GENOME-WIDE ASSOCIATION ANALYSIS OF HOST RESISTANCE TO STEM RUST, LEAF RUST, TAN SPOT, AND SEPTORIA NODORUM BLOTCH IN EMMER WHEAT

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Qun Sun

In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Program:
Genomics and Bioinformatics

April 2015

Fargo, North Dakota

North Dakota State University
Graduate School

Title

GENOME-WIDE ASSOCIATION ANALYSIS OF HOST RESISTANCE TO STEM RUST, LEAF RUST, TAN SPOT, AND SEPTORIA NODORUM BLOTCH IN EMMER WHEAT

By

Qun Sun

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Steven S. Xu
Chair

Phillip E. McClean

Xiwen Cai

Justin D. Faris

Marion O. Harris

Approved:

4/7/2015
Date

Phillip E. McClean
Department Chair

ABSTRACT

Cultivated emmer wheat (*Triticum turgidum* ssp. *dicoccum*) is a good source of genes for resistance to several major diseases of wheat. The objectives of this study were to use genome-wide association analysis to detect genomic regions in cultivated emmer germplasm harboring novel resistance genes to four wheat diseases: stem rust, leaf rust, tan spot, and Septoria nodorum blotch (SNB). A natural population including 180 cultivated emmer accessions with a high level of geographic diversity was assembled as the association-mapping panel. This cultivated emmer panel was evaluated phenotypically by scoring reactions to stem rust, leaf rust, tan spot, and SNB and was genotyped using a 9K SNP Infinium array. After filtering for missing data points and minor allele frequency (MAF), 4,134 SNPs were used for association analysis using 178 emmer accessions. Based on principle component (PC) analysis, five subpopulations strongly associated with geographic origins were suggested by the first three PCs. Genome-wide association analysis revealed that 222, 42, 146, and 42 SNPs were significantly associated with resistance to stem rust, leaf rust, tan spot, and SNB, respectively, at the significant level of 1 percentile. Among the significant SNPs at the significant level of 0.1 percentile, ten, one, nine, and one co-located with known genes or QTL associated with resistance to the four diseases, respectively. The remaining significant SNPs were located in the genomic regions where no known resistance genes have been identified for the four diseases. This evidence suggests that some of the emmer wheat accessions carry novel genes conferring resistance to the four diseases. Additionally, 14, three, eight, and five LD blocks harboring at least one significant SNP were identified and might harbor putative QTL related to resistance to the four diseases, respectively. These studies provide information about the genomic regions in cultivated emmer that are associated with resistance to stem rust, leaf rust, tan spot, and SNB. Results from these studies provide guidance for selecting

emmer accessions when decisions are being made about the parents that will be used for the development of new resistant germplasm and mapping populations for identifying novel genes conferring resistance to major wheat diseases.

ACKNOWLEDGEMENTS

I would like to acknowledge everyone who has assisted me throughout my doctoral studies over the years. I express the deepest appreciation to my major advisor Dr. Steven S. Xu, for agreeing to serve as my advisor and for his encouragement, support, and guidance during my graduate study. I express my sincere appreciation to my graduate committee, Dr. Phillip E. McClean, Dr. Xiwen Cai, Dr. Justin D. Faris, and Dr. Marion O. Harris for their guidance and advice, and also for helping to edit my dissertation. I express my sincere appreciation to Dr. Guangmin Xia at Shandong University for giving me this apportunity to study abroad.

I also express my sincere thanks to Dr. Shiaoman Chao for her help with marker analysis, to Dr. Yue Jin, Dr. Maricelis Acevedo, Dr. Zhaohui Liu, and Dr. Timothy L. Friesen for their help with wheat disease testing, and to Dr. Samira Mafi Moghaddam, Dr. Sujan Mamidi, and Dr. Guotai Yu for their help with the data analysis. I extend my sincere thanks to Daryl L. Klindworth, Jason E. Axtman, Danielle J. Holmes, and Mary M. Osenga for their technical assistance. My thanks also go to Dr. Zhixia Niu, Dr. Chenggen Chu, and Dr. Qijun Zhang for their technical assistance in the lab. In addition, I am deeply grateful to Department of Plant Sciences, Program of Genomics and Bioinformatics, North Dakota State University (NDSU) for providing me with this great opportunity to pursue the Ph.D. degree. Specifically I wish to thank Dr. Richard D. Horsley, Dr. Michael C. Edwards, and Mrs. Eileen Buringrud for their support over the years. I thank the Chinese Scholarship Council (CSC) for funding my four years at NDSU. I am also grateful to Charles and Linda Moses for providing me with a scholarship.

I especially thank my amazing family for the love, support, and constant encouragement over the years. In particular, I thank my parents and my husband. I undoubtedly could not have done this dissertation without their support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

CHAPTER 1. GENERAL INTRODUCTION

Cultivated wheat is one of the world's three primary crops (i.e. wheat, maize, and rice) and provides food for 35% of the world's population (http://faostat.fao.org/). Due to the continuous growth of world population, it is necessary to improve wheat production to meet the increasing demands for food. The world's production of wheat could be increased by either expanding the area planted to wheat or by improving wheat yields. Because the planting area has decreased slightly in the past 20 years, it is expected that the greatest increase in wheat production will come from increasing yields. Effective control of the diseases and pests that attack wheat will be an important part of improving yield. It is estimated that up to 20% of global wheat yields is lost each year due to disease and pests (Oerke, 2006). There are four major diseases that currently threaten wheat production in many of the world's wheat-growing regions.

Wheat stem rust and leaf rust, caused by biotrophic fungal pathogens, *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & Henn. (*Pgt*) and *Puccinia triticina* Eriks., respectively, are historically important diseases that cause large losses in many of the world's most important wheat-growing regions. In the U.S., stem rust destroyed more than 20% of U.S. wheat crops on a number of occassions between 1917 and 1935 (Leonard and Szabo, 2005). From 2000 to 2004, leaf rust caused yield losses of about three million tons, valued at over $350 million (Huerta-Espino et al., 2011). While there are other strategies for managing stem rust and leaf rust, including eradication of alternate hosts and use of fungicides, resistant cultivars have proven to be the most economical and convenient approach. So far, 57 stem rust resistance (*Sr*) genes and 71 leaf rust resistance (*Lr*) genes have been identified in wheat and its related species (McIntosh et al., 2013). Many *Sr* and *Lr* genes have been deployed in the wheat cultivars and played the major roles in protecting wheat crop from stem rust and leaf rust (e.g. *Sr2*, *Sr13*, *Sr9*, *Sr31*, *Lr12*,

Lr21, *Lr27*, *Lr31*, and *Lr34*). Unfortunately, stem rust and leaf rust pathogens are known for their ability to evolve virulence to *Resistance* genes. This means that new virulence races are constantly emerging and threatening the *Sr* and *Lr* genes deployed in wheat cultivars. For example, the *Pgt* race TTKSK (known as Ug99), which was first detected in Uganda in 1999 (Pretorius et al., 2000), has virulence to most *Sr* genes that are currently deployed in wheat cultivars and therefore constitutes a major threat to global wheat production (Singh et al., 2011). Two new *P. triticina* races, TFBJQ and TFBGQ, both having virulence to *Lr21*, were detected in 2010 in the hard red spring wheat region of the U.S., and now pose a serious threat to wheat production (Kolmer and Anderson, 2011). Clearly, it is necessary to continue our search for new *Sr* and *Lr* genes.

Tan spot and Septoria nodorum blotch (SNB) are important wheat diseases caused by necrotrophic fungal pathogens, *Pyrenophora tritici-repentis* (Died.) Drechs. [anamorph *Drechslera tritici-repentis* (Died.) Shoem.] (Ptr) and *Parastagonospora* (syn. *ana, Stagonospora; teleo, Phaeosphaeria*) *nodorum* (Berk.) Quaedvleig, Verkley & Crous, respectively. Under conditions suitable for the development of pathogens, yield losses due to tan spot or SNB can reach up to 50% (Shabeer and Bockus, 1988; Nelson et al., 1974). Mechanisms of virulence differ for biotropic pathogens and necrotrophic pathogens. The virulence of necrotrophic fungi involves different host-specific/selective toxins (HSTs) (i.e. necrotrophic effectors, NE) and nonspecific toxins (Oliver and Solomon, 2010). The necrotrophic pathogen – wheat interaction system follows an inverse of the classical gene-for-gene relationships (Friesen and Faris, 2010). So far, three Ptr HSTs (Ptr ToxA, Ptr ToxB, and Ptr ToxC) and their corresponding sensitivity genes (*Tsn1*, *Tsc2*, and *Tsc1*) in wheat have been identified (Faris et al., 2010) and seven *P. nodorum* NEs (SnToxA, SnTox1, SnTox2, SnTox3, SnTox4, SnTox5, and

SnTox6) and their corresponding host sensitivity genes (*Tsn1*, *Snn1*, *Snn2*, *Snn3*, *Snn4*, *Snn5*, and *Snn6*) have been detected (Gao et al., 2015). Because Ptr and *P. nodorum* HSTs are major virulent factors, elimination of their sensitivity genes from wheat cultivars is the major strategy to improve wheat for resistance to tan spot and SNB. In addition to the host sensitivity genes, a number of quantitative trait loci (QTL) conferring resistances to tan spot (Faris and Friesen, 2005; Chu et al., 2008) and SNB (see reviews by Xu et al., 2004) have been identified in common wheat and synthetic wheat germplasm. Because the majority of current wheat cultivars are susceptible to both diseases (Lamari et al., 2005), identification of new host sensitivity genes to the Ptr and *P. nodorum* HSTs and novel QTL conferring resistance to the two pathogens in untapped germplasms will be useful for improving durum and common wheat for resistance to tan spot and SNB.

