoter of *TaPHS1.* This SNP segregated in the DH population (Fig. 2.5) and was mapped under the peak of *Qphs.hwwg-3A.1.* It explained the greatest phenotypic variation among all the markers mapped in the QTL region, indicating *Qphs.hwwg-3A.1* is corresponding to *TaPHS1*. Therefore, SNP-222 in the promoter is most likely to be the causal mutation of *TaPHS1* in the DH population. Further analysis of SNP-222 in a panel of 167 U.S. winter wheat accessions divided the panel into two allelic groups: the resistant group with the Danby allele and the susceptible group with the Tiger allele. The resistant group had a significant ($P < 0.05$) lower sprouting rate (23.5%, N = 36) than the susceptible group (33.3%, N = 131) across six testing environments.

To develop user-friendly DNA markers for *Qphs.hwwg-3B.1* and *Qphs.hwwg-5A.1*, five additional KASP markers were developed using the sequences of flanking GBS-SNPs. All the five KASP markers were re-mapped to the corresponding QTL regions in the mapping population (Fig. 2.3).

Discussion

Using GBS-SNPs, we identified four QTLs for PHS resistance in this study. *Qphs.hwwg-3A.1* showed a major effect on PHS resistance and was identified in all three experiments conducted. This QTL was co-localized with the previously cloned PHS resistance gene, *TaPHS1* (Liu and Bai 2010; Liu et al. 2008; Mori et al. 2005). This was expected since one of Danby's parental lines (Trego) was derived from Rio Blanco that was used by Liu et al. (2013) for cloning *TaPHS1*. Although two independent studies confirmed *TaPHS1* as the causal gene for the 3AS QTL, they reported different causal SNPs for the same gene using different wheat materials (red vs. white wheat) grown under different temperatures (low vs. normal temperatures for seed development) (Liu et al. 2013, Nakamura et al. 2011). Nakamura et al. (2011) identified SNP-222 in the promoter as the causal mutation for SD using the seeds grown under a low temperature (13^{o}C); however, Chono et al. (2015) found that SNP-222 also impacted PHS resistance for the seeds developed under field conditions when 324 Japanese wheat cultivars or breeding lines were analyzed, which agreed with our results. The insignificant effect of SNP-222 on PHS in Liu et al. (2013) might be due to a smaller sample size (83 accessions) that might cause sampling bias. It is possible that different alleles of this gene demonstrated different level of resistance in different germplasms. Therefore, it is very likely that the variations in the promoter are also very critical to modify *TaPHS1* resistance to PHS. Further investigation of whether those causal SNPs impact PHS resistance of *TaPHS1* independently or together may identify effective haplotypes that can be used in wheat breeding to improve PHS resistance.

Our study also found two minor QTLs, *Qphs.hwwg-3B.1* and *Qphs.hwwg-5A.1*, that Liu et al. (2008) did not identify in Rio Blanco. Those two minor QTLs might be inherited from other parental lines in Danby's pedigree. *Qphs.hwwg-3B.1* showed a minor effect, but was repeatedly detected in both greenhouse and field experiments. Several previous studies have reported QTLs associated with PHS or SD on chromosome 3B, but they are all located on the long arm near the red grain color gene (*R-B1*) or *Viviparous 1* (Cabral et al. 2014; Chang et al. 2010; Fofana et al. 2009; Groos et al. 2002; Lin et al. 2016; Mares et al. 2009; Somers et al. 2004; Yang et al. 2007a; Yang et al. 2007b). In our study, *Qphs.hwwg-3B.1* was located in a region between 55.8 cM and 61.3 cM on 3BS of the Chinese Spring reference map by blasting the GBS tags of

flanking SNPs to the flow-sorted Chinese Spring survey sequences (Mayer et al. 2014). This QTL region on 3BS is different from all previously reported QTLs. Therefore, *Qphs.hwwg-3B.1* is more likely a novel PHS resistance QTL that does not relate to seed color, which may be valuable for pyramiding with other QTLs to improve PHS resistance in white wheat.

Qphs.hwwg-5A.1 was another minor QTL that was consistently detected in the two greenhouse experiments. The peak of this QTL was 8.8 cM away from the distal end of the long arm of chromosome 5A on the Chinese Spring reference map based on the blasting result against the flow-sorted Chinese Spring survey sequences (Mayer et al. 2014). Several PHS-related QTLs were reported on chromosome 5A, but none of them were near the distal end of 5AL as they were either near the centromere (Iehisa et al. 2014; Nakamura et al. 2010) or on the short arm (Groos et al. 2002). In a cross between common wheat and spelt, Zanetti et al. (2000) found a QTL for alpha-amylase activity on chromosome arm 5AL at the *q* locus, which is also far from *Qphs.hwwg-5A.1*. Genome-wide association studies identified two significant PHS-related QTLs on 5AL, but one near the centromere (Zhu et al. 2016) and the other was more than 20 cM away from *Qphs.hwwg-5A.1* (Lin et al. 2016). Therefore, *Qphs.hwwg-5A.*1 is also likely a novel QTL for PHS resistance.

Although, individually, these QTL showed a minor effect on PHS resistance, a combination of *Qphs.hwwg-5A.1* and *Qphs.hwwg-3B.1* significantly reduced the PHS with or without *Qphs.hwwg-3A.1.* These two minor QTLs, together with *Qphs.hwwg-3A.1,* reduced PHS sprouting by 50% or more in comparison to the genotype with *Qphs.hwwg-3A.1* alone in all the three experiments. This significantly enhanced PHS resistance from the combination of the three QTLs suggests a valuable additive effect among these QTLs. Gene interactions at two or more loci are critical in advanced quantitative genetic models, and assembly of favorable QTL or gene combinations is very important not only for crop breeding but also for understanding the genetic basis underlying crop adaptation and evolution (Allard 1996). QTL interactions have been reported for PHS resistance or SD in many crops including rice (Gu et al. 2004; Guo et al. 2004; Wang et al. 2014), barley (Hickey et al. 2012), and wheat (Imtiaz et al. 2008; Kumar et al. 2009; Liu et al. 2011; Mohan et al. 2009). However, QTL interactions among chromosomes 3A, 3B and 5A have not been documented previously for wheat PHS resistance. Our study is the first

to reveal the significant interactions among these three QTLs. The additive effect detected in this study strongly suggests the genetically complex networks in wheat PHS regulation and the importance in pyramiding a specific combination of QTLs or genes in breeding.

Qphs.hwwg-2A.1 was detected in two environments but with contradicting allelic effects, in which Danby contributed the resistant allele in the greenhouse experiment but susceptible allele in the field experiment. Severe stripe rust infection in the 2015 field experiment was most likely responsible for the allelic effect shift. Using the stripe rust data from the same population, a major resistance QTL contributed by Danby was mapped at the same location as *Qphs.hwwg-2A.1* in the distal end of chromosome 2AS (unpublished data). We noticed that plants infected by stripe rust produced shriveled seeds, which might affect germination during the PHS test. Therefore, the PHS resistance allele on 2A contributed by Tiger in 2015 field experiment was most likely due to the rust susceptible allele in Tiger. It is also possible that the correlation between stripe rust susceptibility and PHS resistance is due to plant hormones triggered by rust infection that could suppress seed germination. Plant hormones such as abscisic acid (ABA) and gibberellic acid (GA), the most important regulator of seed dormancy, might be involved in the responses to many biotic stresses including disease and insect (de Zelicourt et al. 2016; Lee and Luan 2012; Pieterse et al. 2012; Skubacz et al. 2016; Verslues and Zhu 2005). Therefore, the effect of *Qphs.hwwg-2A.1* on PHS resistance is unclear and needs to be further explored in experiments without confounding effects from stripe rust infection.

Conclusion

Our study identified four PHS resistance QTLs on chromosome arms 2AS, 3AS, 3BS and 5AL in a white wheat DH population by evaluating the population in both greenhouse and field experiments. We observed that a SNP in the promoter region of *TaPHS1* likely responsible for the greater variation in PHS in this mapping population. We developed a new KASP marker for this SNP for MAS. This KASP-SNP marker was validated in an association panel. Two minor QTLs on 3BS and 5AL appear to be novel QTLs for PHS resistance, and showed additive effects when they were present together with or without *Qphs.hwwg-3A.1*. The results indicate that pyramiding these three QTLs can greatly reduce PHS. Five breeder-friendly KASP markers were developed for the two minor QTLs and validated in this study. Those KASP markers could be a valuable breeder tool for genetic improvement of PHS resistance in white wheat.

References:

Albrecht T, Oberforster M, Kempf H, Ramgraber L, Schacht J, Kazman E, Zechner E, Neumayer A, Hartl L, Mohler V (2015) Genome-wide association mapping of preharvest sprouting resistance in a diversity panel of European winter wheats. J Appl Genet 56:277-285

Allard RW (1996) Genetic basis of the evolution of adaptedness in plants. Euphytica 92:1-11

Anderson JA, Sorrells ME, Tanksley SD (1993) Rflp Analysis of Genomic Regions Associated with Resistance to Preharvest Sprouting in Wheat. Crop Sci 33:453-459

Barnard A, Bona L (2004) Sprout damage and falling number in South African and Hungarian wheats. Cereal Res Commun 32:259-264

Barrero JM, Cavanagh C, Verbyla KL, Tibbits JFG, Verbyla AP, Huang BE, Rosewarne GM, Stephen S, Wang PH, Whan A, Rigault P, Hayden MJ, Gubler F (2015) Transcriptomic analysis of wheat near-isogenic lines identifies PM19-A1 and A2 as candidates for a major dormancy QTL. Genome Biol 16

Bi HH, Sun YW, Xiao YG, Xia LQ (2014) Characterization of DFR allelic variations and their associations with pre-harvest sprouting resistance in a set of red-grained Chinese wheat germplasm. Euphytica 195:197-207

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633-2635

Cabral AL, Jordan MC, McCartney CA, You FM, Humphreys DG, MacLachlan R, Pozniak CJ (2014) Identification of candidate genes, regions and markers for pre-harvest sprouting resistance in wheat (Triticum aestivum L.). Bmc Plant Biol 14

Cao LZ, Hayashi K, Tokui M, Mori M, Miura H, Onishi K (2016) Detection of QTLs for traits associated with pre-harvest sprouting resistance in bread wheat (Triticum aestivum L.). Breeding science 662:260-270

Chang C, Zhang HP, Feng JM, Yin B, Si HQ, Ma CX (2010) Identifying alleles of Viviparous-1B associated with pre-harvest sprouting in micro-core collections of Chinese wheat germplasm. Mol Breeding 25:481-490

Chapman JA, Mascher M, Buluc A, Barry K, Georganas E, Session A, Strnadova V, Jenkins J, Sehgal S, Oliker L, Schmutz J, Yelick KA, Scholz U, Waugh R, Poland JA, Muehlbauer GJ, Stein N, Rokhsar DS (2015) A whole-genome shotgun approach for assembling and anchoring the hexaploid bread wheat genome. Genome Biol 16

Clarke FR, Knox RE, DePauw RM (2005) Expression of dormancy in a spring wheat cross grown in field and controlled environment conditions. Euphytica 143:297-300

de Zelicourt A, Colcombet J, Hirt H (2016) The Role of MAPK Modules and ABA during Abiotic Stress Signaling. Trends Plant Sci 21:677-685

DePauw RM, Knox RE, Singh AK, Fox SL, Humphreys DG, Hucl P (2012) Developing standardized methods for breeding preharvest sprouting resistant wheat, challenges and successes in Canadian wheat. Euphytica 188:7-14

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285-294

Fakthongphan J, Bai GH, St Amand P, Graybosch RA, Baenziger PS (2016) Identification of markers linked to genes for sprouting tolerance (independent of grain color) in hard white winter wheat (HWWW). Theor Appl Genet 129:419-430

Flintham J, Adlam R, Bassoi M, Holdsworth M, Gale M (2002) Mapping genes for resistance to sprouting damage in wheat. Euphytica 126:39-45

Flintham JE (2000) Different genetic components control coat-imposed and embryo-imposed dormancy in wheat. Seed Sci Res 10:43-50

Fofana B, Humphreys DG, Rasul G, Cloutier S, Brule-Babel A, Woods S, Lukow OM, Somers DJ (2009) Mapping quantitative trait loci controlling pre-harvest sprouting resistance in a red x white seeded spring wheat cross. Euphytica 165:509-521

Gao F, Ayele BT (2014) Functional genomics of seed dormancy in wheat: advances and prospects. Front Plant Sci 5:458

Gao X, Hu CH, Li HZ, Yao YJ, Meng M, Dong J, Zhao WC, Chen QJ, Li XY (2013) Factors Affecting Pre-Harvest Sprouting Resistance in Wheat (Triticum Aestivum L.): A Review. J Anim Plant Sci 23:556-565

Graybosch RA, St Amand P, Bai GH (2013) Evaluation of genetic markers for prediction of preharvest sprouting tolerance in hard white winter wheats. Plant Breeding 132:359-366

Groos C, Gay G, Perretant MR, Gervais L, Bernard M, Dedryver F, Charmet D (2002) Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a whitexred grain bread-wheat cross. Theor Appl Genet 104:39-47

Gu XY, Kianian SF, Foley ME (2004) Multiple loci and epistases control genetic variation for seed dormancy in weedy rice (Oryza sativa). Genetics 166:1503-1516

Guo LB, Zhu LH, Xu YB, Zeng DL, Wu P, Qian Q (2004) QTL analysis of seed dormancy in rice (Oryza sativa L.). Euphytica 140:155-162

Hareland GA (2003) Effects of pearling on falling number and alpha-amylase activity of preharvest sprouted spring wheat. Cereal Chem 80:232-237

Hickey LT, Lawson W, Arief VN, Fox G, Franckowiak J, Dieters MJ (2012) Grain dormancy QTL identified in a doubled haploid barley population derived from two non-dormant parents. Euphytica 188:113-122