Relatives and ancestors of wheat have proven to be good places to find novel resistance to pathogens of wheat. In particular, cultivated emmer wheat (*Triticum turgidum* ssp. *dicoccom*) has proven a good source of resistance to stem rust and leaf rust. Many genes/alleles for resistance to stem rust (i.e. *Sr2*, *Sr9d*, *Sr9e*, *Sr13*, *Sr14*, and *Sr17*) and leaf rust (i.e. *Lr14a*, and *Lr27*) have been identified in cultivated emmer and transferred into common wheat and/or durum wheat (McIntosh et al., 2013). The fact that these *Sr* and *Lr* genes/alleles were derived from a small proportion of available cultivated emmer accessions suggests a strategy of exploration of the remaining larger proportion of cultivated emmer accessions, which are maintained in various genetic resource centers worldwide. For tan spot and SNB, several studies on cultivated emmer have shown a large degree of variation in resistance. However, no susceptibility and/or resistance genes for tan spot or SNB have been identified from cultivated emmer germplasm. In the U.S., there are 631 cultivated emmer accessions that are maintained in

the USDA-ARS National Plant Germplasm System (http://www.ars-grin.gov/cgi-bin/npgs/html/tax_acc.pl?taxno=314587&unavail=on&rownum=0). Clearly, these cultivated emmer accessions are a valuable genetic resource for improving wheat's resistance to biotic stress.

In recent years, association mapping, also named as linkage disequilibrium mapping or association analysis, has become an important strategy for identifying major genes and quantitative trait loci (QTL) in various crop species such as rice, maize, and soybean. Association mapping utilizes linkage disequilibrium among alleles at different loci to analyze the association between markers and characters and it could be used to identify functional genes/alleles controlling phenotypic traits (Gupta et al., 2005). I believe that the cultivated emmer accessions maintained in the USDA-ARS National Plant Germplasm System could be a useful population for association analysis of the resistance to stem rust, leaf rust, tan spot, SNB, and other major diseases of wheat.

The objectives of this study are to: 1) phenotypically evaluate a cultivated emmer wheat panel, including 180 accessions from USDA-ARS National Plant Germplasm System, for reactions to stem rust, leaf rust, tan spot, and SNB pathogens; 2) genotypically evaluate the emmer panel with 9K SNP array and analyze the linkage disequilibrium (LD) and population structure; 3) for the emmer panel, use genome-wide association analysis to identify SNPs that are significantly associated with phenotypic variation for the four diseases; and 4) identify LD blocks associated with disease resistance and allelic combinations of significant markers useful for improvement of wheat cultivars or varieties in wheat breeding activity.

Literature Cited

Chu, C.G., T.L. Friesen, S.S. Xu and J.D. Faris. 2008. Identification of novel tan spot resistance loci beyond the known host-selective toxin insensitivity genes in wheat. Theoretical and Applied Genetics 117: 873-881.

Faris, J.D. and T.L. Friesen. 2005. Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theoretical and Applied Genetics 111: 386-392.

Faris, J.D., Z. Zhang, H. Lu, S. Lu, L. Reddy, S. Cloutier, et al. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proceedings of the National Academy of Sciences 107: 13544-13549.

Friesen, T.L. and J.D. Faris. 2010. Characterization of the wheat-*Stagonospora nodorum* disease system: what is the molecular basis of this quantitative necrotrophic disease interaction? Canadian Journal of Plant Pathology 32: 20-28.

Gao, Y., J.D. Faris, Z. Liu, Y. Kim, R.A. Syme, et al. 2015. Identification and characterization of the SnTox6-*Snn6* interaction in the *Parastagonospora nodorum* - wheat pathosystem. Molecular Plant-Microbe Interactions. DOI: 10.1094/MPMI-12-14-0396-R.

Gupta, P.K., S. Rustgi and P.L. Kulwal. 2005. Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Molecular Biology 57: 461-485.

Huerta-Espino, J., R.P. Singh, S. Germán, B.D. McCallum, R.F. Park, W.Q. Chen, et al. 2011. Global status of wheat leaf rust caused by *Puccinia triticina*. Euphytica 179: 143-160.

Kolmer, J. A. and J.A. Anderson. 2011. First detection in North America of virulence in wheat leaf rust (*Puccinia triticina*) to seedling plants of wheat with *Lr21*. Plant Disease 95: 1032-1032.

Lamari, L., B.D. McCallum and R.M. dePauw. 2005. Forensic pathology of Canadian bread wheat: The case of tan spot. Phytopathology 95: 144-152.

Leonard, K.J. and L.J. Szabo. 2005. Stem rust of small grains and grasses caused by *Puccinia graminis*. Molecular Plant Pathology 6: 99-111.

McIntosh, R.A., Y. Yamazaki, J. Dubcovsky, J. Rogers, C. Morris, R. Appels, et al. 2013. Catalogue of gene symbols for wheat. 12th International Wheat Genetics Symposium, Yokohama, Japan

Nelson, L.R., D.D. Morey and A.R. Brown. 1974. Wheat cultivar responses to severe glume blotch in Georgia. Plant Disease Reporter 58: 21-23.

Oerke, E.-C. 2006. Crop losses to pests. The Journal of Agricultural Science 144: 31-43.

Oliver, R.P. and P.S. Solomon. 2010. New developments in pathogenicity and virulence of necrotrophs. Current Opinion in Plant Biology 13: 415-419.

Pretorius, Z.A., R.P. Singh, W.W. Wagoire and T.S. Payne. 2000. Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis*. f. sp. *tritici* in Uganda. Plant Disease 84: 203-203.

Shabeer, A. and W.W. Bockus. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. Plant Disease 72:599–602.

Singh, R.P., D.P. Hodson, J. Huerta-Espino, Y. Jin, S. Bhavani, P. Njau, et al. 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annual Review of Phytopathology 49: 465-481.

Xu, S.S., Friesen, T.L., and Cai, X. 2004. Sources and genetic control of resistance to Stagonospora nodorum blotch in wheat. In: Recent Research Development in Genetics and Breeding 1:449-469. Research Signpost, Kerala, India.

CHAPTER 2. LITERATURE REVIEW

Modern Cultivated Wheat

Taxonomic Classification

Modern cultivated wheat is classified in the genus *Triticum*, Tribe Triticeae, Family Poaceae. Based on the classification of van Slageren (1994), the genus *Triticum* includes six species, two species each for diploid, tetraploid, and hexaploid wheat (Table 2.1). There are a total of 17 subspecies having one or more of the four A, B, D, and G genomes (Table 2.1). Modern wheat crops consist primarily of two species, tetraploid durum wheat (*T. turgidum* ssp. *durum*, 2n = 4x = 28, AABB genomes) and hexaploid common wheat (*T. aestivum* L. ssp. *aestivum*, 2n = 6x = 42, AABBDD), which occupy 4% and 96% of wheat acreage, respectively (Gill et al., 2004).

Durum wheat and common wheat originated from interspecific or intergeneric hybridization of two or more genomes from different species. A spontaneous hybridization of diploid species *T. urartu* (AA) with a goatgrass species *Aegilops speltoides* Tausch (2*n* = 2*x* = 14, SS), followed by spontaneous chromosome doubling ca. 0.5 million years ago, formed tetraploid wild emmer wheat (*T. turgidum* ssp. *dicoccoides* (Körn.) Thell), which is the ancestor of modern durum wheat (Dvorak and Zhang, 1990; Dvorak et al., 1993). At about 8,000 years ago, common wheat arose from hybridization between tetraploid wheat with AB genomes (*T. turgidum*, 2n = 4x = 28, AABB) and the goatgrass *Aegilops tauchii* (Coss.) Schmal. (2n = 2x = 14, DD) (Faris, 2014).

Table 2.1. Classification of *Triticum* species according to van Slageren (1994).

Section and species	Ploidy	Genome
Section *Monococca* Flaksb.		
Triticum monococcum L.		
ssp. *monococcum*	2x	A^m
ssp. *aegilopoides* (Link) Thell.	2x	A
Triticum urartu Tumanian ex Gandilyan	2x	A
Section *Dicoccoidea* Flaksb.		
Triticum turgidum		
ssp. *turgidum*	4x	AB
ssp. *carthlicum* (Nevski in Kom.) Á.Löve & D.Löve	4x	AB
ssp. *dicoccum* (Schrank ex Schübler) Thell.	4x	AB
ssp. *durum* (Desf.) Husnot	4x	AB
ssp. *paleocolchicum* (Menabde) Á.Löve & D.Löve	4x	AB
ssp. *polonicum* (L.) Thell.	4x	AB
ssp. *turanicum* (Jakubz.) Á.Löve & D.Löve	4x	AB
ssp. *dicoccoides* (Körn. ex Asch. & Graebner) Thell.	4x	AB
Triticum timopheevii (Zhuk.) Zhuk.		
ssp. *timopheevii*	4x	AG
ssp. *armeniacum* (Jakubz.) MacKey	4x	AG
Section *Triticum*		
Triticum aestivum L.		
ssp. *aestivum*	6x	ABD
ssp. *compactum* (Host) MacKey	6x	ABD
ssp. *macha* (Dekapr. & Menabde) MacKey	6x	ABD
ssp. *spelta* (L.) Thell.	6x	ABD
ssp. *sphaerococcum* (Percival) MacKey	6x	ABD
Triticum zhukovskyi Menabde & Ericzjan	6x	A^mAG

Importance and Challenges

Cultivated wheat is one of the world's three primary food crops (i.e. wheat, maize, and rice). Among the three crops, wheat has the largest area of cultivation (220 million ha in 2012) and the most extensive geographical distribution (http://faostat.fao.org/). As the world's third largest crop (670 million tonnes in 2012), wheat provides food for 35% of the world's population (http://faostat.fao.org/). Due to the continuous growth of the world population, world wheat production must be increased to meet increasing food demand. This could be done by expanding acreage and/or by improving yield. Genetic improvement and modified breeding methods have

improved yields, from 25k Hg/Ha in 1993 to 31k Hg/Ha in 2012. On the other hand, acreage has slightly decreased in the past 20 years, from 223 million Ha in 1993 to 215 million Ha in 2012 (http://faostat.fao.org/). Clearly, recent increases in yield have come from improvements in wheat production rather than increases in acreage.

Increasing future wheat yields will be challenged by several factors, including climate change, reduced genetic diversity, and various abiotic and biotic stresses. For example, it has been estimated that, at the global scale, wheat production decreased by 5.5% due to climate change between the years of 1980 and 2008 (Lobell et al., 2011). As has also been the case for other major crops, wheat has undergone a dramatic reduction of genetic variability in the past century due to extensive utilization of elite cultivars (Niu et al., 2011). This has made it difficult to find new genes from adapted germplasm for high yield and many other important traits such as resistance to newly emerging diseases. The loss of genetic diversity in wheat germplasm also makes wheat vulnerable to the mutations in pest populations and environmental changes that induce critical crop losses (Reif et al., 2005). Major abiotic stresses such as drought, salinity, anoxia, low or high temperatures, mineral toxicities and deficiencies, and low nutrients all influence wheat production to different degrees (Collins et al., 2008). Biotic stresses include various pests and diseases. Collectively pests and diseases are major factors reducing yield worldwide. It is estimated that up to 20% (i.e. 140 million tonnes) of the global wheat yield is lost each year due to diseases and pests (Oerke, 2006). Although wheat diseases and pests can be controlled by deploying resistant cultivars, resistance can be defeated if the enemy evolves virulence. This makes it necessary for wheat scientists to continue their search for novel resistance genes in the untapped germplam of wheat and its relatives, and then deploy these genes into wheat cultivars to protect wheat yield.

Cultivated Emmer Wheat

An Ancient Cereal

Emmer wheat (*T. turgidum* ssp. *dicoccom*), known as cultivated emmer, is a hulled tetraploid wheat subspecies. It was domesticated from wild emmer wheat (*T. turgidum* ssp. *dicoccoides*) 10,000 years ago (Willcox, 1998) and is the oldest staple crop in the world. The geographical location of this first domestication has not been confirmed. Based on 226 wild and domesticated lines, Ozkan et al. (2002, 2005) suggested that the emmer wheat domestication first occurred either in the Karacadag region of southeastern Turkey at the northern Levant of Fertile Crescent and/or in the Sulaimaniya region of Iraq. Luo et al. (2007) suggested the domestication first occurred in the Karacadag region.

According to the archaeological evidence, cultivated emmer spread from its original site of domestication to Central Asia, Southwest Asia, Europe, Northeast Africa, the Arabian Peninsula, and the Indian Sub-continent (Zaharieva et al., 2010). The spread of cultivated emmer began in the seventh millennium B.C., when emmer spread to eastern Anatolia, northern Iraq, and southwestern Iran (Zaharieva et al., 2010). In the sixth millennium B.C., wild emmer was widely cultivated in the plains of Mesopotamia and western Anatolia. In the middle of the sixth millennium B.C., cultivated emmer spread to Turkmenistan (Harris et al., 1993). In the fifth millennium B.C., cultivated emmer spread to Egypt, Mediterranean Basin, Europe, and Central Asia. In the fourth millennium B.C., it reached India and Ethiopia (Feldman et al., 1976). Cultivated emmer was widely planted in Georgia by the end of the fourth millennium B.C., and in Armenia, Azerbaijan and Russia by the end of the third millennium B.C. (Dorofeev et al., 1979). While cultivated emmer was a major crop in the past, today it accounts for only a small proportion of the world's total wheat acreage and is mainly cultivated in regions that suffer from

drought, including mountainous areas of the former Yugoslavia, the Volga Basin, Iran, Yemen, India, Spain, Italy, Ethiopia, Eastern Turkey, Central Europe, and Transcaucasia (Stallknecht et al., 1996).

A Modern Cereal with Quality Advantage

Cultivated emmer can be used for making pasta product, which has an acceptable quality with rare stickiness, sufficient firmness, rare bulkiness, and light dark color (Cubbada and Marconi, 1996). However, the bread made from cultivated emmer has lower loaf volume and quality than common wheat (Cubbada and Marconi, 1996). In Italy, cultivated emmer is also called "*farro*" or "*farro medio*", which is characterized by high starch, mineral, protein, and fiber concentration, but having low fat concentration, so it is a healthy food source for patients with colonitis, hypersensitivity, and other diseases (Galterio et al., 2001; 2003).

The cultivated emmer grains generally have higher protein content (up to 18-23%) (Stehno, 2007; Damania et al., 1992); higher content of Li, Mg, P, Se, and Zn (Piergiovanni, 1997); higher contents of ashes, crude fiber, carbohydrate, and β-carotene (Bhuvaneshwari, 2001); and lower amylase content (Rodriguez-Quijano et al., 2003) than durum and common wheat. Therefore, the cultivated emmer possesses unique genes controlling high quality and is valuable genetic resource for improvement of quality traits in durum and common wheat breeding. Galterio et al. (2001) reported that the advanced lines (F_6 generations) derived from a cross between a emmer cultivar 'Molise' and a durum cultivar 'Ofanto' had increased protein and gluten contents and decreased starch content, which made these lines more suitable for making bread than their parents. They found that the advanced lines (F_6 generations) derived from a cross between 'Molise' and a durum cultivar 'Simeto' were more suitable for making pasta than their parents and '*farro*', the most used emmer material for making pasta. From the

cross between ‘Molise’ and ‘Simeto’, Galterio et al. (2003) obtained three new varieties, Davide, Mosè, and Padre Pio, which all had lower plant heights, higher yield, and larger seed weight than their parents. Among the three cultivars, Davide had lower SDS (sodium dodecyl sulphate) sedimentation value than its parents and it is appropriate for making soup and cookies, but Mosè and Padre Pio had much higher SDS sedimentation values than their parents and they are suitable for making pasta and bread (Galterio et al., 2003). These results suggest that cultivated emmer is an excellent genetic resource to broaden the genetic diversity of modern durum for quality and multiple uses.

A Good Source of Genes for Resistance to Biotic and Abiotic Stress

Cultivated emmer, having the same genomes as durum wheat, is an alternative genetic resource for durum wheat improvement. Because of this, the collections of cultivated emmer that are maintained in the world’s various gene banks have been extensively evaluated for resistance to various biotic and abiotic stresses. A number of cultivated emmer accessions have been shown to have resistance to various wheat diseases such as stinking smut, dusty smut, common bunt, stem rust, leaf rust, stripe or yellow rust, powdery mildew, Fusarium head blight, Septoria nodorum blotch, and tan spot (Corazza et al., 1986; Mithal and Kopper, 1990; Damania et al., 1992; Boguslavskij et al., 2000; Beteselassie et al., 2007; Oliver et al., 2008b; Chu et al., 2008a; Olivera et al., 2012). A number of major genes, such as *Sr2*, *Sr9d, Sr9e*, *Sr13, Sr14,* and *Sr17* for stem rust resistance, *Lr14a* and *Lr27* for leaf rust resistance, and *Pm4a* and *Pm5a* for powdery mildew resistance, have been successfully transferred into durum and common wheat (McFadden, 1930; Knott, 1962; McIntosh et al., 1967; Sunderwirth and Roelfs, 1980; Bennett, 1984). Several of these genes, such as *Sr2*, *Sr9d*, *Sr13*, *Lr14a*, and *Lr27,* have been extensively used in durum and common wheat breeding and production.

In addition to resistance to the major wheat diseases, emmer is also a source for resistance to insects and tolerance to abiotic stresses. Lage et al. (2004) produced a synthetic hexaploid wheat line with resistance to Russian wheat aphid (Homoptera: Aphididae) from a cross between a resistant cultivated emmer accession and a susceptible *Aegilops tauschii* accession. Liu et al. (2005) transferred a Hessian fly resistance gene, designated as *Hdic*, from cultivated emmer accession PI 94641 to common wheat. For tolerance to abiotic stress, Sayed (1985) evaluated 37 cultivated emmer accessions for salt tolerance and found that cultivated emmer had a higher proportion (16%) of tolerant accessions than any other wheat species tested under conditions of a high NaCl concentration. Xiong et al. (2006) evaluated two cultivated emmer accessions for drought tolerance and found that both had intermediate levels of drought tolerance compared with four other diploid and hexaploid wheat accessions.

Wheat Stem Rust

Wheat Stem Rust and Its Pathogen

Wheat stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & Henn. (*Pgt*), is a serious disease of wheat in almost all wheat-growing regions of the world. In Europe and Australia, stem rust epidemics have occurred several times in the 20th century and caused serious yield losses. In the U.S., stem rust destroyed more than 20% of U.S. wheat crops several times between 1917 and 1935. Losses reached 10% in the spring wheat region of Minnesota, North Dakota, and South Dakota in eight of 40 years between 1920 and 1960 (Leonard and Szabo, 2005).

In 1794, Christiaan H. Persoon designated the stem rust pathogen as *Puccinia graminis* Pers. (Schafer et al., 1984). The wheat stem rust pathogen is *P. graminis* f. sp. *tritici* (*Pgt*), which is one of the six formae speciales (f. sp.) of stem rust for different host plant species (Leonard

and Szabo, 2005). The wheat stem rust pathogen includes different races or pathotypes, which were originally designated using three letters by Roelfs and Martens (1988). Jin et al. (2008a) modified the nomenclature system to five letters to accommodate newly-identified races. In the new nomenclature system, 20 differential lines carrying individual *Sr* genes are divided into five differential sets with four differentials in each set. A letter code is given to a certain infection type (IT) to the four differentials in each set. The letter code indicates levels of virulence to the differentials in each set, for example, for the same differential set a race with a higher letter code T can infect more differentials than that with a lower letter code B (Roelfs and Martens, 1988). So, each race is represented as a combination of five letter codes (Jin et al., 2008a). For example, the Ug99 was designated as TTKSK and the first letter T indicates a high IT to the first differential set.

Puccinia graminis f. sp. *tritici* is an obligate biotrophic and heteroecious fungus (Roelfs, 1985; Roelfs et al., 1992; Leonard and Szabo, 2005). Its life cycle on wheat is a non-sexual reproductive cycle. In its life cycle, the pathogen has five spore stages, including urediniospores, teliospores, basidiospores, pycniospores, and aeciospores (Roelfs, 1985; Leonard and Szabo, 2005). *Puccinia graminis* f. sp. *tritici* has an 88.6 Mb-sized genome (Duplessis et al., 2011), which includes 18 chromosomes (Boehm et al., 1992). In the *Pgt* genome, the *Avirulence* gene (*Avr*) controls the phenotype of avirulence or virulence to wheat, and the *Avr* genes are inherited based on Mendelian genetics (Johnson and Newton, 1940; Johnson, 1954; Loegering and Powers, 1962; Zambino et al., 2000). Many *Avr* genes have been mapped using a large number of molecular markers developed from the *Pgt* genome (Zambino et al., 2000; Zhong et al., 2009). The interactions between the *Pgt Avirulence* genes and the *Sr* genes in wheat fit the classical gene-for-gene model established by Flor (1971).