Imtiaz M, Ogbonnaya FC, Oman J, van Ginkel M (2008) Characterization of quantitative trait loci controlling genetic variation for preharvest sprouting in synthetic backcross-derived wheat lines. Genetics 178:1725-1736

Kato K, Nakamura W, Tabiki T, Miura H, Sawada S (2001) Detection of loci controlling seed dormancy on group 4 chromosomes of wheat and comparative mapping with rice and barley genomes. Theor Appl Genet 102:980-985

King RW, Richards RA (1984) Water-Uptake in Relation to Pre-Harvest Sprouting Damage in Wheat - Ear Characteristics. Aust J Agr Res 35:327-336

Kulwal P, Ishikawa G, Benscher D, Feng ZY, Yu LX, Jadhav A, Mehetre S, Sorrells ME (2012) Association mapping for pre-harvest sprouting resistance in white winter wheat. Theor Appl Genet 125:793-805

Kumar A, Kumar J, Singh R, Garg T, Chhuneja P, Balyan HS, Gupta PK (2009) QTL analysis for grain colour and pre-harvest sprouting in bread wheat. Plant Sci 177:114-122

Lee SC, Luan S (2012) ABA signal transduction at the crossroad of biotic and abiotic stress responses. Plant Cell Environ 35:53-60

Lei L, Zhu XK, Wang SW, Zhu MR, Carver BF, Yan LL (2013) TaMFT-A1 Is Associated with Seed Germination Sensitive to Temperature in Winter Wheat. Plos One 8

Li C, Ni P, Francki M, Hunter A, Zhang Y, Schibeci D, Li H, Tarr A, Wang J, Cakir M, Yu J, Bellgard M, Lance R, Appels R (2004) Genes controlling seed dormancy and pre-harvest sprouting in a rice-wheat-barley comparison. Funct Integr Genomics 4:84-93

Lin M, Cai SB, Wang S, Liu SB, Zhang GR, Bai GH (2015) Genotyping-by-sequencing (GBS) identified SNP tightly linked to QTL for pre-harvest sprouting resistance. Theor Appl Genet 128:1385-1395

Lin M, Zhang DD, Liu SB, Zhang GR, Yu JM, Fritz AK, Bai GH (2016) Genome-wide association analysis on pre-harvest sprouting resistance and grain color in US winter wheat. Bmc Genomics

17

Liu SB, Bai GH (2010) Dissection and fine mapping of a major QTL for preharvest sprouting resistance in white wheat Rio Blanco. Theor Appl Genet 121:1395-1404

Liu SB, Bai GH, Cai SB, Chen CX (2011) Dissection of genetic components of preharvest sprouting resistance in white wheat. Mol Breeding 27:511-523

Liu SB, Cai SB, Graybosch R, Chen CX, Bai GH (2008) Quantitative trait loci for resistance to pre-harvest sprouting in US hard white winter wheat Rio Blanco. Theor Appl Genet 117:691-699

Lohwasser U, Arif MAR, Borner A (2013) Discovery of loci determining pre-harvest sprouting and dormancy in wheat and barley applying segregation and association mapping. Biol Plantarum 57:663-674

Mares D, Rathjen J, Mrva K, Cheong J (2009) Genetic and environmental control of dormancy in white-grained wheat (Triticum aestivum L.). Euphytica 168:311-318

Mares DJ, Mrva K (2001) Mapping quantitative trait loci associated with variation in grain dormancy in Australian wheat. Aust J Agr Res 52:1257-1265

Mares DJ, Mrva K (2014) Wheat grain preharvest sprouting and late maturity alpha-amylase. Planta 240:1167-1178

Mayer KFX, Rogers J, Dolezel J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski AJ, Sourdille P, Endo TR, Dolezel J, Kubalakova M, Cihalikova J, Dubska Z, Vrana J, Sperkova R, Simkova H, Rogers J, Febrer M, Clissold L, McLay K, Singh K, Chhuneja P, Singh NK, Khurana J, Akhunov E, Choulet F, Sourdille P, Feuillet C, Alberti A, Barbe V, Wincker P, Kanamori H, Kobayashi F, Itoh T, Matsumoto T, Sakai H, Tanaka T, Wu JZ, Ogihara Y, Handa H, Pozniak C, Maclachlan PR, Sharpe A, Klassen D, Edwards D, Batley J, Olsen OA, Sandve SR, Lien S, Steuernagel B, Wulff B, Caccamo M, Ayling S, Ramirez-Gonzalez RH, Clavijo BJ, Steuernagel B, Wright J, Pfeifer M, Spannagl M, Mayer KFX, Martis MM, Akhunov E, Choulet F, Mayer KFX, Mascher M, Chapman J, Poland JA, Scholz U, Barry K, Waugh R, Rokhsar DS, Muehlbauer GJ, Stein N, Gundlach H, Zytnicki M, Jamilloux V, Quesneville H, Wicker T, Mayer KFX, Faccioli P, Colaiacovo M, Pfeifer M, Stanca AM, Budak H, Cattivelli L, Glover N, Martis MM, Choulet F, Feuillet C, Mayer KFX, Pfeifer M, Pingault L, Mayer KFX, Paux E, Spannagl M, Sharma S, Mayer KFX, Pozniak C, Appels R, Bellgard M, Chapman B, Pfeifer M, Pfeifer M, Sandve SR, Nussbaumer T, Bader KC, Choulet F, Feuillet C, Mayer KFX, Akhunov E, Paux E, Rimbert H, Wang SC, Poland JA, Knox R, Kilian A, Pozniak C, Alaux M, Alfama F, Couderc L, Jamilloux V, Guilhot N, Viseux C, Loaec M, Quesneville H, Rogers J, Dolezel J, Eversole K, Feuillet C, Keller B, Mayer KFX, Olsen OA, Praud S, Iwgsc (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345

Miao XL, Zhang YJ, Xia XC, He ZH, Zhang Y, Yan J, Chen XM (2013) Mapping quantitative trait loci

for pre-harvest sprouting resistance in white-grained winter wheat line CA 0431. Crop Pasture Sci 64:573-579

Miura H, Sato N, Kato K, Amano Y (2002) Detection of chromosomes carrying genes for seed dormancy of wheat using the backcross reciprocal monosomic method. Plant Breeding 121:394-399

Mohan A, Kulwal P, Singh R, Kumar V, Mir RR, Kumar J, Prasad M, Balyan HS, Gupta PK (2009) Genome-wide QTL analysis for pre-harvest sprouting tolerance in bread wheat. Euphytica 168:319-329

Mori M, Uchino N, Chono M, Kato K, Miura H (2005) Mapping QTLs for grain dormancy on wheat chromosome 3A and the group 4 chromosomes, and their combined effect. Theor Appl Genet 110:1315-1323

Nakamura S, Abe F, Kawahigashi H, Nakazono K, Tagiri A, Matsumoto T, Utsugi S, Ogawa T, Handa H, Ishida H, Mori M, Kawaura K, Ogihara Y, Miura H (2011) A Wheat Homolog of MOTHER OF FT AND TFL1 Acts in the Regulation of Germination. Plant Cell 23:3215-3229

Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM (2012) Hormonal Modulation of Plant Immunity. Annu Rev Cell Dev Bi 28:489-521

Poland JA, Brown PJ, Sorrells ME, Jannink JL (2012) Development of High-Density Genetic Maps for Barley and Wheat Using a Novel Two-Enzyme Genotyping-by-Sequencing Approach. Plos One 7

Ramirez-Gonzalez RH, Uauy C, Caccamo M (2015) PolyMarker: A fast polyploid primer design pipeline. Bioinformatics 31:2038-2039

Rasul G, Humphreys DG, Brule-Babel A, McCartney CA, Knox RE, DePauw RM, Somers DJ (2009) Mapping QTLs for pre-harvest sprouting traits in the spring wheat cross 'RL4452/AC Domain'. Euphytica 168:363-378

Roy JK, Prasad M, Varshney RK, Balyan HS, Blake TK, Dhaliwal HS, Singh H, Edwards KJ, Gupta PK (1999) Identification of a microsatellite on chromosomes 6B and a STS on 7D of bread wheat showing an association with preharvest sprouting tolerance. Theor Appl Genet 99:336-340

Shorinola O, Bird N, Simmonds J, Berry S, Henriksson T, Jack P, Werner P, Gerjets T, Scholefield D, Balcarkova B, Valarik M, Holdsworth MJ, Flintham J, Uauy C (2016) The wheat Phs-A1 pre-harvest sprouting resistance locus delays the rate of seed dormancy loss and maps 0.3 cM distal to the PM19 genes in UK germplasm. Journal of experimental botany

Silva Lda C, Wang S, Zeng ZB (2012) Composite interval mapping and multiple interval mapping: procedures and guidelines for using Windows QTL Cartographer. Methods in molecular biology

871:75-119

Skubacz A, Daszkowska-Golec A, Szarejko L (2016) The Role and Regulation of ABI5 (ABA-Insensitive 5) in Plant Development, Abiotic Stress Responses and Phytohormone Crosstalk. Front Plant Sci 7

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (Triticum aestivum L.). Theor Appl Genet 109:1105-1114

Steber CM, Carter AH, and Pumphrey MO (2014) Reduce Falling Numbers Risk. http://wagrains.org/on-going-falling-numbers-reseach. Accessed 28 June 2017

Toojinda T, Baird E, Booth A, Broers L, Hayes P, Powell W, Thomas W, Vivar H, Young G (1998) Introgression of quantitative trait loci (QTLs) determining stripe rust resistance in barley: an example of marker-assisted line development. Theor Appl Genet 96:123-131

Verslues PE, Zhu JK (2005) Before and beyond ABA: upstream sensing and internal signals that determine ABA accumulation and response under abiotic stress. Biochem Soc T 33:375-379

Wang L, Cheng JP, Lai YY, Du WL, Huang X, Wang ZF, Zhang HS (2014) Identification of QTLs with additive, epistatic and QTL x development interaction effects for seed dormancy in rice. Planta 239:411-420

Xiao SH, Zhang HP, You GX, Zhang XY, Yan CS, Chen X (2012) Integration of marker-assisted selection for resistance to pre-harvest sprouting with selection for grain-filling rate in breeding of white-kernelled wheat for the Chinese environment. Euphytica 188:85-88

Yang Y, Ma YZ, Xu ZS, Chen XM, He ZH, Yu Z, Wilkinson M, Jones HD, Shewry PR, Xia LQ (2007a) Isolation and characterization of Viviparous-1 genes in wheat cultivars with distinct ABA sensitivity and pre-harvest sprouting tolerance. J Exp Bot 58:2863-2871

Yang Y, Zhao XL, Xia LQ, Chen XM, Xia XC, Yu Z, He ZH, Roeder M (2007b) Development and validation of a Viviparous-1 STS marker for pre-harvest sprouting tolerance in Chinese wheats. Theor Appl Genet 115:971-980

Zanetti S, Winzeler M, Keller M, Keller B, Messmer M (2000) Genetic analysis of pre-harvest sprouting resistance in a wheat x spelt cross. Crop Sci 40:1406-1417

Zhu YL, Wang SX, Zhang HP, Zhao LX, Wu ZY, Jiang H, Cao JJ, Liu K, Qin M, Lu J, Sun GL, Xia XC, Chang C, Ma CX (2016) Identification of major loci for seed dormancy at different post-ripening stages after harvest and validation of a novel locus on chromosome 2AL in common wheat. Mol Breeding 36

Table 2.1 Variance analysis and its expected mean square of pre-harvest sprouting for the DH population evaluated in two greenhouse experiments and one field experiment

Source	DF	Type III SS	Mean square	Expected mean square	F Value	Pr > F
Environments (E)	2	106961.6	53480.8		173.6	< 0.0001
Genotypes (G)	191	370284.1	1928.6	$V_e+rV_{ge}+reV_g$	6.3	< 0.0001
G*E	382	203781.7	533.5	V_e+rV_{ge}	1.7	< 0.0001
Error	573	158347.9	308.1	V_e		

V_e is the variance due to error; V_{ge} is the variance due to interaction between genotype and environment; V_g is the variance due to genotype; *r* is number of replications and *e* is the number of environments.