Stem Rust Resistance in Wheat

The host plants of stem rust include 28 species in eight genera under natural conditions and 78 species in 34 genera under artificial inoculation (Anikster, 1984). There are two types of host resistances, race-specific and race non-specific resistance. The genetic basis of race-specific resistance follows the gene-for-gene model, i.e., a single *Sr* gene in the host corresponds with a single *Avr* gene in the pathogen (Dyck and Kerber, 1985; Singh et al., 2011b). The race non-specific resistance is conferred by multiple genes and is considered to be the 'adult plant resistance (APR)' (Roelfs et al., 1992; Singh et al., 2011b), which shows lightly susceptible reactions in both of the seedling and adult stages (Singh et al., 2011a). So far, 57 *Sr* genes, designated *Sr1* through *Sr57*, have been identified in wheat and its related species (McIntosh et al., 2013). Except for the four genes conferring 'adult plant resistance' (*Sr2*, *Sr55*, *Sr56*, and *Sr57*), most of the *Sr* genes are race-specific.

Deployment of *Sr* genes into wheat cultivars is an effective method for controlling stem rust (Roelfs, 1985; Roelfs et al., 1992; Leonard and Szabo, 2005). Since the 1950s, several groups of wheat cultivars carrying different numbers of *Sr* genes were used but were eventually replaced by other higher-yielding resistant cultivars. The utilized cultivars included 'Selkirk' (carrying *Sr2*, *Sr6*, *Sr7b*, *Sr9d*, *Sr17*, and *Sr23*) (Roelfs, 1985), 'Era' (carrying *Sr5*, *Sr 6*, *Sr12*, and *Sr17*) (Roelfs et al., 1992), and 'Waldron' (carrying *Sr5*, *Sr11*, and *SrWld-1*) (Riede et al., 1995a), which were all considered to be landmarks in the history of wheat resistance to stem rust. Many other important *Sr* genes were also utilized, for example, *Sr26* deployed in Australia since 1972 (Roelfs et al., 1992), *Sr24*, *Sr31*, and *Sr42* deployed in bread wheat worldwide, and *Sr13* deployed in durum cultivars in the United States.

Ug99 Race Group and Its Control

Wheat stem rust had been effectively controlled for almost 30 years by the deployment of *Sr* genes in the cultivars worldwide until a new *Pgt* race virulent to *Sr31* was detected in Uganda in 1999. The race was initially designated as Ug99 based on the country and year of the first detection (Pretorius et al., 2000). After it was tested for virulence to the differentials, it was re-designated as TTKSK based on the North American nomenclature system (Jin et al., 2008a). Ug99 spread rapidly from the highlands of East Africa to other wheat-growing regions such as Yemen (Jin et al., 2008a) and Iran (Nazari et al., 2009). Several new variants of TTKSK have been detected in the past 15 years, such as TTKST (virulent to *Sr24*), TTTSK (virulent to *Sr36*), TTKSF (virulent to *Sr21*), TTKSP (virulent to *Sr31*, *Sr21*, and *Sr24*), PTKSK, PTKST, and TTKSF+ (Jin et al., 2008, 2009; Hodson et al., 2012). Therefore, the Ug99 race group is evolving virulence against many of the *Sr* genes that are currently deployed in wheat cultivars around the world (Singh et al., 2011; Jin and Singh, 2006). Only a small number of the wheat cultivars that were developed before 2007 were found to be resistant to the Ug99 race group (Singh et al., 2011a; Jin and Singh, 2006).

The Ug99 race group poses a serious threat to global wheat production, both in Africa and in other continients where Ug99 eventually spread (Singh et al., 2011a). To address the Ug99 threat, a global effort, under the coordination of the Borlaug Global Rust Initiative (http://www.globalrust.org/traction), was initiated to search for Ug99-effective *Sr* genes in wheat and its relatives (Yu et al., 2015). So far, at least 31 *Sr* genes catalogued in wheat have been identified as conferring resistance against TTKSK and/or its variants (Singh et al., 2011a; McIntosh et al., 2013) (Table 2.2). Among them, 15 *Sr* genes, including *Sr2, Sr9h/SrWeb, Sr13*, *Sr22*, *Sr28*, *Sr29*, *Sr33*, *Sr35*, *Sr42*, *Sr45*, *Sr46*, *Sr48*, *Sr55*, *Sr56*, and *Sr57*, were derived from

the wheat A, B, and D genomes in durum wheat, common wheat, and their progenitors such as *T. turgidum* ssp. *dicoccum*, *T. monococcum*, and *Ae. tauschii* in the primary gene pool. The other 16 *Sr* genes, including *Sr24*, *Sr25*, *Sr26*, *Sr27*, *Sr32*, *Sr36*, *Sr37*, *Sr39*, *Sr40*, *Sr43*, *Sr44*, *Sr47*, *Sr50*, *Sr51, Sr52*, and *Sr53*, were transferred from alien genomes in the wild grass species in the secondary and tertiary gene pools of wheat (Dundas et al., 2007; Anugrahwati et al., 2008; Liu et al., 2011a, b, 2013; Niu et al., 2011, 2014; Qi et al., 2011; Klindworth et al., 2012; Mago et al., 2013; McIntosh et al., 2013).

Table 2.2. Chromosome location and original source of *Sr* genes resistant to TTKSK.

Sr gene	Chr loc[a]	Original source	References
Sr2	3BS	*T. turgidum* ssp. *dicoccum*	Knott (1968)
Sr9h/SrWeb	2BL	*T. aestivum* ssp. *aestivum*	Rouse et al. (2014)
Sr13	6AL	*T. turgidum* ssp. *dicoccum*	Knott (1962)
Sr22	7AL	*T. monococcum* ssp. *monococcum*	The (1973)
Sr24	3DL	*Th. ponticum*	McIntosh et al. (1976)
Sr25	7DL	*Th. ponticum*	McIntosh et al. (1976)
Sr26	6AL	*Th. ponticum*	McIntosh et al. (1976)
Sr27	3A	*S. cereale*	Marais and Marais (1994)
Sr28	2BL	*T. aestivum* ssp. *aestivum*	Rouse et al. (2012)
Sr29	6DL	*T. aestivum* ssp. *aestivum*	Dyck and Kerber (1977)
Sr32	2A, 2B, 2D	*Ae. speltoides*	McIntosh et al. (1995)
Sr33	1DS	*Ae. tauschii*	Jones et al. (1991)
Sr35	3AL	*T. monococcum* ssp. *monococcum*	Saintenac et al. (2013)
Sr36	2BS	*T. timopheevii* ssp. *timopheevii*	McIntosh et al. (1995)
Sr37	4BL	*T. timopheevii* ssp. *timopheevii*	McIntosh et al. (1995)
Sr39	2BS	*Ae. speltoides*	Niu et al. (2011)
Sr40	2BS	*T. timopheevii* ssp. *armeniacum*	Dyck (1992)
Sr42/SrCad	6DS	*T. aestivum* ssp. *aestivum*	Hiebert et al. (2011)
Sr43	7DS	*Th. ponticum*	Niu et al. (2014)
Sr44	7DS	*Th. intermedium*	Liu et al. (2013)
Sr45	1DS	*Ae. tauschii*	Marais et al. (1998)
Sr46	2DS	*Ae. tauschii*	Yu et al. (2015)
Sr47	2BL	*Ae. speltoides*	Klindworth et al. (2012)
Sr48	2AL	*T. aestivum* ssp. *aestivum*	Bansal et al. (2009)
Sr50	1DS	*S. cereale*	Anugrahwati et al. (2008)
Sr51	3A, 3B, 3D	*Ae. searsii*	Liu et al. (2011a)
Sr52	6AS	*D. villosum*	Qi et al. (2011)
Sr53	5D	*Ae. geniculata*	Liu et al. (2011b)
Sr55	4DL	*T. aestivum* ssp. *aestivum*	Herrera-Foessel et al. (2014)
Sr56	5BL	*T. aestivum* ssp. *aestivum*	Bansal et al. (2008)
Sr57	7DS	*T. aestivum* ssp. *aestivum*	Singh et al. (in preparation)

[a]Chromosomal location.

In addition to the wheat cultivars and lines carrying the known TTKSK-effective *Sr* genes, a large number of Ug99-resistant lines with unknown *Sr* genes have been identified in durum wheat, bread wheat, and related species. Xu et al. (2009) evaluated 62 wheat-alien species derivatives from the crosses between common or durum wheat and eight alien species, including *Thinopyrum junceum*, *Th. intermedium*, *Th. bessarabicum*, *Th. elongatum*, *Th. ponticum*, *Elymus rectisetus*, *Ae. caudata*, and *Ae. speltoides,* for seedling reactions to TTKSK, TTKST, and TTTSK, and identified 30 lines that were resistant to all the three races. They inferred that 12 of the 30 lines may have novel *Sr* genes. Rouse et al. (2011b) evaluated 456 *Ae. tauschii* accessions with several *Pgt* races, including TTKSK, and identified 98 accessions with resistance to TTKSK. Several other germplasm evaluation studies also showed that there are rich sources of TTKSK resistance in the collections of *T. monococcum, T. urartu*, *T. turgidum* subsp. *dicoccoides*, *T. turgidum* subsp. *dicoccum*, *T. timopheevii*, *Ae. speltoides*, *Ae. sharonensis*, triticale, and various *Thinopyrum* species (Steffenson et al., 2007; Jin et al., 2009; Olivera et al., 2012; Zheng et al., 2014).

Wheat cultivars and lines carrying the known and unknown Ug99-effective *Sr* genes have provided important genetic resources for wheat breeding programs to develop Ug99-resistant cultivars for target regions. Since 2007, the major wheat breeding programs in CIMMYT, Eastern Africa, Southern Asia, and the Middle East have been actively utilizing the available Ug99-resistance genes in their wheat breeding. From 2009 to 2010, CIMMYT released several Ug99 resistant varieties such as ‘Koshan 09’, ‘Muqawim 09’, ‘Baghlan 09’, and ‘Chonte #1’ in Afghanistan and ‘Danda’ and ‘Kakaba’ in Ethiopia. (Singh et al., 2011a). However, these varieties, which have *Sr2* only, or *Sr2* combined with either *SrTmp* or *Sr25*, cannot provide wheat cultivars with adequate resistance to many other *Pgt* races. In order to overcome this

problem, new Ug99-resistant varieties have now been developed by the gene-pyramiding strategy and have been released in several African countries (Njau et al., 2013).

As the ongoing global effort to combat Ug99 threat continues, it is expected that other *Sr* genes and their linked markers will soon be identified (Bowden, 2013). This will make possible the development of additional Ug99-resistant cultivars with novel combinations of Ug99 resistance genes. In summary, it is anticipated that the African stem rust epidemic caused by Ug99 will be controlled simply by growing Ug99-resistant cultivars, with this also eliminating the global threat from Ug99.

Wheat Leaf Rust

Wheat Leaf Rust and Its Pathogen

Leaf rust or brown rust, caused by *Puccinia triticina* Eriks., is one of the most common and destructive diseases of wheat (Anikster et al., 1997). Although leaf rust is less damaging than stem rust and stripe rust, it causes higher yield losses than the other two rust diseases due to its frequent and widespread occurrence in most of the wheat growing areas in the world (Huerta-Espino et al., 2011). In the U.S., leaf rust caused yield losses of about three million tons (worth over $350 million) from 2000 to 2004 (Huerta-Espino et al., 2011). It was estimated that losses owing to leaf rust in Canada could have reached up to 10% of total yield per year between 2000 and 2009 (Huerta-Espino et al., 2011). In northwestern Mexico, the recent outbreaks of leaf rust caused by a new race, BBG/BN, resulted in heavy yield losses of durum wheat, which were worth about $32 million during from 2000 to 2003 (Singh et al., 2004a).

Like wheat stem rust pathogen, *P. triticina* is also a biotrophic and heteroecious fungus, which obtains nutrients from living host cells and needs two taxonomically unrelated hosts in a complete life cycle (Mendgen and Hahn, 2002; Kolmer, 2013). In its life cycle, *P. triticina* has

three stages (urediniospores, teliospores, and basidiospores) on wheat and two stages (pycniospores and aeciospores) on its alternative host *Thalictrum speciosissimum* L. (Kolmer, 2013). Similar to stem rust pathogen, *P. triticina* isolates have been grouped into different races according to their reactions to a number of differential lines carrying different combinations of resistance genes (Huerta-Espino et al., 2011). However, unlike stem rust pathogen, a common nomenclature system of races or pathotypes has not been established for *P. triticina*, and several different nomenclature systems have been utilized for race designations in different countries or regions (Huerta-Espino et al., 2011). A system commonly used in the U.S. and Canada is similar to the system of stem rust pathogen (Long and Kolmer, 1989). This system originally included 12 differential lines, which were grouped into three differential sets, with each set containing four lines (Long and Kolmer, 1989). According to this system, a *P. triticina* race can be designated using a three-letter code, which represents the pathogenicity of the particular race to the three differential sets. The letter codes included letters from a lower letter code B to a higher letter code T without the vowels. The lower letter code indicates a lower infection type to a differential set, and vice versa (Long and Kolmer, 1989). As more races have been identified, supplementary differential sets have been added to this system. In different countries, different supplementary differential sets were added to the original three sets. So far, two supplementary differential sets have been added to the original U.S. nomenclature system.

Globally, *P. triticina* populations have a high degree of genetic diversity (Huerta-Espino, 1992). For instance, up to 70 races have been identified annually in the U.S. (Kolmer et al., 2007), with an average of 35 races detected annually in Canada (Wang et al., 2010). The genetic diversity and relationship of *P. triticina* populations have been extensively analyzed using various molecular markers such as random amplified DNA polymorphism (RAPD), amplified

fragment length polymorphism (AFLP), and simple sequence repeat (SSR) polymorphism (Kolmer et al., 1995; Kolmer, 2001; Szabo and Kolmer, 2007). The *P. triticina* races in North America were classified into six groups, including NA-1 to NA-6, based on the SSR marker genotyping analysis (Ordoñez and Kolmer, 2009). Ordoñez and Kolmer (2007) also found that the isolate collections of *P. triticina* from durum wheat from Europe, South America, and North America had similar virulence phenotypes, indicating that isolates from these regions might have a common origin.

Leaf Rust Resistance in Wheat

Ausemus (1946) designated the first three leaf rust resistance (*Lr*) genes, *Lr1, Lr2*, and *Lr3*. Subsequently, extensive effort in the wheat community has been devoted to identify and map new *Lr* genes. To date, over 100 *Lr* genes and numerous QTL have been identified in wheat and related species such as *Ae. tauschii*, *Th. elongatum*, *Ae. umbellulata*, and *S. cereale* (Kolmer, 2013). Seventy-one *Lr* genes have been designated *Lr1- Lr71* (McIntosh et al., 2013). Most of the *Lr* genes have been mapped with molecular markers and four genes (i.e. *Lr1, Lr10, Lr21*, and *Lr34*) have been cloned (Cloutier et al., 2007; Feuillet et al., 2003; Huang et al., 2003; Krattinger et al., 2009). Most of the *Lr* genes are effective in both seedling and adult stages, such as the race specific genes *Lr1, Lr10,* and *Lr21*. But there are also some *Lr* genes that only express resistance during the adult plant stage, such as race-specific genes *Lr12, Lr13, Lr22a,* and *Lr37*, and race non-specific genes *Lr34, Lr46, Lr67,* and *Lr68* (Kolmer, 2013).

To utilize the *Lr* genes in control leaf rust, the most effective approach is to pyramid several *Lr* genes. In contrast to cultivars having only race-specific *Lr* genes, which can lose their resistances in a few years, cultivars with combinations of race non-specific *Lr* genes are more durable, having a longer period over which resistance remains effective (Kolmer, 2013). The

race non-specific genes *Lr34*, *Lr46*, *Lr67*, and *Lr68* are also known as "slow rusting genes". These genes only confer partial resistance but it is more durable and includes resistance during the adult plant stage to all known races of *P. triticina* (Caldwell, 1968; Kolmer, 2013). The "slow rusting genes" are valuable in breeding programs because *P. triticina* is always evolving virulence to race specific genes (Huerta-Espino et al., 2011). On the other hand, the resistance conferred by race specific genes is much stronger than that conferred by "slow rusting" genes (Caldwell, 1968). Thus, a single "slow rusting" gene cannot provide long-lasting resistance. Therefore, the better strategy is to combine several "slow rusting" genes together to achieve a high level of resistance, or to add "slow rusting" genes as a complement to race-specific genes for durable resistance (Kolmer, 2013). Many wheat cultivars around world currently carry *Lr34* and several other race-specific *Lr* genes (Zhang et al., 2008) and these cultivars will be useful for developing new cultivars by introducing additional *Lr* genes.

Tan Spot and Septoria Nodorum Blotch

Based on the mode of the infection, fungal pathogens can be divided into two groups: biotrophic and necrotrophic pathogens (Oliver and Ipcho, 2004). Necrotrophic pathogens kill plant tissue during colonization and then feed on nutrients from the dead tissues that they have helped to create. Biotrophic pathogens get their nutrition from the living host cells and do not kill the host cells during colonization or while feeding (Oliver and Ipcho, 2004). Recently, necrotrophic fungal pathogens have caused increased losses globally. This increase is associated with the increased use of reduced tillage. Necrotrophic pathogens overwinter in wheat stubble and therefore benefit from reduced tillage (Faris et al., 2013; Oliver and Solomon, 2010). Another problem is the lack of known *Resistance* genes for necrotrophic pathogens (Oliver and Solomon, 2010). Clearly it is of great importance to identify new sources of resistance to

necrotrophic pathogens. The following part of this review will focus on two necrotrophic fungal pathogens that cause important wheat diseases, i.e., tan spot and Septoria nodorum blotch (SNB).

Tan Spot and Its Pathogen

In wheat, tan spot (also named yellow spot or yellow leaf blotch) is caused by *Pyrenophora tritici-repentis* (Died.) Drechs (Ptr). [anamorph *Drechslera tritici-repentis* (Died.) Shoem.] (Krupinsky, 1982; De Wolf et al., 1998; Faris et al., 2013; Singh et al., 2010). Schilder and Bergstrom (1992) showed that the tan spot life cycle involves a sexual stage producing ascospores and an asexual stage producing conidia. On the leaves of susceptible genotypes, the fungus induces an eye spot-like lesion, having a tan colored necrotic spot in the center, which is surrounded by a chlorotic zone that is yellow (Singh et al., 2010). Based on a two-year field experiment, tan spot pathogens were shown to cause 5-10% yield losses on average per year; however, under conditions suitable for disease development, losses reached up to 50% (Shabeer and Bockus, 1988).

Different tan spot isolates induced either necrotic or chlorotic symptoms that were genetically distinct on different host genotypes (Lamari and Bernier, 1989a, b). Lamari et al. (1995, 2003) assigned the tan spot isolates to eight races (numbered 1 to 8). This was based on the host-selective toxins (HSTs) that they produce and the necrotic or chlorotic symptoms they produced on a differential set of wheat cultivars that included Salamouni, Glenlea, 6B662, and 6B365 (Table 2.3). Except for the eight races in Table 2.3, the isolates that caused different necrotic or chlorotic symptoms on some of the differentials also were reported (Ali and Francl, 2002; Ali et al., 2002; Manning et al., 2002). Moreno et al. (2008) identified the isolates producing chlorosis on Glenlea.

Table 2.3. The eight current *P. tritici-repentis* races and the host-selective toxins that they produce (Source: Faris et al., 2013).

Race	HSTs produced	Symptoms on host differentials			
		Salamouni	Glenlea	6B662	6B365
1	Ptr ToxA PtrToxC	-	Necrosis	-	Chlorosis
2	Ptr ToxA	-	Necrosis	-	-
3	Ptr ToxC	-	-	-	Chlorosis
4	None	-	-	-	-
5	Ptr ToxB	-	-	Chlorosis	-
6	Ptr ToxB Ptr ToxC	-	-	Chlorosis	Chlorosis
7	Ptr ToxA Ptr ToxB	-	Necrosis	Chlorosis	-
8	Ptr ToxA Ptr ToxB Ptr ToxC	-	Necrosis	Chlorosis	Chlorosis

Septoria Nodorum Blotch and Its Pathogen

Septoria nodorum blotch (SNB), caused by *Parastagonospora* (syn. *ana, Stagonospora; teleo, Phaeosphaeria*) *nodorum* (Berk.) Quaedvleig, Verkley & Crous, is a second necrotrophic worldwide disease of wheat. It causes substantial yield losses due to the damage caused by leaf and glume blotch (Weber, 1922; Machacek, 1945; Quaedvlieg et al., 2013). It has been estimated that SNB causes yield losses of up to 30-50%, which happened in the southeastern U.S. (Scharen and Krupinsky, 1969; Nelson et al., 1974). In Australia, especially Western Australia, it can cause 5% average losses (>AUD $100 million) per year (Murray and Brennan, 2009). *P. nodorum* is a heterothallic species, with sexual reproduction controlled by a diallelic mating-type locus (Bennett et al., 2003). Because of gene flow and sexual recombination, there are high levels of genetic and genotypic diversity between and within geographic populations of *P. nodorum* (McDonald et al., 1994; Keller et al., 1997a, b). The genome sequence of *P. nodorum* was published in 2007 (Hane et al., 2007), with estimates of 12,382 nuclear genes (Oliver et al., 2012). Because of the less specificity in the wheat - *P. nodorum* pathosystem compared with

other fungi (Eyal, 1999), there has not been a taxonomic system to group *P. nodorum* isolates or strains into physiological races.