Table 2.2 Quantitative trait loci for pre-harvest sprouting resistance detected in 2014 (2014GH) and 2015 (2015GH) greenhouse experiments, and 2015 field experiment (2015FLD)

Experiment	Chromosome arm	QTL peak location (cM [a])	LOD [b]	PVE [c] (%)	Additive effect
2014GH	3AS	3.8	31.7	41.0	-16.7
	3BS	18.5	4.0	4.7	-5.7
	5AL	10.1	5.9	6.4	-6.4
2015GH	2AS	115.5	3.62	4.7	-5.1
	3AS	3.8	20.2	35.8	-13.8
	5AL	15.6	3.4	5.4	-5.3
2015FLD	2AS	115.5	10.3	15.3	8.9
	3AS	1.6	16.7	21.6	-10.8
	3BS	22.8	4.6	4.7	-4.8

[a] CentiMorgans

[b] Logarithm of odds

[c] Phenotypic variation explained by QTL

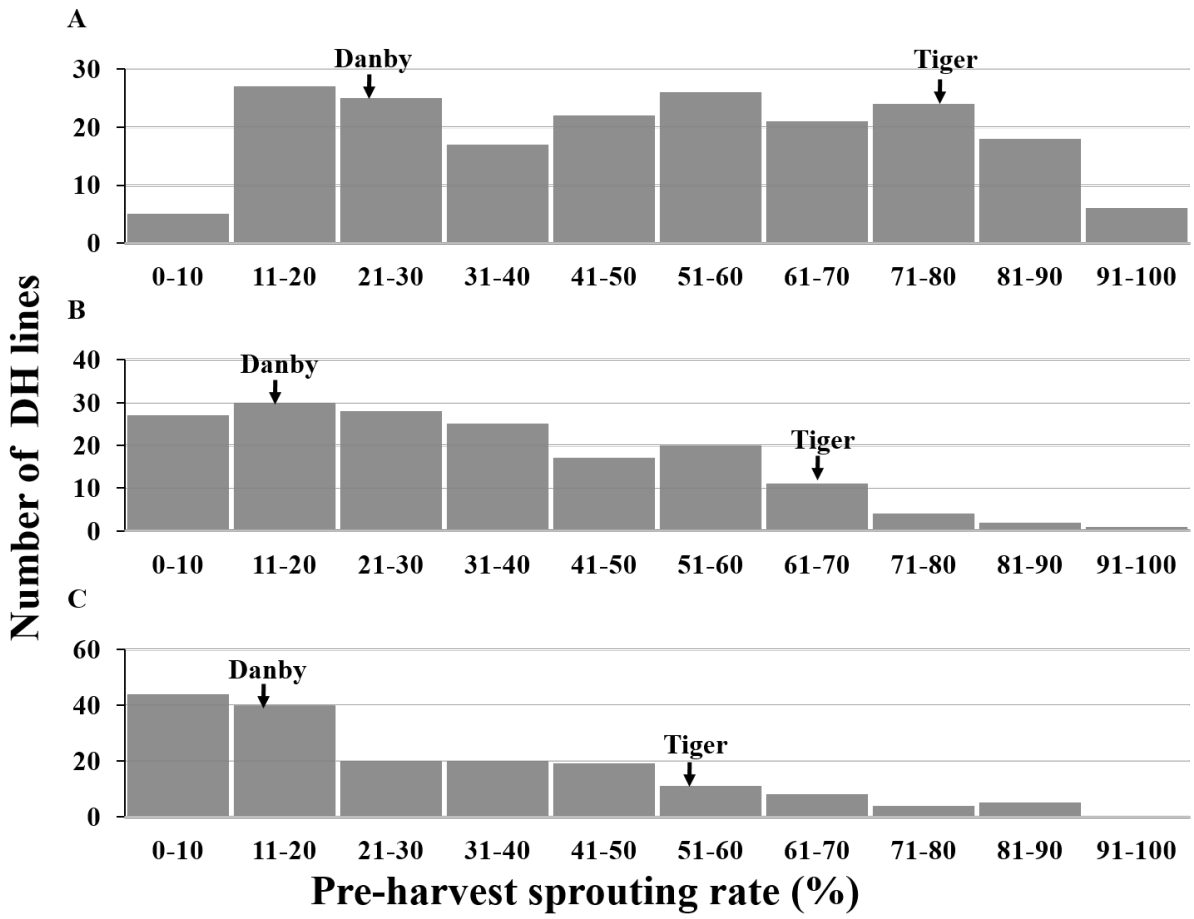

Figure 2.1 Frequency distributions of pre-harvest sprouting (PHS) rates in the DH population.

The PHS rates are the mean values of the two replications in each experiment. Black arrows point to the PHS rates of the parental lines. (A) PHS rate from 2014 greenhouse experiment. (B) PHS rate from 2015 greenhouse experiment. (C) PHS rate from 2015 field experiment.

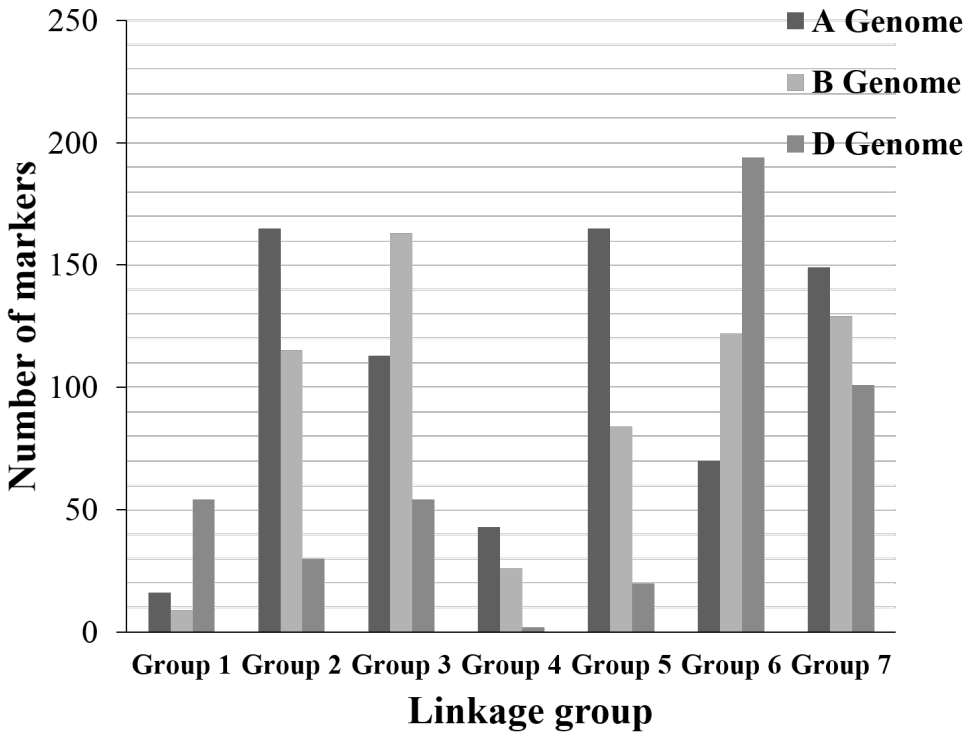

Figure 2.2 Distribution of GBS markers among wheat linkage groups.

A total of 1811 GBS-SNPs and 13 SSR markers were mapped across all the 21 wheat linkage groups. The marker distribution was uneven across three genomes with 721 markers on A genome, 648 markers on B genome, and 455 markers on D genome.

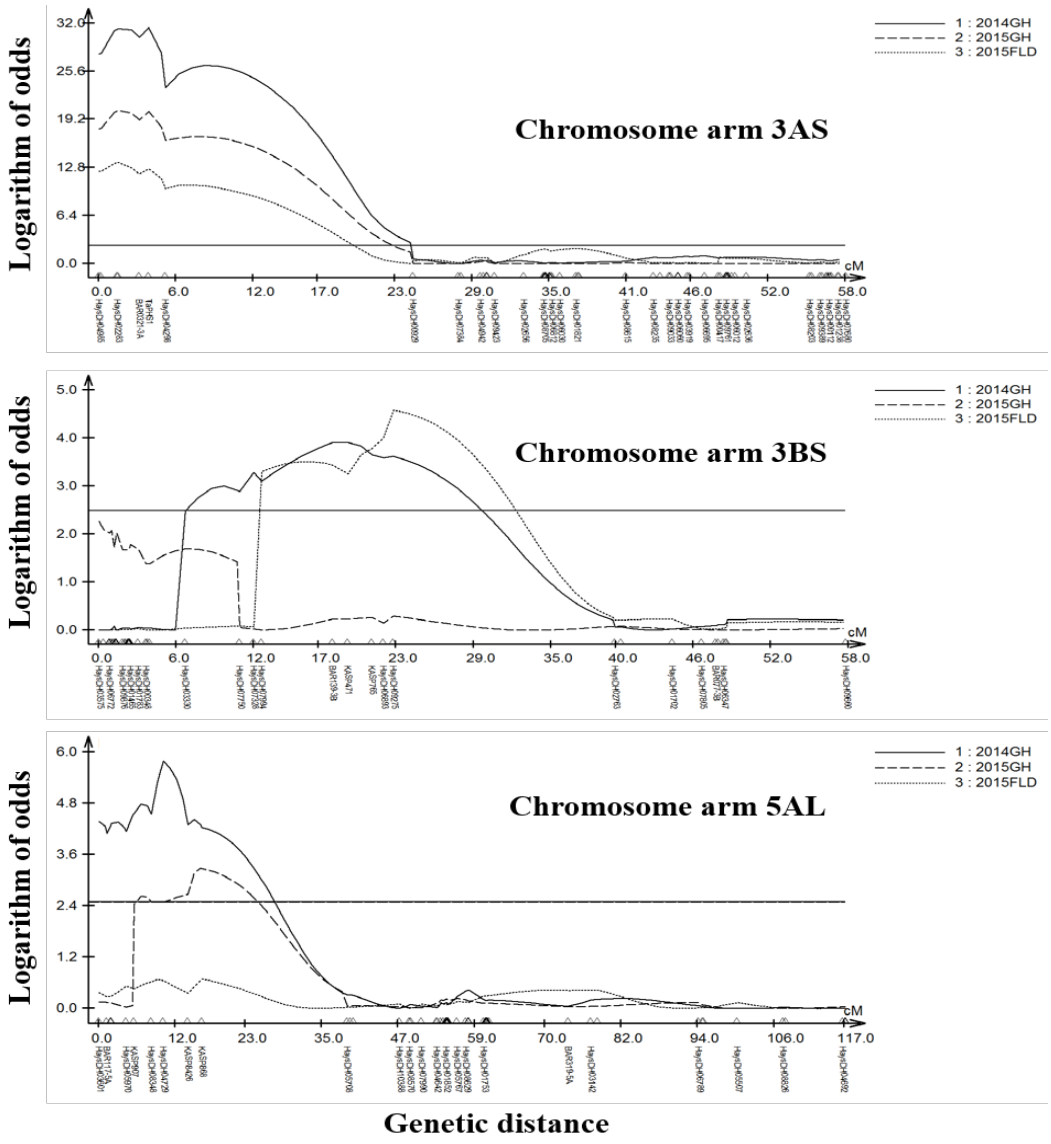

Figure 2.3 Composite interval mapping of a major QTL on chromosome arm 3AS, and two minor QTLs on chromosome arms 3BS and 5AL for pre-harvest sprouting resistance in 2014 greenhouse (2014GH), 2015 greenhouse (2015GH) and 2015 field (2015FLD) experiments.

Line parallel to the X-axis is the threshold for claiming significant QTLs with a logarithm of odds at 2.5. Genetic distances of molecular markers are shown in centiMorgans (cM) along the X-axis.

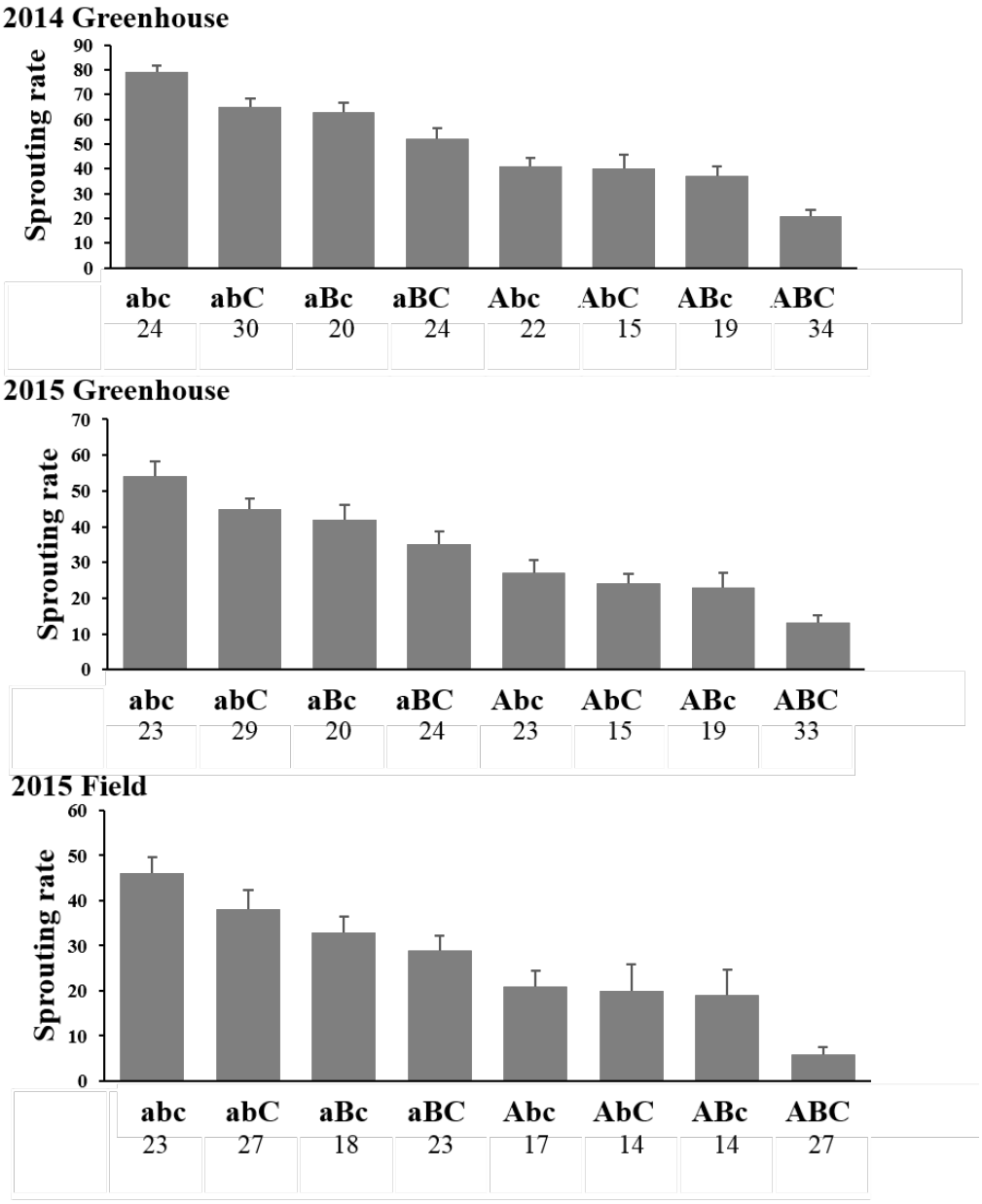

Figure 2.4 Comparisons of pre-harvest sprouting rates (%) among different QTL combinations.