Host Selective Toxins (HSTs)

The virulence of necrotrophic fungi is related to host-specific/selective toxins and nonspecific toxins (Oliver and Solomon, 2010). The identifications of HSTs, also called necrotrophic effectors (NEs), was a significant advance toward understanding the necrotrophic pathogen-wheat interaction system, which follows a relationship that is the inverse of the classical gene-for-gene relationship between a host and a biotrophic pathogen (Friesen and Faris, 2010). A compatible interaction between necrotrophic pathogens and wheat involves the recognition of HSTs by the product of a host susceptibility gene, and results in the susceptible reaction of the host plant (Friesen and Faris, 2010). However, as more non-race-specific resistance QTL have been detected (Faris and Friesen, 2005; Faris et al., 2012; Francki et al., 2011), it has become clear that the inverse gene-for-gene model does not fully explain all of the interactions that occur between wheat and necrotrophic pathogens.

So far, three Ptr HSTs have been identified and designated as Ptr ToxA, Ptr ToxB, and Ptr ToxC (Tomás et al., 1990; Orolaza et al., 1995; Strelkov et al., 1999; Effertz et al., 2002). Ptr ToxA induces necrosis in susceptible wheat genotypes while Ptr ToxB and Ptr ToxC induce chlorosis (Table 2.3) (Strelkov and Lamari, 2003). Ptr ToxA and Ptr ToxB have been isolated and well-characterized, and the fungal genes that are responsible for the production of the two HSTs have been cloned; while Ptr ToxC has not been purified and the controlled gene(s) have not been cloned (Ballance et al., 1996; Ciuffetti et al., 1997; Martinez et al., 2001; Ciuffetti et al., 2010). Ptr ToxA produced by fungal gene *ToxA* in race 1, 2, 7 and 8 is a small 13.2-kDa protein (Lamari et al., 2003; Ballance et al., 1996; Ciuffetti et al., 1997). Host sensitivity to Ptr ToxA is

conferred by a single dominant gene *Tsn1* (Lamari and Bernier, 1989b) on chromosome 5BL (Faris et al., 1996, 2010; Anderson et al., 1999). Ptr ToxB is a small 6.6-kDa protein secreted by races 5, 6, 7, and 8, and is encoded by a multicopy gene *ToxB* (Martinez et al. 2001; Lamari et al., 2003). Ptr ToxB sensitivity gene *Tsc2,* which accounted for 69% of the phenotypic variance caused by race 5, has been mapped to chromosome 2BS (Friesen and Faris, 2004). Unlike the proteinaceous toxins Ptr ToxA and Ptr ToxB, Ptr ToxC from a race 1 isolate is a nonionic, polar, and low molecular weight molecule (Effertz et al., 2002). The host Ptr ToxC sensitivity gene, designated as *Tsc1,* was mapped to the short arm of chromosome 1A (Effertz et al., 2002). The mode of inheritance of *Tsc1* is not known (Faris et al., 2013).

For *P. nodorum*, seven proteinaceous necrosis-inducing HSTs (SnToxA, SnTox1, SnTox2, SnTox3, SnTox4, SnTox5, and SnTox6) have been identified (Liu et al., 2004a; Friesen et al., 2006, 2007, 2008, 2012; Abeysekara et al., 2009; Gao et al., 2015). All seven HSTs are small proteinaceous molecules with an estimated size of 10 to 30 kD (Oliver et al., 2012; Gao et al., 2015). The major genes for three NEs, SnToxA, SnTox1, and SnTox3, have been cloned, and were identified in estimated 36%, 85%, and 59% of worldwide *P. nodorum* isolates, respectively (Liu et al., 2009, 2012). Seven host genes, *Tsn1*, *Snn1*, *Snn2*, *Snn3*, *Snn4*, *Snn5*, and *Snn6,* confer sensitivity to the seven HSTs so far identified in wheat. Friesen et al. (2006) found that *SnToxA* gene for the SnToxA of *P. nodorum* has a 99.7% sequence similarity to the Ptr *ToxA* gene for the Ptr ToxA of tan spot. Like Ptr ToxA, SnToxA also interacts with the wheat gene *Tsn1* following the inverse gene-for-gene model. They deduced that Ptr virulence was derived from an interspecific gene transfer of *ToxA* from *P. nodorum* to Ptr (Friesen et al., 2006). For toxin SnTox3, two sensitivity genes *Snn3-B1* and *Snn3-D1* were identified in wheat and *Ae. tauschii*, respectively, and they were mapped to chromosome arm 5BS and 5DS, respectively (Friesen et

al., 2008; Zhang et al., 2011). The other five sensitivity genes (*Snn1, Snn2, Snn4, Snn5,* and *Snn6*) were mapped to chromosome arm 1BS, 2DS, 1AS, 4BL, and 6AL, respectively (Oliver et al., 2012; Friesen et al., 2012; Gao et al., 2015). The eight interactions, including SnToxA-*Tsn1*, SnTox1-*Snn1*, SnTox2-*Snn2*, SnTox3-*Snn3-B1*, SnTox3-*Snn3-D1*, SnTox4-*Snn4*, SnTox5-*Snn5*, and SnTox6-*Snn6* explained up to 95%, 58%, 47%, 18%, 100%, 41%, 63%, and 27% of the observed phenotypic variation, respectively (Oliver et al., 2012; Friesen et al., 2012; Gao et al., 2015).

Tan Spot and Septoria Nodorum Blotch Resistance in Wheat

Tan spot and Septoria nodorum blotch have been an increasing problem in recent years in many wheat-growing regions of the world (Xu et al., 2004). Because the majority of past and current wheat cultivars are susceptible to the two necrotrophic pathogens (Lamari et al., 2005; Singh et al., 2006c, d; Tadesse et al., 2006b), numerous efforts have been made by the wheat community to search for sources of resistance. High levels of resistance to the two diseases have been detected in numerous bread wheat (Mergoum et al., 2007; Rees and Platz, 1990; Singh et al., 2006c, d; Tadesse et al., 2006b), tetraploid wheat (Chu et al., 2008a), *Ae. tauschii* (Cox et al., 1992; Siedler et al., 1994), and synthetic hexaploid wheat (SHW) (Xu et al., 2004; Friesen et al., 2008; Morris et al., 2010) germplasm collections and various wheat-alien species derivatives (Oliver et al., 2008).

Resistance to tan spot in wheat can be qualitatively or quantitatively inherited (Singh et al., 2010). Except for the three HST insensitivity genes, *tsn1*, *tsc1*, and *tsc2*, qualitative inheritance involves several other genes (e.g. *tsr2 to tsr5*) conferring resistance to tan spot in wheat (Faris et al., 2013). Genes having the designation '*Tsr*' for '*Tan spot resistance*' that were identified through conidial inoculation are different from '*Tsn*' genes for '*Tan spot necrosis*' and

'*Tsc*' for '*Tan spot chlorosis*' (McIntosh et al., 2013). In the '*Tsr*' designation system, *tsr1*and *tsr6* are synonymous with *tsn1* and *tsc2*, respectively, but *Tsc1* does not have a synonymous *Tsr* designation because of its unknown mode of inheritance (Faris et al., 2013). Other four *tsr* genes, *tsr2* through *tsr5*, are all recessive and are located on chromosome arms 3BL, 3DS, 3AS, and 3BL, respectively (see review by Faris et al., 2013). The genes *tsr2* and *tsr5* confer resistance to necrosis caused by races 3 and 5, respectively, in tetraploid wheat (Gamba and Lamari, 1998; Singh et al., 2006b). Genes *tsr3* and *tsr4* confer resistance to race 1 and were found in a synthetic hexaploid wheat line and hexaploid landrace 'Salamouni', respectively (Tadesse et al., 2006a, 2006b, 2007). The inheritance mode of tan spot resistance has also been reported as being quantitative (Elias et al., 1989; Nagle et al., 1982). Several QTL conferring race specific or race non-specific resistance have been identified in the wheat genome. Faris et al. (1997) identified a QTL (*QTsc.ndsu-1A*) conferring resistance to chlorosis induced by race 1 and 3. Faris and Friesen (2005) identified two QTL (*QTs.fcu-1B* and *QTs.fcu-3B*) conferring resistance to four races (1, 2, 3, and 5) that were tested.

Resistance to SNB can also be inherited qualitatively or quantitatively (Oliver et al., 2012). As reviewed above for wheat resistance to tan spot, the responses of wheat to SNB are mainly governed by the NE-host sensitivity gene interactions, which follow an inverse gene-for-gene interaction in an qualitative fashion (Oliver et al., 2012). In addition to the seven host sensitivity genes reviewed above, several QTL for seedling resistance and adult plant resistance have been reported. Arseniuk et al. (2004) identified a QTL on chromosome 6AL (*QSnl.ihar-6A*), which explained 36% of phenotypic variation for disease severity at the seedling stage in a doubled haploid (DH) population derived from a cross between two wheat cultivars, 'Alba' and 'Begra. Liu et al. (2004b) identified a major QTL on 1BS and six minor QTL on 3AS, 4AL,

5AL, 4BL, 7BL, and 5DL for seedling resistance by analyzing International Triticeae Mapping Initiative (ITMI) mapping population. Schnurbusch et al. (2003) identified two QTL (*QSng.sfr-3BS* and *QSng.sfr-4BL*) responsible for increased resistance to glume blotch using a RIL (recombinant inbred line) population from the cross 'Arina' × 'Forno'. Tommasini et al. (2007) increased the marker resolution in the region of *QSng.sfr-3BS*, and detected a marker SUN2-3B that has a strong association with the glume blotch resistance.