Letter combinations abc, abC, aBc, aBC, Abc, AbC, ABc, ABC were designated for eight possible allele combinations of the three consistent QTLs, *Qphs.hwwg-3A.1.* (A), *Qphs.hwwg-3B.1* (B) and *Qphs.hwwg-5A.1* (C). On the x-axis, capital letters, A, B and C, were designated as resistance alleles, and a, b and c were susceptibility alleles for the three QTLs, respectively. The number below the letters is the sample size for each genotype. Error bar is the standard error of the group mean.

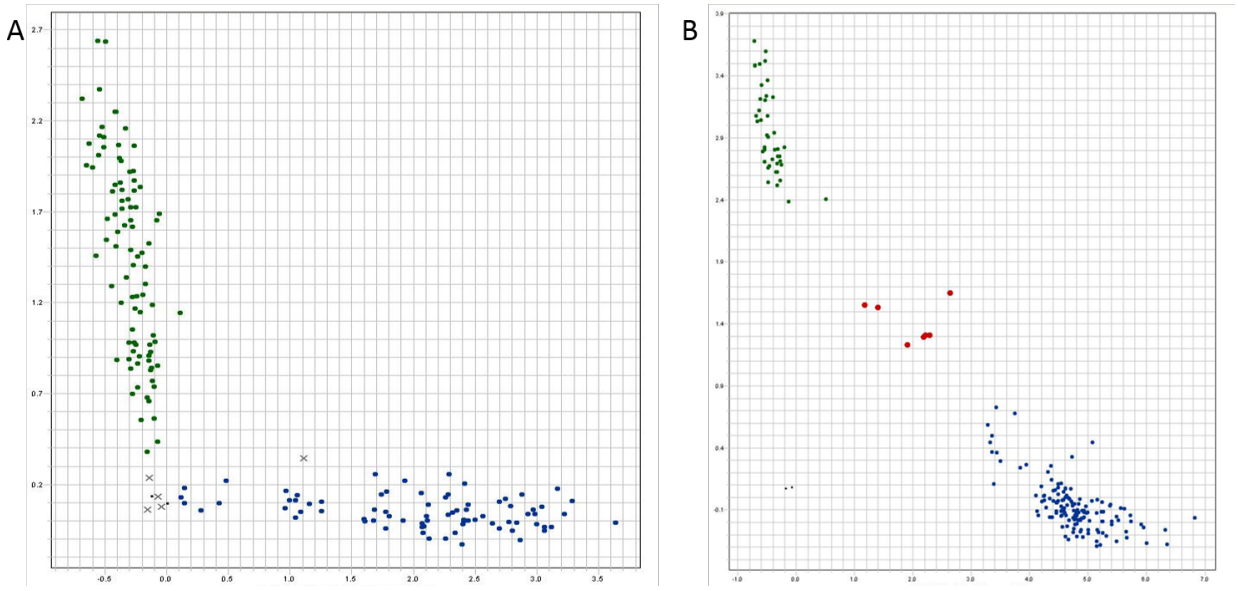

Figure 2.5 KASP assays of the causal single nucleotide polymorphism (SNP) at the position of 222 bp in the promoter of *TaPHS1* in the double haploid mapping population.

(A) and an association panel of 167 wheat accessions (B). Green dots are the resistant genotypes with T nucleotide. Blue dots are the susceptible genotypes with C nucleotide. Red dots represent heterozygous genotypes. The black dots are blank controls and cross symbols represent undetermined genotypes because of unsuccessful PCR reactions.

Chapter 3 - Molecular Mapping of Stripe Rust Resistance in Hard White Winter Wheat Danby and Tiger

Abstract

Stripe rust, caused by *Puccinia striiformis* f.sp. tritici, is one of the most important foliar diseases in wheat. To investigate the genetic basis of stripe rust resistance in Danby and Tiger, a double haploid (DH) population derived from these two cultivars was genotyped with simple sequence repeats (SSR) markers and single nucleotide polymorphism (SNP) markers, and evaluated for infection type (IT) and disease severity (DS) in greenhouse and fields. Three major quantitative trait loci (QTLs) were identified for IT and DS evaluated at adult plant stage. Danby contributed the resistant allele for *QYr.hwwg-2AS1* and *QYr.hwwg-4BL1*. *QYr.hwwg-2AS1* explained up to 28.4% of the phenotypic variance (PVE) for IT and 60.5% for DS. *QYr.hwwg-4BL1* explained up to 15.7% phenotypic variance of IT and 7.9% of DS. The third QTL, *QYr.hwwg-3BS1* had PVE values up to 14.7% for IT and 22.9% for DS with the resistant allele contributed by Tiger. *QYr.hwwg-2AS1* and *QYr.hwwg-4BL1* are likely the same resistance genes reported previously. However, *QYr.hwwg-3BS1* might be different from the reported gene cluster near the distal end harboring *Yr57*, *Yr4*, *Yr30* and *Sr2* because it was located in the proximal end of 3BS chromosome arm. None of these major QTLs was detected in the seedling assessments against two predominant isolates collected from Kansas, suggesting that these QTLs were only effective at adult plant stage. Significant additive effects were observed among the three major QTLs on reducing IT and DS. Several user-friendly Kompetitive allele specific PCR (KASP) markers were developed for *QYr.hwwg-2AS1* and *QYr.hwwg-4BL1*, which will be useful for marker-assisted gene pyramiding to improve durable stripe rust resistance in wheat breeding.

Introduction

Wheat stripe rust, also called yellow rust caused by *Puccinia striiformis* f.sp. tritici, is one of the most damaging diseases in bread wheat (*Triticum aestivum* L.), especially for those growing areas under cool conditions during flowering (Brown and Hovmoller 2002; Thach et al. 2016; Wellings 2011). Stripe rust epidemics have become more frequent and severe in the major wheat growing areas, including China, Europe and North America, since 2000 including many warm wheat growing areas where stripe rust was previously rare (Chen 2005; Hovmoller et al. 2010; Wellings 2011). Yield losses can be as much as 50%, and even up to 100%, when severe epidemics occur (Sorensen et al. 2016).

In the United States, stripe rust epidemics have been more frequent and widespread in the last 15 years, causing significant yield losses. It has caused significant damage in the western states almost every year and has become increasingly important in the Great Plains and eastern regions of the United States (Chen 2005, 2007; Chen et al. 2002, 2010; Line 2002; Wan and Chen 2014). Yearly losses exceeding 1 million metric t from 2000 to 2010 were reported in 2001, 2003, 2005 and 2010 (http://striperust.wsu.edu). Following the widespread epidemics throughout the country in 2010, when the resistance in Danby was overcome, stripe rust also caused significant damage in 2011 and 2012 (Wan and Chen 2014). In 2012, stripe rust epidemics spread across more than 25 states including the entire Great Plains. The yield losses were estimated at 4.0% in Colorado, 5.7% in Kansas, 5.0% in Oklahoma, and 2.4% for the entire United States (http://www.ars.usda.gov/main/docs.htm?docid=10123). The most severe epidemics occurred in 2015, which resulted in an estimated nation-wide winter wheat yield loss of 12.7%.

Growing resistant cultivars is considered to be the most economical and environment-friendly means for stripe rust management in wheat (Chen, 2007). Genetic resistance to stripe rust can be categorized into two types, race-specific (all-stage) resistance and non-race-specific resistance or adult-plant resistance (APR) (Chen et al. 2014; Chen 2005; Ellis et al. 2014). Race-specific resistance mostly functions from seedling to adult stages and is most often controlled by a single gene (Chen 2005; Chen and Line 1992). Cultivars with race-specific resistance genes

usually lose their resistance in only a few years after release (Chen 2005; Losert et al. 2016). In contrast, non-race-specific resistance provides resistance to a broad spectrum of races, and tends to be more durable than race-specific resistance (Chen et al. 2014; Chen 2005; Ellis et al. 2014). Although a single non-race-specific resistance gene only provides a low level of resistance, combining several non-race-specific genes together will provide sufficient protection against rust epidemics (Chen et al. 2014; Miedaner and Korzun 2012).

Characterizing resistance QTLs or genes and developing user-friendly molecular markers linked to these APR QTLs is essential for achieving a higher level and more durable resistance through marker-assisted gene pyramiding. To date, there are 76 designated genes or quantitative trait loci (QTLs) (*Yr1*–*Yr76*) and more than 40 temporarily designated QTLs for stripe rust resistance in bread wheat (McIntosh et al. 2013; Rosewarne et al. 2013) (Basnet et al., 2013; McIntosh et al., 2012). Some of them have been characterized with relatively clear gene structures and functions, such as *Yr10* and *Yr38* (Spielmeyer and Lagudah, 2003), *Yr39* (Coram et al., 2008a), *Yr18* (Lagudah et al. 2009; Lagudah et al. 2006; Lillemo et al. 2008; Suenaga et al. 2003) and *Yr36* (Fu et al., 2009). However, most of the genes or QTLs identified to date confer race-specific resistance and only a few of them confer APR including *Lr34*/*Yr18*, *Lr46*/*Yr29,* and *Lr67*/*Yr46* (Dyck, 1987; Herrera-Foessel et al., 2011; Hiebert et al., 2011; Krattinger et al., 2009; Singh et al., 1998; William et al., 2003). Therefore, identification of novel APR genes and investigating their combined effects in rust resistance will provide valuable breeding tools to minimize stripe rust damage.

Two hard white winter wheat (HWWW) cultivars, Danby (PI 648010) and Tiger (PI 661995, Martin et al., 2013), released by Kansas State University, have been supplying adequate resistance to stripe rust for many years in the Central Plains of the United States. They were also frequently used as crossing parents in new cultivar developments. However, their genetic mechanisms of stripe rust resistance are still unknown. We hypothesize that Danby might have different resistance genes from Tiger since they demonstrated different levels of resistance in different years. Mapping resistance genes from these two cultivars would help us to understand their different resistance mechanisms and might discover new resistance genes. Therefore, the objective of this study was to map stripe rust resistance QTLs in Danby and Tiger and develop

user-friendly markers for the major QTLs identified.

Materials and Methods

Plant materials and disease evaluation

A population of 211 doubled haploid (DH) lines was developed from a cross between Danby and Tiger by Heartland Plant Innovation (Manhattan, KS). The DH population and its parents were evaluated for resistance to two important stripe rust isolates PST2010 and PST2012 that were collected in Kansas during the epidemics of 2010 and 2012, respectively. Stripe rust was evaluated at both seedling and adult plant stages. The APR was evaluated in both 2014 and 2015 greenhouse experiments at Manhattan, KS, with temperatures set at 22°C day/17°C night and supplemental daylight of 12 h. In the 2014 greenhouse experiment, only one replicate was used due to limited seed availability. Five seedlings per line were transplanted into a 13 x 13 cm plastic pot and the experiments used a randomized complete block design. At the heading stage, flag leaves were separately inoculated with the two stripe rust isolates, PST2010 in 2014 and PST2012 in 2015. The infection type (IT) and disease severity (DS) were evaluated 18 and 25 d after inoculation, respectively. IT was visually scored using a 0-9 scale (Line and Qayoum, 1991). DS was visually scored as percentage of flag leaf infected using the modified Cobb Scale of 0 to 100% (Peterson et al., 1948).

Seedling tests were conducted in a growth chamber with two replications. About eight seeds per entry were planted in small pots (6 x 6 x 6 cm). The seedlings were inoculated at the two-leaf stage the PST2010 and PST2012 isolates. Inoculated seedlings were first kept in a dark and humid chamber at 10°C for about 24 h and then moved to another growth chamber with 16 h light and diurnal temperatures gradually changing from 12°C to 15°C for disease development. IT was recorded at 21 d after inoculation using a 0-9 scale.

The DH population and its parents were also evaluated for stripe rust resistance in the field at two Kansas locations (Rossville and Hays, KS) in 2015 and 2016. In Rossville, stripe rust was evaluated by artificial inoculation of mixed isolates of PST2010 and PST2012, whereas stripe rust was evaluated under natural infection in Hays. All the lines were planted in single-row (1.5 m long and 30 cm apart) plots with two replications using a random complete block design in all the field experiments except the 2016 Hays field experiment, which was planted in six-row plots

(3.0 m-long row with 25.4 cm between rows) using an augmented design.

In Rossville, stripe rust infection was induced by planting every third drill pass (1.5 m wide) with a highly susceptible breeding line and also planting seven rows of the susceptible breeding line in the border of the field as spreader rows. The spreader rows were inoculated with the mixture of PST2010 and PST2012 by transplanting greenhouse cultured rust-infected seedlings of the susceptible cultivar Morocco into the spreader rows in mid-April and also by inoculating the highly susceptible spreader rows with an oil (Soltrol 170, Chevron Phillips Chemical Company LP, The Woodlands, TX) suspension of urediniospores. This field inoculation was done using an ultralow volume sprayer every week during early jointing and early boot stages (Zadoks 31–41).

IT and DS were recorded at the early to medium milk stage (Zadoks 73–75) in the field experiments. IT was visually scored using a 0-9 scale (Line and Qayoum, 1991). DS was visually scored as percentage of the upper leaf canopy infected using the modified Cobb Scale of 0 to 100% (Peterson et al., 1948).

Data analysis

All statistical analyses, including descriptive statistics, Pearson's correlation coefficient, analysis of variance, transgressive segregation test and multiple comparisons were performed using SAS 9.4 (SAS 9.4, SAS Institute, 2012).

Linkage map construction and QTL analysis

The same set of linkage maps constructed with GBS SNPs and SSR markers described in the methods of chapter 2 were used for QTL analysis.

Composite interval mapping analyses (Silva Lda et al. 2012) were performed for each experiment based on line means or adjusted line values using QTL Cartographer V2.5 (Wang et al. 2007) to locate QTLs for stripe rust resistance . Significant QTLs were claimed if the LOD scores were above the threshold that was derived from 1,000 permutations (Doerge and Churchill 1996).