Association Mapping

Association Mapping versus Bi-parental Mapping

Association mapping, also called linkage disequilibrium mapping or association analysis, is a method of genetic mapping based on linkage disequilibrium (Gupta et al., 2005; Zondervan and Cardon, 2004). Association mapping utilizes linkage disequilibrium among alleles at different loci to first analyze the association between markers and characters and then identify functional alleles, DNA sequences, and genotypes highly associated with the phenotypic traits (Gupta et al., 2005). According to the scale of the research, there are two strategies for association analysis: genome-wide association studies (GWAS) and candidate-gene association studies (Zhu et al., 2008). Procedures for both strategies include assembling, phenotyping, and genotyping a natural population, analyzing the population structure and kinship of the population, choosing an appropriate statistical model to estimate the significance level of associations between molecular markers and the evaluated characters, and discovering the targeted genes and QTL (Breseghello and Sorrells, 2006). The following section is a review of the strategy of genome-wide association studies (GWAS), which I used for my research.

Compared to traditional bi-parental mapping, GWAS has several advantages. First, because it uses a natural population, there is no need to develop mapping populations. Second,

because traditional linkage mapping uses only two parents, only two alleles are analyzed at one locus in the linkage mapping population. In contrast, GWAS allows the simultaneous study of all the alleles at one locus in the natural population (Kraakman et al., 2006). Third, compared to the limited recombination that occurs in traditional mapping populations, many more recombinations and mutations have accumulated over time in the natural population that is used in GWAS. This means that GWAS can produce higher resolution than traditional mapping, allowing QTL is be fine-mapped to the level of the gene (Flint-Garcia et al., 2003; Remington et al., 2001). Fourth, GWAS can analyze a number of different complicated quantitative traits simultaneously, in contrast to traditional mapping which develops mapping populations for only one target trait (Kraakman et al., 2006). Fifth, for some plants having a whole-genome sequence, GWAS usually uses the physical positions to locate molecular markers, while linkage mapping uses the genetic positions based on the recombination rate in the genetic segregation population to locate molecular markers or develop genetic maps. This makes it easier for GWAS to refer and combine the results from different mapping populations.

Linkage Disequilibrium

Linkage disequilibrium (LD) or gametic disequilibrium is used to indicate nonrandom association among the alleles that are located at different loci in a population (Flint-Garcia et al., 2003). When the frequency of association of two alleles at different loci is significantly larger than that of random association of the two alleles in a population, these two loci are considered in LD (Flint-Garcia et al., 2003). Linkage disequilibrium can occur among the loci on the same or different chromosomes. Although close linkages could increase the level of LD among loci, LD and genetic linkage are different concepts. Genetic linkage represents the associated inheritance between the linked loci on the same chromosome, whereas LD represents the

correlated relationships among alleles in a population (Flint-Garcia et al., 2003). The effective GWAS is largely based on the knowledge of the strength and structure of LDs in a population (Pritchard et al., 2001). Generally, at the chromosome regions with low LD levels, more markers would be needed for GWAS, but it is easier to identify markers highly associated with target genes or QTL. In contrast, at the genomic regions with high LD levels, relatively fewer markers are needed, but the identified markers might not be highly associated with target genes or QTL (Neale and Savolainen, 2004; Buckler et al., 2006). Therefore, for GWAS, it is critical to learn the LD levels in both the whole genome and single chromosomes.

The LD is measured based on the difference between the observed frequency of haplotype and the expected frequency under random assortment. Generally, for the markers with two types of alleles, like single nucleotide polymorphism (SNP), *D'* and r^2 are calculated to measure LD (Flint-Garcia et al., 2003). It is assumed that there are two loci represented as 'A' and 'B', each of which has two alleles 'A', 'a' and 'B', 'b', respectively. The two loci each with two alleles could produce four haplotypes 'AB', 'Ab', 'aB', and 'ab'. If $f(x)$ is used to stand for the frequency of each allele and haplotype, then the level of LD between these loci could be indicated as in equation 2.1 (Lewontin and Kojima, 1960).

$$D = f(AB) - f(A)f(B) \quad \text{(Eq. 2.1)}$$

When D equals to zero, these two loci are in complete linkage equilibrium, otherwise they are in LD. D' and r^2 are the normalized forms of *D*, and they have a value between 0 and 1 (Wang et al., 2005). They can be calculated as in equations 2.2, 2.3, 2.4, and 2.5 (Lewontin, 1964; Hill and Robertson, 1968; Wang et al., 2005).

$$r^2 = \frac{D^2}{f(A)f(a)f(B)f(b)} \quad \text{(Eq. 2.2)}$$

$$D' = \left|\frac{D}{D_{max}}\right| \quad \text{(Eq. 2.3)}$$

$$D_{max} = \min(f(A)f(b), f(a)f(B)), \text{ for } D \geq 0 \quad \text{(Eq. 2.4)}$$

$$D_{max} = \min(f(A)f(B), f(a)f(b)), \text{ for } D < 0 \quad \text{(Eq. 2.5)}$$

r^2 can also be considered as the squared value of the correlation coefficient between two loci. Two loci will be in complete LD with D' or r^2 having a value of 1, and in complete linkage equilibrium with D' or r^2 having a value of 0. As the value of D' and r^2 is larger, the level of LD is higher. r^2 is used more frequently than D' because r^2 can reflect the LD level among loci more objectively (Zondervon et al., 2004). The distribution of LD at genome or single chromosomes can be indicated in scatterplot of LD decay or matrix of pairwise LD. The former shows the rate of LD decline as the increase of physical or genetic distances between loci, and the latter shows the LD levels between any two loci in genome or single chromosomes using different colors of spots in the matrix (Gaut et al., 2003).

The LD extent varies in different species, different populations of the same species, and different genomic regions in the same population. This is because LD levels can be influenced by many factors such as recombination, genetic drift, selection, mating pattern, mutation, and gene flow (Flint-Garcia et al., 2003). Recombination can increase genetic diversity and decrease the LD levels among loci and LD extent, and it also helps association analysis reach a higher level of mapping resolution. Genomic regions with low recombination usually have high LD levels and large LD extent, whereas genomic regions with high recombination have relatively low LD levels and short LD extent (Flint-Garcia et al., 2003). Genetic drift and selection can change allele frequencies, make populations stratified, and then create new nonrandom associations among unlinked loci (Flint-Garcia et al., 2003).

The mating systems of plants have a major impact on LD, making LD extent distinct in different species (Flint-Garcia et al., 2003). The LD extent in outcrossing plants is largely shorter

than that in self-pollinated plants. Wheat, being a self-pollinated crop, has a LD that decays more slowly than that of maize, which is an outcrossing crop. Based on 205 elite U.S. wheat breeding lines, Zhang et al. (2010b) estimated that the average genome-wide LD extent was about 10 cM, with a r^2 value larger than 0.1. They also found that the LD extents for different regions of genomes were highly variable (Zhang et al., 2010b). Using 95 soft winter wheat cultivars, Breseghello and Sorrells (2006) estimated the LD extents of chromosome 2D and part of 5A to 1 cM and 5 cM, with an r^2 critical value of 0.065, respectively. However, in a population with 170 CIMMYT elite spring wheat lines, Crossa et al. (2007) found that the genome-wide LD extent was 40 cM, with an r^2 critical value of 0.115.

Genotyping and SNP Assay

Several molecular marker systems, such as simple sequence repeat (SSR), diversity arrays technology (DArT), and single nucleotide polymorphism (SNP), have been utilized for association mapping analysis. In many plant species, SNP has become the most popular marker system for association mapping due to its high throughput, high stability, and detection of genetic polymorphisms on the level of functional gene sequence (Ganal et al., 2012; Wang et al., 2014). For SNP genotyping, many high-throughput SNP assay methods have been developed, including dual-labeled hydrolysis probes from TaqMan (Salvi et al., 2001), Affymetrix SNP assay (Matsuzaki et al., 2004), Invader assay (Olivier, 2005), Multiplex MassARRAY spectrometry (iPLEX) (Wright et al., 2008), competitive allele-specific PCR (KASPar) (Nijman et al., 2008), Illumina GoldenGate assay (Fan et al., 2003) and Illumina Infinium assay (Gunderson, 2009). The Illumina GoldenGate assay with up to 3,072-plex SNPs per sample has proven to be effective in high-throughput SNP genotyping in several species that have a complex and polyploid genome, including common wheat (Akhunov et al., 2009; Chao et al., 2010),

maize (Jones et al., 2009), and soybean (Hyten et al., 2008). Among these methods, the Infinium assay combined with BeadChip platform could reach up to highest multiplex level up to 1M SNPs per sample (Gunderson, 2009) and have been successfully used to genotype complex genomes like wheat (Cavanagh et al., 2013) and maize (Ganal et al., 2011). The commercial SNP arrays are now available in several major crop plants, including maize 60K, rice 44K, rye 5K, sunflower 10K, and wheat 9K and 90K arrays (Ganal et al., 2012)

Design and development of the high-throughput SNP assay in wheat was initiated by Akhunov et al. (2009). By using 135 SNPs from the Wheat SNP Database (http://wheat.pw.usda.gov/SNP/new/index.shtml) established from 32 tetraploid and hexaploid wheat lines, Akhunov et al. (2009) proved that the Illumina GoldenGate assay was a powerful approach of high-throughput SNP genotyping for polyploid wheat. A set of 1,536 SNPs were then chosen from the same Wheat SNP Database for developing a wheat oligonucleotide pool assay (OPA), which was later used to genotype 478 spring and winter wheat line (Chao et al., 2010). The genotyping and genotype calling were carried out using the Illumina GoldenGate assay and BeadStudio software, respectively. Cavanagh et al. (2013) developed wheat 9K iSelect array based on the Illumina Infinium I assay technology. The wheat 9K iSelect array includes 8,632 functional SNPs in which 7,504 SNPs were mapped to hexploid wheat genome with a marker density of 1.9 ± 1.0 SNP/cM. Then a wheat 90K iSelect array, based on Infinium I and Infinium II assay technology, was developed with 81,587 functional SNPs, in which 46,977 SNPs were genetically located on a consensus map (Wang et al., 2014). The wheat 9K and 90K iSelect arrays have been extensively utilized in mapping wheat genes and genome through linkage and association analysis (Gurung et al., 2014; Mackay et al., 2014).