Combined allelic effects of QTLs

To investigate the combined allelic effects of consistently detected QTLs, all DH lines were grouped based on different allele combinations of the QTLs identified. The markers most closely linked to the QTLs were selected to represent the alleles of these QTLs. IT and DS of each allelic group was compared using Tukey's multiple comparison tests.

Development of KASP markers

User-friendly KASP markers associated with major resistance QTLs were developed from GBS based markers in this study. GBS-SNP tag sequences were used to design primers using the same software and website based tools as described in the methods of chapter two. The newly designed KASP primers were first used to screen parents for polymorphisms and the polymorphic SNPs were used to genotype the mapping population. KASP assays were also performed following the same manufacturer’s instruction as described in chapter two. The GBS-SNPs were then replaced by KASP markers for linkage map re-construction to check the linkage between new KASP markers and their corresponding QTLs.

Results

Adult plant resistance in greenhouse experiments

At the adult stage, parental lines had different reactions to these two isolates inoculated. Danby and Tiger had the same IT of 5 to isolate PST2010, but different IT to the isolate PST2012 (Table 3.1) with Tiger being more resistant (IT = 4) than Danby (IT=7). The DH population segregated for IT to both isolates with IT scores ranging from 0 to 9. As for the DS, Danby was significantly ($P < 0.05$) higher than Tiger for both isolates. For the DS of isolate PST2010, Danby was 70% and Tiger was 30%. For the DS of isolate PST2012, Danby was 40% and Tiger was 5%. The DS of the DH population ranged from 0 to 100% for both isolates inoculated (Table 3.2).

Seedling resistance in growth chamber experiments

In the seedling tests, both parents and the DH lines showed intermediate reactions (IT from 4 to 6) to both isolates on the first leaf from the bottom, but susceptible reaction (IT from 6 to 8) on the second leaf. The difference in IT was not significant ($P > 0.05$) for either leaf between parental lines or among DH lines.

Adult plant resistance in field experiments

Stripe rust infection was well established in all the four field experiments. In general, Danby showed more resistance than Tiger. Similar ITs were observed at Rossville for Danby and Tiger after inoculation with the mixed isolates of PST2010 and PST2012 (Table 3.1), but Danby had much lower DS than Tiger in both years (14.4% vs. 65.0% in 2015, and 1.2% vs. 99.6% in 2016, Table 3.2). The same trend was observed under natural infection at Hays (Table 3.1 and 3.2). Danby had an IT of 5 in 2015 and 3 in 2016 and DS of 20% in 2015 and 12.8% in 2016 while Tiger had an IT of 8 in both years and DS of 75% in 2015 and 95% in 2016 in Hays.

Correlations of the rust reactions among experiments

IT was significantly correlated with DS in all greenhouse and field experiments with correlation coefficients ranging from 0.54 to 0.83 ($p < 0.01$). The field experiments conducted in Hays had the highest correlation coefficient between IT and DS ($r = 0.84$ in 2015 and $r = 0.83$ in 2016).

Significant correlations were also observed for both IT and DS among experiments (Table 3.3). In the greenhouse experiments, significant correlations ($P < 0.01$) were observed between isolates PST2010 and PST2012 for both IT (r = 0.72) and DS (r = 0.56). In the field, the correlations were slightly higher between years in the same location (r ranged from 0.70 to 0.83) for IT or DS than those between locations in the same year (r values ranged from 0.50 to 0.59). This might be due to the larger difference in predominant races of pathogen population between locations than between years.

QTL analysis

In total, 12 sets of IT and DS data collected from greenhouse and field experiments were analyzed separately to identify QTLs (Table 3.6 and 3.7). Only QTLs that were consistently detected in at least six of 12 experiments and explained more than 5% of the phenotypic variation on average were considered as major QTLs. Only three QTLs met this standard. They were located on chromosome arms 2AS (*QYr.hwwg-2AS1*), 3BS (*QYr.hwwg-3BS1*) and 4BL (*QYr.hwwg-4BL1*) (Table 3.4 and 3.5 and Fig. 3.1, 3.2, 3.3). Danby carries the resistant alleles of *QYr.hwwg-2AS1* and *QYr.hwwg-4BL1,* whereas Tiger carries the resistant allele of *QYr.hwwg-3BS1*.

QYr.hwwg-2AS1 was significant in all eight experiments for IT and DS. The PVE for IT ranged from 7.1 to 28.4% and the PVE for DS was high, up to 50.3% in 2015 Hays and 60.5% in 2016 Rossville, however it was not significant in all greenhouse experiments (Table 3.4 and 3.5 and Fig. 3.1). *QYr.hwwg-4BL* was also consistently detected in the field experiments for both IT and DS, but with a much smaller effect than *QYr.hwwg-2AS1* (Table 3.4 and 3.5 and Fig. 3.3). The PVE of this QTL ranged from 7.1% to 11.8% for IT and 5.3 to 7.3 for DS. *QYr.hwwg-3BS,* was consistently detected in the greenhouse and Rossville field experiments, but not in Hays field experiments (Table 3.4 and 3.5 and Fig. 3.2). Phenotypic variance explained by this QTL was intermediate to the previous two QTLs, ranging from 10.8% to 14.7% for IT and 6% to 22.9% for DS.

In addition, several minor QTLs were also detected on chromosomes 1A, 1D, 2B, 3A, 3D, 5A, 5B, 6A, 6B, 6D and 7B (Table 3.4 and 3.5). Among those minor QTLs, three has resistant alleles

contributed by Tiger (*QYr.hwwg-3DS1, QYr.hwwg-5B* and *QYr.hwwg-6AL*) with all others contributed by Danby. Among the minor QTLs, *QYr.hwwg-3DS1*, located near the distal end of 3DS (Fig. 3.4), is the most stable, consistently explaining 10.1% to 11.9% of the phenotypic variances for IT in three experiments (two greenhouse experiments and 2015 Rossville field experiment) and one greenhouse experiment for DS inoculated by isolate PST2010 (Table 3.4 and 3.5).

Combined effects of three major QTLs

Three major QTLs, *QYr.hwwg-2AS1*, *QYr.hwwg-3BS1*, and *QYr.hwwg-4BL1* were used to investigate their combined effects on IT and DS. The DH lines were grouped into eight groups (abc, aBc, abC, aBC, Abc, AbC, ABc, ABC) based on allele combinations of those three QTLs, and the averaged IT and DS values from all the experiments were compared among these groups (Fig. 3.5 and 3.6).

For both IT and DS, significant ($P < 0.001$) differences were found among QTL groups (Fig. 3.5 and Fig. 3.6). Additive effects on IT and DS were significant among these three QTLs ($P < 0.001$). When abc and ABC groups were compared, IT was reduced from 7.6 to 4.7 and DS was reduced from 66.3% to 27.5%. DH lines with two major QTLs also had relatively lower IT and DS than those with only one QTL, except the aBC group for DS. In general, pyramiding multiple major QTLs had significant effects in reducing IT and DS.

Development of KASP markers

To develop useful markers for marker-assisted selection, four flanking GBS-SNP makers were successfully converted into user-friendly KASP markers that flank *QYr.hwwg-3BS1* and *QYr.hwwg-4BL1*. These KASP markers mapped near the peak of respective QTLs (Fig. 3.2 and 3.3) are useful for selecting the QTLs and can be used to pyramid these QTLs with QTLs or genes to improve stripe rust resistance in wheat breeding.

Discussion

Three consistent QTLs, *QYr.hwwg-2AS1*, *QYr.hwwg-3BS1* and *QYr.hwwg-4BL1*, and several minor QTLs were detected in multiple experiments on various chromosomes.

QYr.hwwg-2AS, the most stable and effective major QTL, was consistently detected in all field environments for both IT and DS in two years. The effect was especially strong for DS, with PVE as high as 50.3% in 2015 at Hays field and 60.5% in 2016 at Rossville (Table 3.6 and 3.7, and Fig. 3.1). Based on field screening results in this study, this QTL can still provide resistance to predominant races in two tested field locations of Kansas. Therefore, it might be still useful to stack with other resistance genes to improve rust resistance in new cultivars.

QYr.hwwg-2AS was mapped on the distal end of chromosome arm 2AS where many other stripe rust resistance QTLs have been reported. This region harbors the 2AS-2NS translocated fragment from *T. ventricosum*, which carries *Yr17* (Helguera et al. 2003) and *Yr56* (Wheat Catalogue).. Other QTLs mapped to 2AS include *QYr.tam-2AS* (Basnet et al. 2014a), *QYr.uga-2AS* (Hao et al. 2011), *QYr.ucw-2A.2* (Maccaferri et al. 2015), *QYr.ufs-2A* (Agenbag et al. 2012), *QYr.sun-2A* (Bansal et al. 2014), *QYr.inra-2AS.1* (Dedryver et al. 2009), *QYrva.vt-2AS* (Christopher et al. 2013), *QYrst.orr-2AS* (Vazquez et al. 2012), and *QYr.ucw-2A* (Lowe et al. 2011). All of these genes or QTLs located within the similar region suggesting there is a resistance gene cluster responsible for different races or diseases.

Another major QTL, *QYr.hwwg-3BS1*, was consistently detected for the two separate isolates in the greenhouse and Rossville field experiments, but not in the Hays field experiment (Table 3.6 and 3.7 and Fig. 3.2). That implies its resistance might be effective to multiple races because it was effective to both isolates in greenhouse adult plant test but not detected in the seedling assessment experiments for the same two isolates. This QTL was mapped near the proximal end of the 3BS, between 58.9 and 78.8 cM, on the Chinese Spring reference genome. Several stripe rust resistance QTLs, including *QYr.cim-3B* (Rosewarne et al. 2012), *QYr.inra-3Bcentr* (Dedryver et al. 2009), *QYrpi.vt-3BL* the same region as *QYr.hwwg-3BS1*. However, , this region is far away from the genes located at the distal end of 3BS such as *Yr57*, *Yr4*, *Yr30*, *Yrns-B1,* and *Sr2* (Spielmeyer et al. 2003). This QTL is most likely responsible for the stripe resistance in Tiger that

made this cultivar more resistant than Danby in the epidemics of 2010 and 2011, but was defeated in 2012. The stripe rust reaction of Tiger changed greatly in 2012 and can most likely be explained by the observation that there was only major QTL present in this cultivar.

The third QTL, *QYr.hwwg-4BL,* was consistently detected in both field locations in both 2015 and 2016 (Table 3.6 and 3.7 and Fig. 3.2). It was located near the centromere region between 74.6 and 87.5 cM on the Chinese Spring reference genome. This region contains many reported QTLs associated with stripe rust, such as *Yr50* (Liu et al. 2013), *Yr62* (Lu et al. 2014), *QYr.sun-4B* (Zwart et al. 2010), *QYr.ufs-4B* (Agenbag et al. 2012), *QYr-4B* (William et al. 2006), *QYr.jic-4B* (Jagger et al. 2011), *QYr.caas-4BL* (Lu et al. 2009), *QYr.ui-4B* (Chen et al. 2012), *QYr.vt-4BL* (Christopher et al. 2013), *QYr.jic-4B* (Melichar et al. 2008). It is hard to differentiate *QYr.hwwg-4BL1* from the QTLs previously reported in this region.

Unlike these three major QTLs that were mapped in the extremely similar chromosome regions to many previously reported QTLs or genes, *QYr.hwwg-3DS1,* the most stable minor QTL, was detected on the distal end of chromosome arm 3DS where only two previous QTLs, *Yr66* (McIntosh et al. 2013) and *QYr.tam-3D* (Basnet et al. 2014b), are reported. Additional allelic tests are needed to differentiate it from these two reported QTLs. This QTL could be valuable specially to reduce IT because it was repeatedly detected in three screening experiments with more than 10% PVE for IT.

Three stable major QTLs were used to investigate their combinational effect on resistance enhancement. The resistance to stripe was significantly enhanced after combining two or three QTLs together demonstrating significant additive effects on both IT and DS (Fig.3.5 and 3.6). So these major QTLs will be very valuable to improve stripe rust resistance in wheat breeding by pyramiding them together or with other reported resistance QTLs.

Conclusion

This study revealed the genetic basis of stripe rust resistance in two hard white winter wheat cultivars Danby and Tiger for the first time. Three major QTLs, *QYr.hwwg-2AS1*, *QYr.hwwg-3BS1* and *QYr.hwwg-4BL1* were detected in multiple experiments. Among them, *QYr.hwwg-2AS1* and *QYr.hwwg-4BL1* were found to be the major resistance components in Danby, and *QYr.hwwg-3BS1* in Tiger. Although additional allelic tests are in need to differentiate these QTLs from previously reported QTLs, the stability of their resistance in different experiments and significant combinational effects in reducing IT and DS, indicate their values in development of high level and more durable stripe rust resistance through gene pyramiding assisted by the new KASP markers developed in this study.

References:

Agenbag GM, Pretorius ZA, Boyd LA, Bender CM, Prins R (2012) Identification of adult plant resistance to stripe rust in the wheat cultivar Cappelle-Desprez. Theor Appl Genet 125:109-120

Bansal UK, Kazi AG, Singh B, Hare RA, Bariana HS (2014) Mapping of durable stripe rust resistance in a durum wheat cultivar Wollaroi. Mol Breeding 33:51-59

Basnet BR, Ibrahim AMH, Chen XM, Singh RP, Mason ER, Bowden RL, Liu SY, Hays DB, Devkota RN, Subramanian NK, Rudd JC (2014a) Molecular Mapping of Stripe Rust Resistance in Hard Red Winter Wheat TAM 111 Adapted to the U.S. High Plains. Crop Sci 54:1361-1373

Basnet BR, Singh RP, Ibrahim AMH, Herrera-Foessel SA, Huerta-Espino J, Lan C, Rudd JC (2014b) Characterization of Yr54 and other genes associated with adult plant resistance to yellow rust and leaf rust in common wheat Quaiu 3. Mol Breeding 33:385-399

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633-2635

Brown JKM, Hovmoller MS (2002) Epidemiology - Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. Science 297:537-541

Chapman JA, Mascher M, Buluc A, Barry K, Georganas E, Session A, Strnadova V, Jenkins J, Sehgal S, Oliker L, Schmutz J, Yelick KA, Scholz U, Waugh R, Poland JA, Muehlbauer GJ, Stein N, Rokhsar DS (2015) A whole-genome shotgun approach for assembling and anchoring the hexaploid bread wheat genome. Genome Biol 16

Chen JL, Chu CG, Souza EJ, Guttieri MJ, Chen XM, Xu S, Hole D, Zemetra R (2012) Genome-wide identification of QTL conferring high-temperature adult-plant (HTAP) resistance to stripe rust (Puccinia striiformis f. sp tritici) in wheat. Mol Breeding 29:791-800

Chen W, Wellings C, Chen X, Kang Z, Liu T (2014) Wheat stripe (yellow) rust caused by Puccinia striiformis f. sp.tritici. Mol Plant Pathol 15:433-446

Chen XM (2005) Epidemiology and control of stripe rust [Puccinia striiformis f. sp tritici] on wheat. Can J Plant Pathol 27:314-337

Chen XM, Line RF (1992) Inheritance of Stripe Rust Resistance in Wheat Cultivars Used to Differentiate Races of Puccinia-Striiformis in North-America. Phytopathology 82:633-637

Christopher MD, Liu SY, Hall MD, Marshall DS, Fountain MO, Johnson JW, Milus EA, Garland-Campbell KA, Chen XM, Griffey CA (2013) Identification and Mapping of Adult Plant Stripe Rust Resistance in Soft Red Winter Wheat VA00W-38. Crop Sci 53:871-879

Dedryver F, Paillard S, Mallard S, Robert O, Trottet M, Negre S, Verplancke G, Jahier J (2009) Characterization of Genetic Components Involved in Durable Resistance to Stripe Rust in the

Bread Wheat 'Renan'. Phytopathology 99:968-973

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285-294

Ellis JG, Lagudah ES, Spielmeyer W, Dodds PN (2014) The past, present and future of breeding rust resistant wheat. Front Plant Sci 5:641

Hao YF, Chen ZB, Wang YY, Bland D, Buck J, Brown-Guedira G, Johnson J (2011) Characterization of a major QTL for adult plant resistance to stripe rust in US soft red winter wheat. Theor Appl Genet 123:1401-1411

Helguera M, Khan IA, Kolmer J, Lijavetzky D, Zhong-qi L, Dubcovsky J (2003) PCR assays for the Lr37-Yr17-Sr38 cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Sci 43:1839-1847

Hovmoller MS, Walter S, Justesen AF (2010) Escalating Threat of Wheat Rusts. Science 329:369-369

Jagger LJ, Newell C, Berry ST, MacCormack R, Boyd LA (2011) Histopathology provides a phenotype by which to characterize stripe rust resistance genes in wheat. Plant Pathol 60:640-648

Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espino J, Spielmeyer W, Brown-Guedira G, Selter LL, Keller B (2009) Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. Theor Appl Genet 119:889-898

Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, Spielmeyer W (2006) Molecular genetic characterization of the Lr34/Yr18 slow rusting resistance gene region in wheat. Theor Appl Genet 114:21-30

Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, Bjornstad A (2008) The adult plant rust resistance loci Lr34/Yr18 and Lr46/Yr29 are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor Appl Genet 116:1155-1166

Line, R.F., and A. Qayoum. 1991. Virulence, aggressiveness, evo- lution, and distribution of races of Puccinia striiformis (the cause of stripe rust of wheat) in North America, 1968–87. USDA Tech. Bull. 1788. U.S. Gov. Print. Office, Washington, DC.

Losert D, Maurer HP, Leiser WL, Wurschum T (2016) Defeating the Warrior: genetic architecture of triticale resistance against a novel aggressive yellow rust race. Theor Appl Genet

Lowe I, Jankuloski L, Chao S, Chen X, See D, Dubcovsky J (2011) Mapping and validation of QTL which confer partial resistance to broadly virulent post-2000 North American races of stripe rust in hexaploid wheat. Theor Appl Genet 123:143-157

Lu YM, Lan CX, Liang SS, Zhou XC, Liu D, Zhou G, Lu QL, Jing JX, Wang MN, Xia XC, He ZH (2009) QTL mapping for adult-plant resistance to stripe rust in Italian common wheat cultivars Libellula and Strampelli. Theor Appl Genet 119:1349-1359

Maccaferri M, Zhang JL, Bulli P, Abate Z, Chao SM, Cantu D, Bossolini E, Chen XM, Pumphrey M, Dubcovsky J (2015) A Genome-Wide Association Study of Resistance to Stripe Rust (Puccinia striiformis f. sp tritici) in a Worldwide Collection of Hexaploid Spring Wheat (Triticum aestivum L.). G3-Genes Genom Genet 5:449-465

Mayer KFX, Rogers J, Dolezel J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski AJ, Sourdille P, Endo TR, Dolezel J, Kubalakova M, Cihalikova J, Dubska Z, Vrana J, Sperkova R, Simkova H, Rogers J, Febrer M, Clissold L, McLay K, Singh K, Chhuneja P, Singh NK, Khurana J, Akhunov E, Choulet F, Sourdille P, Feuillet C, Alberti A, Barbe V, Wincker P, Kanamori H, Kobayashi F, Itoh T, Matsumoto T, Sakai H, Tanaka T, Wu JZ, Ogihara Y, Handa H, Pozniak C, Maclachlan PR, Sharpe A, Klassen D, Edwards D, Batley J, Olsen OA, Sandve SR, Lien S, Steuernagel B, Wulff B, Caccamo M, Ayling S, Ramirez-Gonzalez RH, Clavijo BJ, Steuernagel B, Wright J, Pfeifer M, Spannagl M, Mayer KFX, Martis MM, Akhunov E, Choulet F, Mayer KFX, Mascher M, Chapman J, Poland JA, Scholz U, Barry K, Waugh R, Rokhsar DS, Muehlbauer GJ, Stein N, Gundlach H, Zytnicki M, Jamilloux V, Quesneville H, Wicker T, Mayer KFX, Faccioli P, Colaiacovo M, Pfeifer M, Stanca AM, Budak H, Cattivelli L, Glover N, Martis MM, Choulet F, Feuillet C, Mayer KFX, Pfeifer M, Pingault L, Mayer KFX, Paux E, Spannagl M, Sharma S, Mayer KFX, Pozniak C, Appels R, Bellgard M, Chapman B, Pfeifer M, Pfeifer M, Sandve SR, Nussbaumer T, Bader KC, Choulet F, Feuillet C, Mayer KFX, Akhunov E, Paux E, Rimbert H, Wang SC, Poland JA, Knox R, Kilian A, Pozniak C, Alaux M, Alfama F, Couderc L, Jamilloux V, Guilhot N, Viseux C, Loaec M, Quesneville H, Rogers J, Dolezel J, Eversole K, Feuillet C, Keller B, Mayer KFX, Olsen OA, Praud S, Iwgsc (2014) A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345

Melichar JPE, Berry S, Newell C, MacCormack R, Boyd LA (2008) QTL identification and microphenotype characterisation of the developmentally regulated yellow rust resistance in the UK wheat cultivar Guardian. Theor Appl Genet 117:391-399

Miedaner T, Korzun V (2012) Marker-Assisted Selection for Disease Resistance in Wheat and Barley Breeding. Phytopathology 102:560-566

Peterson, R.F., A.B. Campbell, and A.E. Hannah. 1948. A diagrammatic scale for estimating rust severity on leaves and stems of cereals. Can. J. Res. C. 26:496–500. doi:10.1139/ cjr48c-033

Poland JA, Brown PJ, Sorrells ME, Jannink JL (2012) Development of High-Density Genetic Maps for Barley and Wheat Using a Novel Two-Enzyme Genotyping-by-Sequencing Approach. Plos One 7

Ramirez-Gonzalez RH, Uauy C, Caccamo M (2015) PolyMarker: A fast polyploid primer design

pipeline. Bioinformatics 31:2038-2039

Rosewarne GM, Singh RP, Huerta-Espino J, Herrera-Foessel SA, Forrest KL, Hayden MJ, Rebetzke GJ (2012) Analysis of leaf and stripe rust severities reveals pathotype changes and multiple minor QTLs associated with resistance in an Avocet x Pastor wheat population. Theor Appl Genet 124:1283-1294

Silva Lda C, Wang S, Zeng ZB (2012) Composite interval mapping and multiple interval mapping: procedures and guidelines for using Windows QTL Cartographer. Methods in molecular biology 871:75-119

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (Triticum aestivum L.). Theor Appl Genet 109:1105-1114

Sorensen CK, Thach T, Hovmoller MS (2016) Evaluation of Spray and Point Inoculation Methods for the Phenotyping of Puccinia striiformis on Wheat. Plant Dis 100:1064-1070

Suenaga K, Singh RP, Huerta-Espino J, William HM (2003) Microsatellite markers for genes Lr34/Yr18 and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93:881-890

Thach T, Ali S, de Vallavieille-Pope C, Justesen AF, Hovmoller MS (2016) Worldwide population structure of the wheat rust fungus Puccinia striiformis in the past. Fungal Genetics and Biology 87:1-8

Vazquez MD, Peterson CJ, Riera-Lizarazu O, Chen XM, Heesacker A, Ammar K, Crossa J, Mundt CC (2012) Genetic analysis of adult plant, quantitative resistance to stripe rust in wheat cultivar 'Stephens' in multi-environment trials. Theor Appl Genet 124:1-11

Wellings CR (2011) Global status of stripe rust: a review of historical and current threats. Euphytica 179:129-141

William HM, Singh RP, Huerta-Espino J, Palacios G, Suenaga K (2006) Characterization of genetic loci conferring adult plant resistance to leaf rust and stripe rust in spring wheat. Genome 49:977-990

Zwart RS, Thompson JP, Milgate AW, Bansal UK, Williamson PM, Raman H, Bariana HS (2010) QTL mapping of multiple foliar disease and root-lesion nematode resistances in wheat. Mol Breeding 26:107-124

Table 3.1 The stripe rust infection type (0-9 scales) for parents and the double haploid population evaluated in six experiments.

	Parents		Population			
Experiment[a]	Danby	Tiger	Minimum	Maximum	Mean	SD[b]
GH1	5	5	0	9	5.73	2.24
GH2	7	4	0	9	5.83	2.58
RS15	7	6	1.5	9	6.79	1.96
RS16	5	5	1	8.5	5.38	2.22
HY15	5	8	1	9	6.04	2.37
HY16	3	8	2	9	6.09	2.39

[a]Experiments conducted in different environments (GH1, greenhouse inoculated with isolate PST-2010; GH2, greenhouse inoculated with isolate PST-2012; RS15 and RS16, field inoculation with mixed isolates of PST-2010 and PST-2012 in Rossville, KS in 2015 and 2016; HY15 and HY16, field natural inoculation in Hays, KS in 2015 and 2016).

[b]SD, standard deviation.

Table 3.2 The stripe rust disease severities (%) in parents and the double haploid population in different experiments.

	Parents		Population			
Experiment[a]	Danby	Tiger	Minimum	Maximum	Mean	SD[b]
GH1	70	30	0	100	31.57	30.23
GH2	40	5	0	100	34.90	31.61
RS15	14.38	65	0.5	90	36.02	28.30
RS16	1.17	99.5	1	100	43.43	42.11
HY15	20	75	5	90	48.06	28.11
HY16	12.8	100	0	100	47.62	33.33

[a]Experiments conducted in different environments (GH1, greenhouse inoculated with isolate PST-2010; GH2, greenhouse inoculated with isolate PST-2012; RS15 and RS16, field inoculation with mixed isolates of PST-2010 and PST-2012 in Rossville, KS in 2015 and 2016; HY15 and HY16, field natural inoculation in Hays, KS in 2015 and 2016).
[b]SD, standard deviation.

Table 3.3 Pearson's correlation coefficients for infection type (IT) and disease severity (DS) of the double haploid mapping population in different experiments.

IT / DS	GH1	GH2	RS15	RS16	HY15	HY16
GH1	-	0.72*	0.56*	0.44*	0.27	0.19
GH2	0.56*	-	0.60*	0.43*	0.20	0.02
RS15	0.34	0.23	-	0.70*	0.50*	0.22
RS16	0.06	0.16	0.70*	-	0.73*	0.59*
HY15	0.01	0.11	0.66*	0.83*	-	0.83*
HY16	0.00	0.10	0.51*	0.71*	0.83*	-

*Significant correlation coefficients (P <0.05). The correlation coefficients were calculated for infection type (upper right half of the table) and disease severity (lower left half of the table) for all six adult plant screening experiments (GH1, greenhouse inoculated with isolate PST-2010; GH2, greenhouse inoculated with isolate PST-2012; RS15 and RS16, field inoculation with mixed isolates of PST-2010 and PST-2012 in Rossville, KS in 2015 and 2016; HY15 and HY16, field natural inoculation in Hays, KS in 2015 and 2016).

Table 3.4 Quantitative trait loci analysis of stripe rust infection type in the Danby/Tiger double haploid population evaluated in different experiments.

Chr[a]	Experiment[b]	Position (cM[c])	Flanking markers	LOD[d]	PVE[e] (%)	Additive effect [f]
2AS	Hays_2015	115.42	DH09463-DH10011	25.36	28.43	-1.32
2AS	Hays_2016	115.36	DH09463-DH10011	10.70	16.02	-1.08
2AS	Ross_2015	115.42	DH09463-DH10011	7.46	9.30	-0.63
2AS	Ross_2016	115.44	DH09463-DH10011	4.45	7.10	-0.62
3BS	GH1	11.99-23.80	DH07328-KASP765	6.07	10.72	0.79
3BS	GH2	11.99-23.80	DH07328-KASP765	5.99	10.79	0.89
3BS	Ross_2015	11.99-23.80	DH07328-KASP765	9.87	14.40	0.80
3BS	Ross_2016	11.99-23.80	DH07328-KASP765	8.86	14.67	0.89
3DS	GH1	0.01	DH10568-DH09616	4.23	10.11	0.77
3DS	GH2	0.01	DH10568-DH09616	4.75	11.02	0.91
3DS	Ross_2015	0.01	DH10568-DH09616	6.98	10.62	0.69
3D	GH1	32.09	DH09616-DH08496	4.57	9.74	-0.87
3D	Hays_2015	76.77	DH10261-DH08109	4.79	4.41	-0.51
3D	Hays_2016	70.41	DH10261-DH08109	3.94	5.99	-0.62
4BL	Hays_2015	38.26	KASP8247-DH10018	11.79	12.13	-0.86
4BL	Hays_2016	38.26	KASP8247-DH10018	8.92	13.94	-0.93
4BL	Ross_2015	39.26	KASP8247-DH10018	7.13	9.34	-0.61
4BL	Ross_2016	38.26	KASP8247-DH10018	9.25	15.68	-0.91
5AL	GH1	4.32	BAR117-KASP907	3.24	5.49	-0.55
5B	Hays_2015	1.58	DH04453-DH10371	2.61	2.43	0.38
5B	Ross_2015	1.58	DH04453-DH10371	6.56	9.28	0.61
6AS	Hays_2015	3.70	DH06957-DH06872	3.45	3.15	-0.42

6AS	Hays_2016	0.01	DH06957-DH06872	3.01	4.15	-0.52
6B	Hays_2016	34.15	DH08556-DH01762	4.24	6.05	-0.63
7BL	Hays_2016	1.61	DH05181-DH06252	4.26	5.98	-0.61

[a] Chromosome arm of corresponding QTL. [b] Experiments conducted in different environments (GH1, greenhouse inoculated with isolate PST-2010; GH2, greenhouse inoculated with isolate PST-2012; RS15 and RS16, field inoculation with mixed isolates of PST-2010 and PST-2012 in Rossville, KS in 2015 and 2016; HY15 and HY16, field natural inoculation in Hays, KS in 2015 and 2016). [c]QTL position (cM) in the partial map of corresponding chromosome arm. [d] LOD, logarithm of odds. [e] Percent phenotypic variation explained by the QTL. [f]The negative (−) sign indicates that resistance is contributed by Danby.

Table 3.5 Quantitative trait loci analysis of stripe rust severity in the Danby/Tiger double haploid population evaluated in different experiments.

Chr[a]	Experiment[b]	Position(cM[c])	Flanking markers	LOD[d]	PVE[e] (%)	Additive effect [f]
1AS	Hays_2015	18.49	DH02869-DH01759	2.86	1.49	-3.52
1AS	Hays_2016	4.95	DH07872-CFA2153	3.88	4.47	-7.35
1D	Hays_2016	9.01	DH03472-DH05141	3.19	3.63	-6.44
1D	Ross_2016	9.97	DH03472-DH05141	5.69	2.65	-7.05
2AS	Hays_2015	115.44	DH09463-DH10011	53.88	50.31	-21.78
2AS	Hays_2016	115.44	DH09463-DH10011	22.05	32.19	-22.22
2AS	Ross_2015	115.42	DH09463-DH10011	19.70	25.22	-14.88
2AS	Ross_2016	115.42	DH09463-DH10011	58.06	60.46	-34.03
2BS	Hays_2015	12.70	DH00697-DH05592	3.48	1.81	-3.98
2BS	Hays_2016	12.55	DH00697-DH05592	3.58	4.03	-7.28
3AL	GH1	23.29	DH04199-DH10274	2.68	5.13	-7.16
3BS	GH1	11.99-23.80	DH07328-KASP765	3.12	6.04	7.91
3BS	GH2	11.99-23.80	DH07328-KASP765	6.36	13.18	11.91
3BS	Ross_2015	11.99-23.80	DH07328-KASP765	16.89	22.91	14.40
3DS	GH1	0.01	DH10568-DH09616	4.49	11.87	11.26
3D	Hays_2015	77.58	DH10261-DH08109	4.83	2.53	-4.61
4BL	Ross_2015	34.66	KASP8247-DH10018	7.27	7.86	-8.31
4BL	Ross_2016	34.66	KASP8247-DH10018	5.29	2.42	-6.80
6AL	GH2	0.53	DH08294-DH06044	4.32	8.71	9.78
6AS	Ross_2015	32.20	DH 02050- DH 06412	3.01	3.28	-5.56
6B	Ross_2016	91.32	DH 03448-DH03467	4.68	2.85	7.43
6DL	Ross_2016	19.78	DH01330-DH08675	3.87	1.75	-5.75

[a] Chromosome arm of corresponding QTL. [b] Experiments conducted in different environments (GH1, greenhouse inoculated with isolate PST-2010; GH2, greenhouse inoculated with isolate PST-2012; RS15 and RS16, field inoculation with mixed isolates of PST-2010 and PST-2012 in Rossville, KS in 2015 and 2016; HY15 and HY16, field natural inoculation in Hays, KS in 2015 and 2016). [c]QTL position (cM) in the partial map of corresponding chromosome arm. [d] LOD, logarithm of odds. [e] Percent phenotypic variation explained by the QTL. [f]The negative (−) sign indicates that resistance is contributed by Danby.

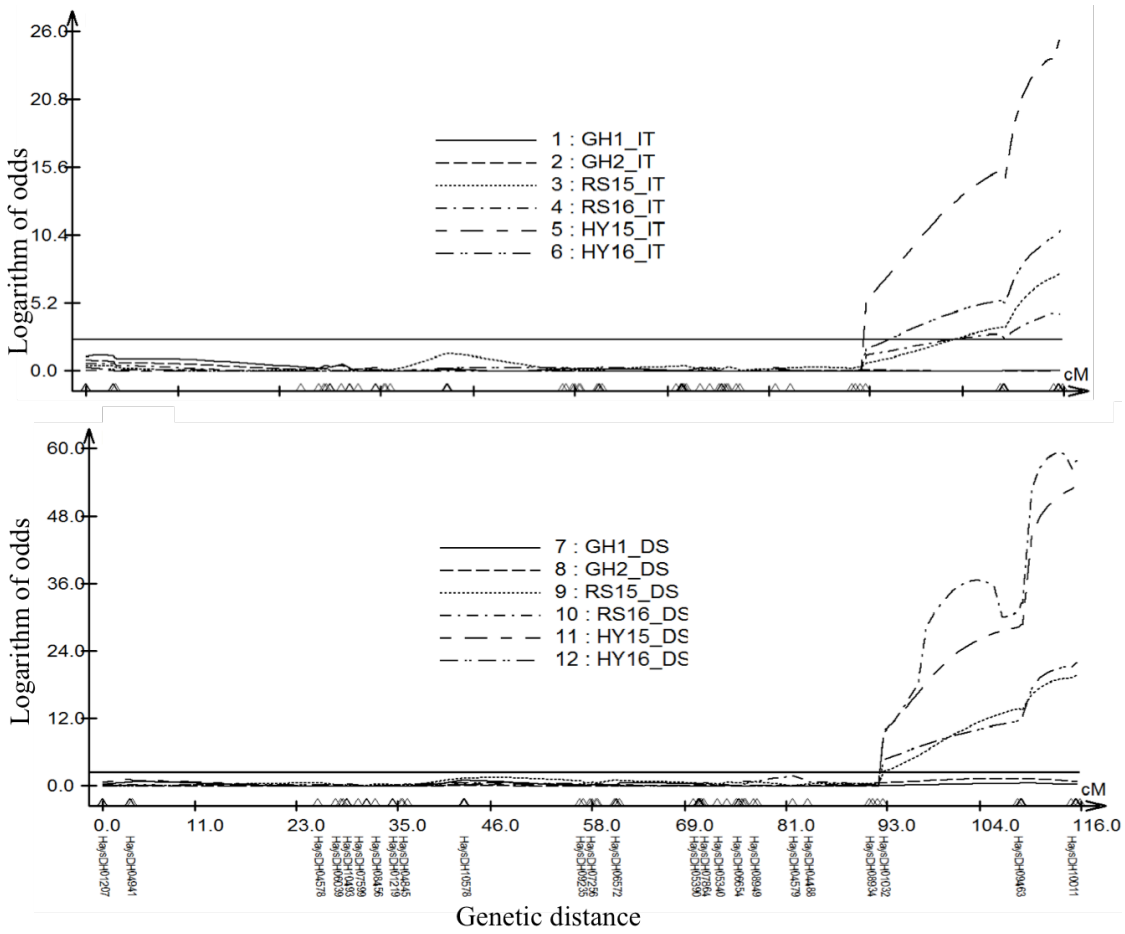

Figure 3.1 Composite interval mapping of infection type (IT) and disease severity (DS) associated QTLs on chromosome 2AS in different experiments.

Line parallel to the X-axis is the threshold for claiming significant QTLs with a logarithm of odds at 2.5. Genetic distances of molecular markers are shown in centiMorgans (cM) along the X-axis. There were six environments in total (GH1, greenhouse inoculated with isolate PST-2010; GH2, greenhouse inoculated with isolate PST-2012; RS15 and RS16, field inoculation with mixed isolates of PST-2010 and PST-2012 in Rossville, KS in 2015 and 2016; HY15 and HY16, field natural inoculation in Hays, KS in 2015 and 2016).

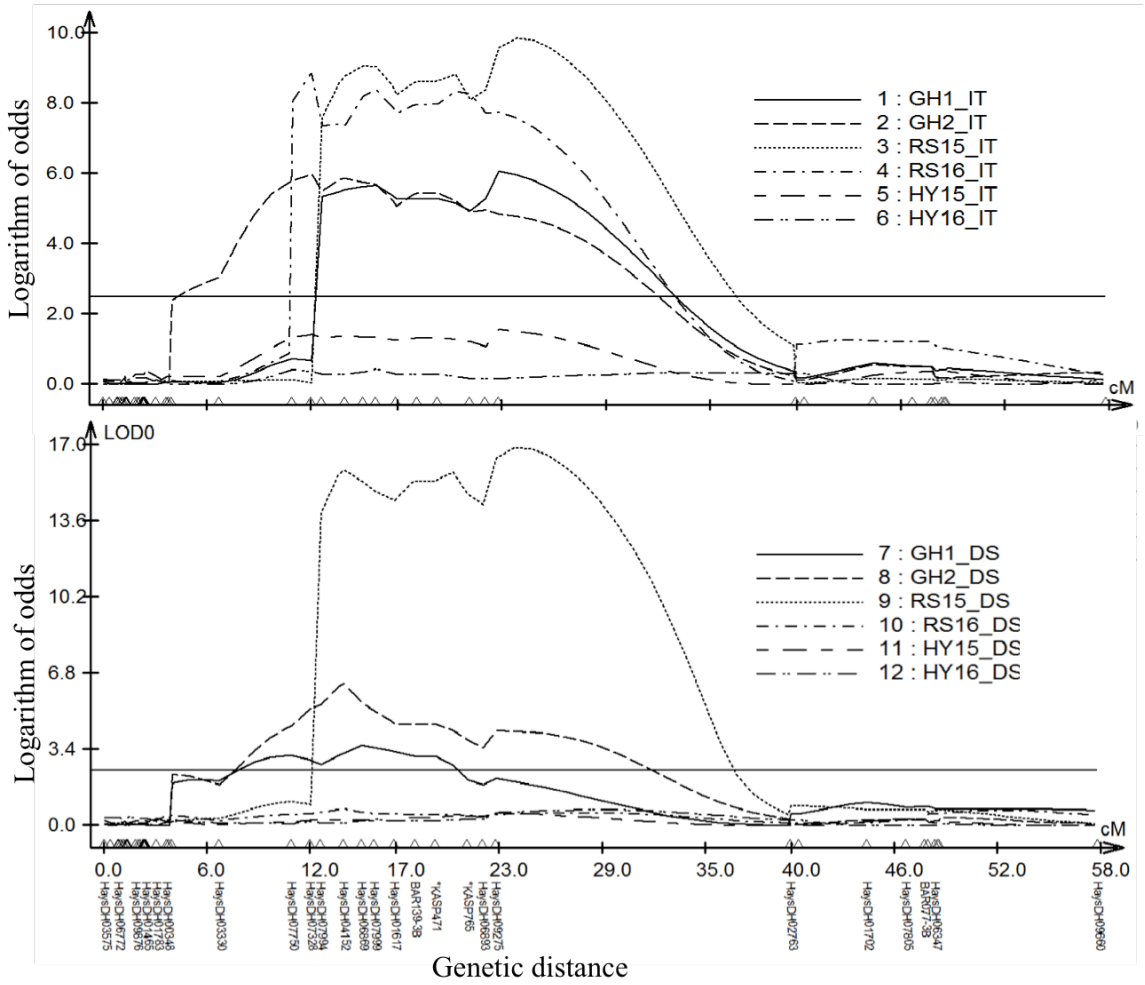

Figure 3.2 Composite interval mapping of infection type (IT) and disease severity (DS) associated QTLs on chromosome 3BS in different experiments.

Line parallel to the X-axis is the threshold for claiming significant QTLs with a logarithm of odds at 2.5. Genetic distances of molecular markers are shown in centiMorgans (cM) along the X-axis. There were six environments in total (GH1, greenhouse inoculated with isolate PST-2010; GH2, greenhouse inoculated with isolate PST-2012; RS15 and RS16, field inoculation with mixed isolates of PST-2010 and PST-2012 in Rossville, KS in 2015 and 2016; HY15 and HY16, field natural inoculation in Hays, KS in 2015 and 2016).

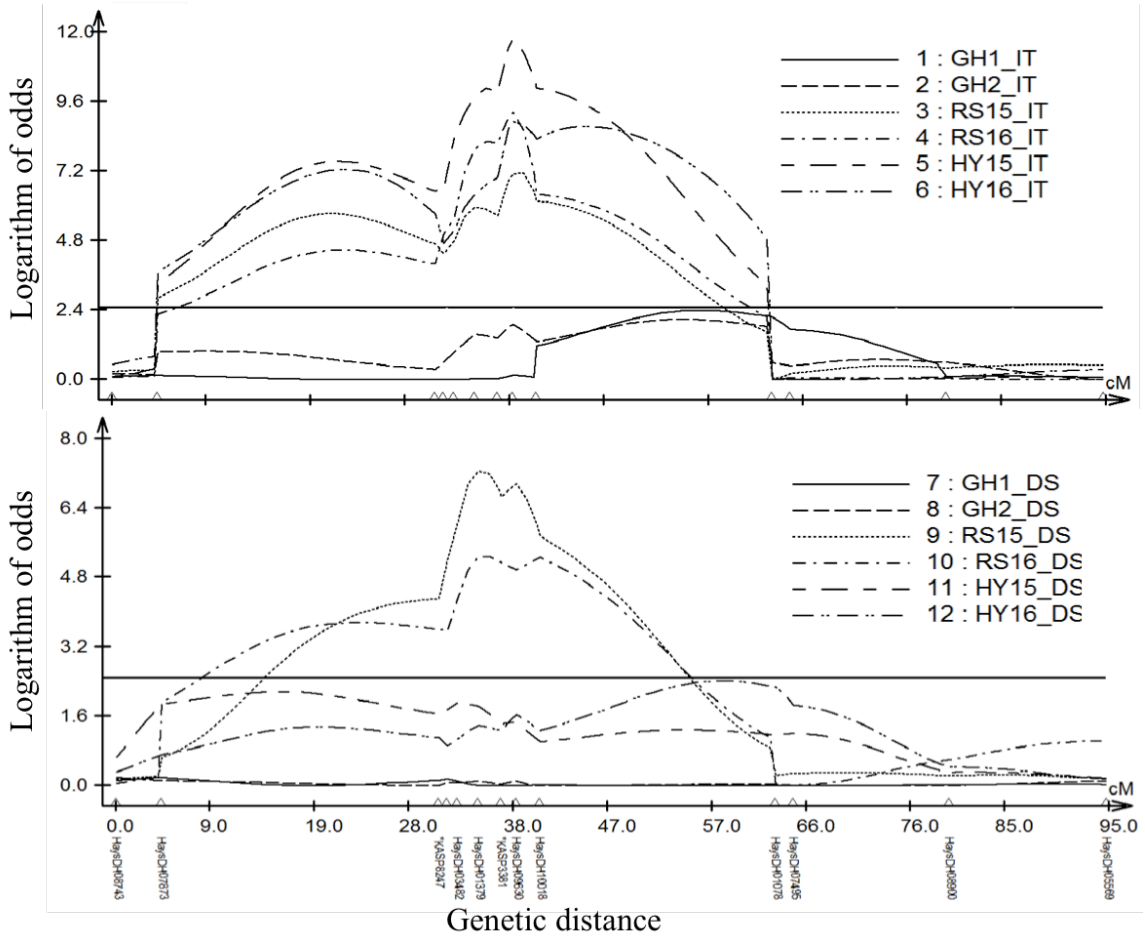

Figure 3.3 Composite interval mapping of infection type (IT) and disease severity (DS) associated QTLs on chromosome 4BL in different experiments.

Line parallel to the X-axis is the threshold for claiming significant QTLs with a logarithm of odds at 2.5. Genetic distances of molecular markers are shown in centiMorgans (cM) along the X-axis. There were six environments in total (GH1, greenhouse inoculated with isolate PST-2010; GH2, greenhouse inoculated with isolate PST-2012; RS15 and RS16, field inoculation with mixed isolates of PST-2010 and PST-2012 in Rossville, KS in 2015 and 2016; HY15 and HY16, field natural inoculation in Hays, KS in 2015 and 2016).

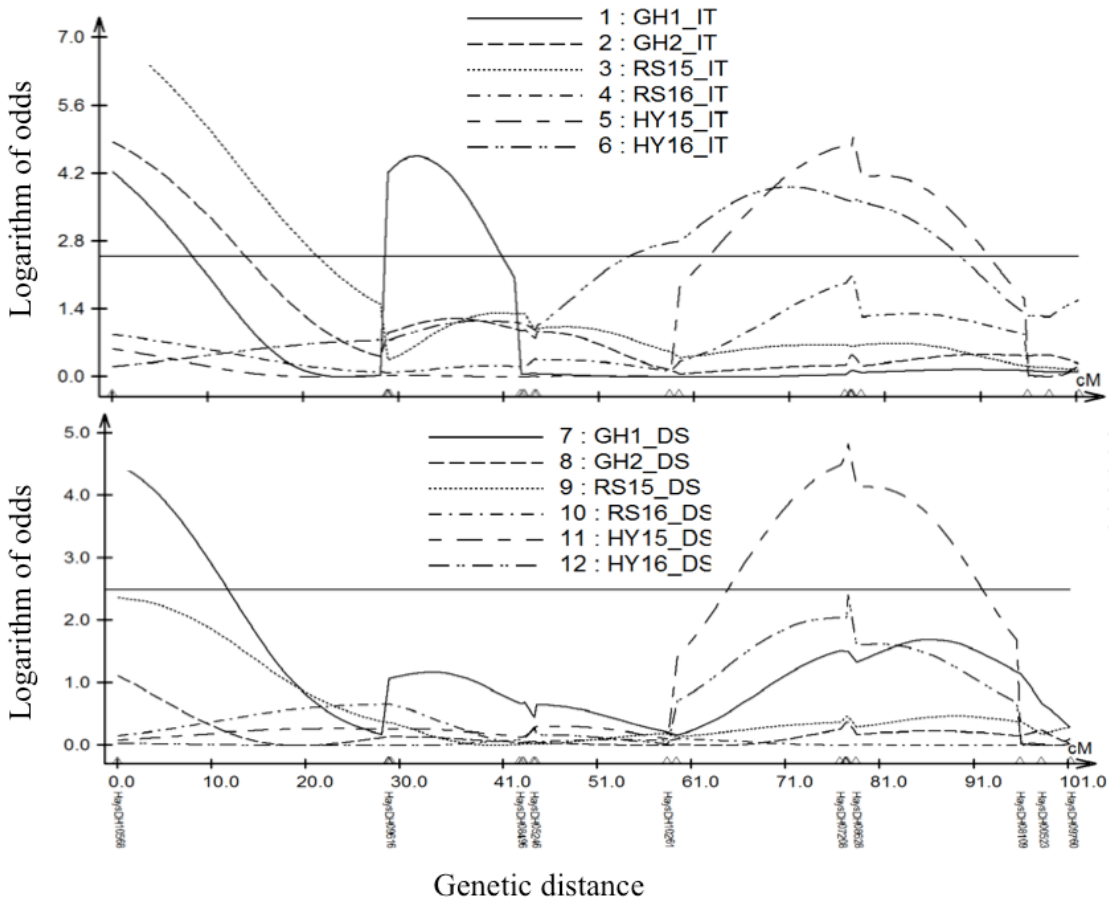

Figure 3.4 Composite interval mapping of infection type (IT) and disease severity (DS) associated QTLs on chromosome 3DS in different experiments.

Line parallel to the X-axis is the threshold for claiming significant QTLs with a logarithm of odds at 2.5. Genetic distances of molecular markers are shown in centiMorgans (cM) along the X-axis. There were six environments in total (GH1, greenhouse inoculated with isolate PST-2010; GH2, greenhouse inoculated with isolate PST-2012; RS15 and RS16, field inoculation with mixed isolates of PST-2010 and PST-2012 in Rossville, KS in 2015 and 2016; HY15 and HY16, field natural inoculation in Hays, KS in 2015 and 2016).

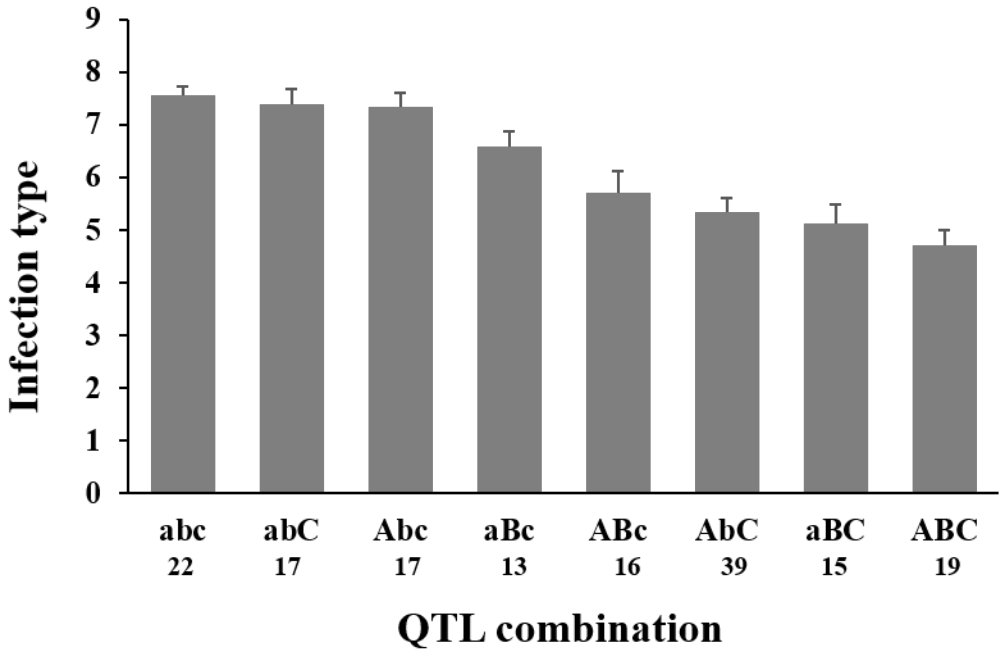

Figure 3.5 The effect of single quantitative trait locus (QTL) and QTL combinations (determined by the most associated flanking markers outlined in Table 3.6) on stripe rust infection type averaged over all six experiments.

Mean and standard errors of the mean are shown. The numbers under the genotypes indicate the number of double haploid lines in each class. The small letters a, b and c represents susceptible alleles of QTL on chromosome arms 2AS, 3BS and 4BL responsively. The capital letters represent the resistant alleles respectively. The number below the letters is the sample size for each genotype group. Error bar is the standard error of the group mean.

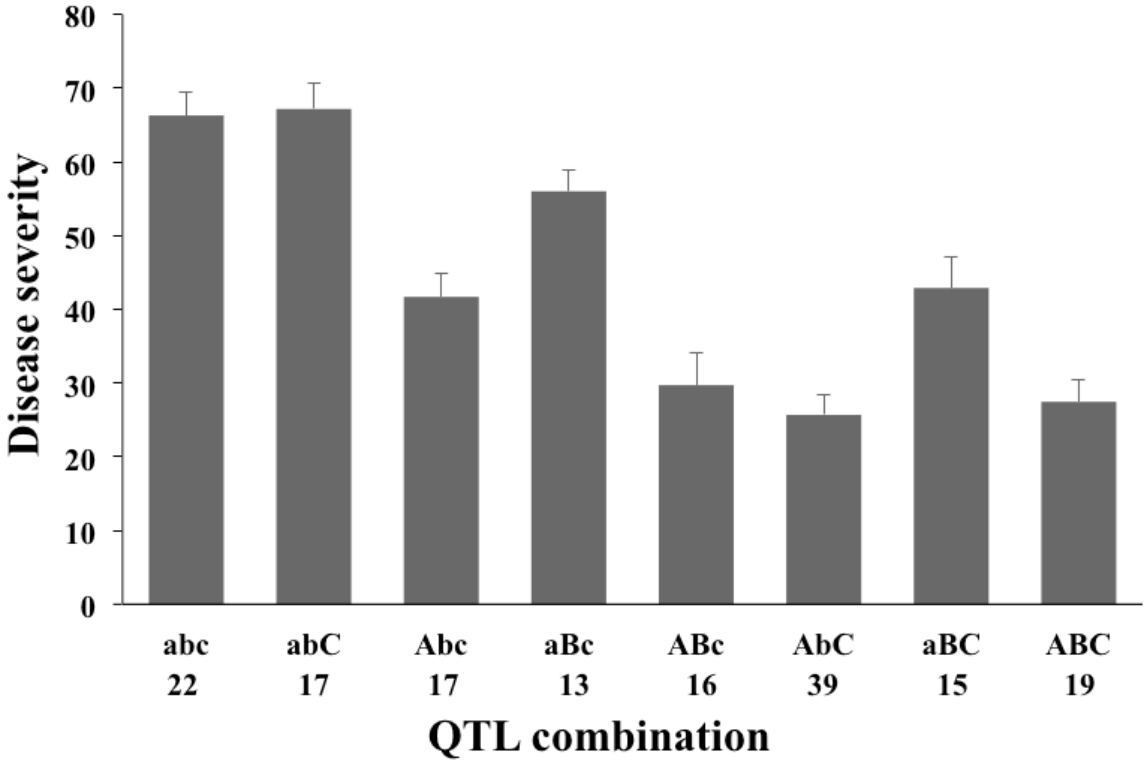

Figure 3.6 The effect of single quantitative trait locus (QTL) and QTL combinations (determined by the most associated flanking markers outlined in Table 3.7) on stripe rust severity averaged over all experiments.

Mean and standard errors of the mean are shown. The numbers under the genotypes indicate the number of double haploid lines in each class. The small letters a, b and c represents susceptible alleles of QTL on chromosome arms 2AS, 3BS and 4BL responsively. The capital letters represent the corresponding resistant alleles respectively. The number below the letters is the sample size for each genotype group. Error bar is the standard error of the group mean.