The Effect of Population Structure

Population structure or population stratification means that the presence of a difference in allele frequencies between sub-populations of a population is due to genetic drift and founder effects (Pritchard and Rosenberg, 1999). The different sub-populations may be from several different breeding programs or geographic regions (Sukumaran and Yu, 2014). The distributions of allele frequencies associated with population structure could increase the LD levels of loci and make spurious associations between target characters and unassociated loci (Yu et al., 2006). The reason that GWAS was rarely used before in plants is the high risk of false positives resulting from population structure (Pritchard et al., 2000a). Therefore, the presence of population structure should be first considered in using GWAS.

Several methods have been used to control for effects from population structure, including Genomic Control (GC), Structured Association (SA), and Principle Component Analysis (PCA). Among the three methods, GC is a relatively nonparametric method, which does not consider the direct effect of environments on the target characters and assumes that the effect from population structure is a constant in whole genome (Devlin and Roeder, 1999). It adjusts the GWAS results of all markers using a unified inflation factor (Devlin et al., 2001). The SA method utilizes the software of STRUCTURE to assign the sampled individuals of population to *K* putative sub-populations with each being 'unstructured'. All loci in the sub-populations are assumed to be in Hardy-Weinberg equilibrium and the GWAS is conducted within sub-populations (Pritchard et al., 2000a; 2000b). The method of PCA focuses on the reduction of dimensions in the analysis of genotypic data (Price et al., 2006). PCA transforms the variables that associated with each other to orthogonal comprehensive variables or components. The first few components are called principle components (PCs), which are used for adjusting

population structure (Price et al., 2006). The number of PCs that should be combined into the GWAS model varies for different traits of interest (Sukumaran and Yu, 2014). This can be determined based on the percentage of variance that the PCs can explain, Bayesian Information Criterion (BIC) values, and Velicer's Minimum Average Partial (MAP) test. BIC is a criterion used for choosing the best-fit model, and the BIC value will be smallest for the GWAS model with the most appropriate number of PCs (Schwarz, 1978). In MAP test, the number of retained PCs is determined by a series of matrices of partial correlations, which are estimated based on a complete principle component analysis (O'Connor, 2000; Velicer, 1976).

Relative kinship or familial relationship among individuals is often taken into account as a random effect to reduce the influence from population structure in GWAS models, and is represented as a matrix of pairwise relatedness of individuals in population (Yu et al., 2006). This matrix combined in GWAS model is calculated from the proportion of loci identical by state (IBS) or identical by descent (IBD) between the pair-wise individuals in a mapping population. Several software packages, such as SPAGeDi (Spatial Pattern Analysis of Genetic Diversity) (Hardy and Vekemans, 2002), GAPIT (Genome Association and Prediction Integrated Tool) (Lipka et al., 2012), and TASSEL (Trait Analysis by aSSociation, Evolution and Linkage) (Bradbury et al., 2007), can be used for this calculation. For SPAGeDi, there are two methods for estimating kinship matrix. One method was developed by Loiselle et al. (1995) to estimate relatedness coefficients for pairwise individuals by calculating the probability of loci within IBD. Another method is to estimate relatedness coefficients based on the proportion of loci within IBD or IBS between two individuals (Ritland, 1996).

Three methods for calculating kinship matrix were built in GAPIT, including the Loiselle method (Loiselle et al., 1995) mentioned above, the method developed by VanRaden (2008), and

the method built in a GWAS method named as efficient mixed-model association (EMMA) (Kang et al., 2008). The method developed by VanRaden (2008) has three approaches to obtain the matrix of pairwise relatedness based on the probability of loci within IBD between two individuals, with the first two approaches being used for weighting the allele frequency of each locus and the third being used for calculating matrix through a regression model. The method in EMMA is to generate a simple IBS allele-sharing matrix, which is ensured to be positive semi-definite (Kang et al., 2008). The simple IBS allele-sharing matrix proved to be effective and better than more complicated methods at adjusting the effect from population structure in GWAS of *Arabidopsis thaliana* (Zhao et al., 2007). In addition, many other methods of calculating kinship matrix have been developed, such as Monte Carlo simulation-based matrix (Wang, 2002), maximum-likelihood kinship matrix (Thomas and Hill, 2000), allele-frequency weighted IBS matrix (Lynch and Ritland, 1999), and scaled IBS matrix (Endelman and Jannink, 2012).

Different Models in GWAS

Linear regression models are typically used for identifying the associations between phenotypes and genotypes, in which the marker genotypes are independent variables and phenotypes are dependent variables. There are several linear models used in GWAS, including a naïve or simple model, general linear models (GLM), and mixed linear models (MLM) (Yu et al., 2006). In a naïve or simple model, the effect from population structure is not adjusted in GWAS while in GLM and MLM, population structure is corrected (Yu et al., 2006). The naïve, GLM, and MLM models are expressed in the equations 2.6, 2.7, and 2.8, respectively (Stich et al., 2008; Weber et al., 2008; Yu et al., 2006).

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{S\alpha} + \boldsymbol{e} \quad \text{(Eq. 2.6)}$$

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Pv} + \boldsymbol{S\alpha} + \boldsymbol{e} \quad \text{(Eq. 2.7)}$$

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Pv} + \boldsymbol{S\alpha} + \boldsymbol{Iu} + \boldsymbol{e} \quad \text{(Eq. 2.8)}$$

In the equations above, $\boldsymbol{y}$ is a vector of phenotypic values, $\boldsymbol{X}$, $\boldsymbol{S}$ and $\boldsymbol{I}$ are identity matrices, $\boldsymbol{\beta}$ is a vector of fixed effects except the effects from markers and population structure, $\boldsymbol{P}$ is the matrix of population structure or principle component vectors (**P** matrix), $\boldsymbol{v}$ represents a vector of fixed effects from population structure, $\boldsymbol{\alpha}$ is a vector of fixed effects from each marker, $\boldsymbol{u}$ is a vector of random effects regarding to recent ancestry, and $\boldsymbol{e}$ is a vector of residual effects. The variances of random effects are expressed in the equations 2.9 and 2.10 (Weber et al., 2008; Yu et al., 2006).

$$Var(\boldsymbol{u}) = 2\boldsymbol{K}\boldsymbol{V_g} \quad \text{(Eq. 2.9)}$$

$$Var(\boldsymbol{e}) = \boldsymbol{I}\boldsymbol{V_R} \quad \text{(Eq. 2.10)}$$

In the equations 2.9 and 2.10, $\boldsymbol{K}$ is the kinship matrix (K matrix), $\boldsymbol{V_g}$ is the genetic variance, $\boldsymbol{I}$ is an identity matrix, and $\boldsymbol{V_R}$ is the residual variance.

There are several methods that can be used to choose an appropriate model for GWAS. One method for choosing an appropriate model for GWAS is based on the BIC values, and a model with lower BIC value is more appropriate for GAWS (Schwarz, 1978). Another method is based on Quantile-Quantile (Q-Q) plots of the expected versus observed *p* values, and the best model has a line of continuous plots nearest to the expected line with a slope of 1 (Sukumaran and Yu, 2014; Riedelsheimer et al., 2012). However, when there are many models needed to be compared in a Q-Q plot, it will be difficult to identify the best one in the figure. In this case, the mean square deviation (MSD) values obtained through the equation 2.11 could be used to select the best model, and a model with lower MSD value is more appropriate for GWAS (Mamidi et al., 2011).

$$MSD = \left\{\sum_{i=1}^{n}\left[p_i - \left({}^{i}/_{n}\right)\right]^2\right\}/n \quad \text{(Eq. 2.11)}$$

Before using the formula to do the calculation, the markers need to be first ranked from the smallest to the largest based on observed p values. In the equation 2.11, p_i is the ith ranked p value, n is the total number of the markers.

As the data size of GWAS exceeds hundreds of millions, a number of new methods or algorithms based on unified MLM were developed to decrease the computing time for GWAS, such as EMMA (Kang et al., 2008) and compressed MLM (Zhang et al., 2010c). In EMMA, the Maximum Likelihood (ML) or REstricted Maximum Likelihood (REML) method used for estimating variance components for each marker becomes time consuming as data size increases. To further save computing time, many approximation methods simplifying the process of estimating variance components in standard MLM have been developed, such as Genome-wide Rapid Association using Mixed Model And Regression (GRAMMAR) (Aulchenko et al., 2007), GRAMMAR-Gamma (Svishcheva et al., 2012), Population Parameters Previously Determined (P3D) method (Zhang et al., 2010c), and EMMA eXpedited (EMMAX) (Kang et al., 2010). Because the accuracy of the approximation methods cannot be guaranteed, two fast and exact methods were recently developed, specially the Genome-wide Efficient Mixed Model Association (GEMMA) (Zhou and Stephens, 2012) and Factored Spectrally Transformed Linear Mixed Models (FaST-LMM) (Lippert et al., 2011). Except for increasing the computational speed of GWAS, MLM was also improved for dealing with complex traits of interest. Multi-Trait Mixed Model (MTMM) (Korte et al., 2012), Multi-Locus Mixed Model (MLMM) (Segura et al., 2012), and multivariate Linear Mixed Models (mvLMMs) (Zhou and Stephens, 2014) were developed for dealing with correlated phenotypes controlled by pleiotropic loci, complex traits controlled by loci with large effects, and multiple correlated phenotypes, respectively.

Several software packages, such as TASSEL, GAPIT, Plink, and ASREML, have been developed based on different models and algorithms for GWAS. TASSEL, developed in Java, is the most commonly used software for GWAS (Bradbury et al., 2007). It incorporates several GWAS methods, including GLM, MLM, compressed MLM, P3D, EMMA, and EMMAX, and therefore performs well with large data sets. TASSEL is updated frequently and its latest version is TASSEL 5.0 (www.maizegenetics.net/tassel). Some statistic software including SAS (SAS Institute, Cary NC) and R (R Development Core Team, 2014) are important genetic tools for GWAS as well. GAPIT is an R package developed for GWAS and genomic prediction and selection (GS) (Lipka et al., 2012). It implements all of the GWAS methods incorporated in TASSEL. GAPIT can produce results with user-friendly tables and figures. Plink is an open-source GWAS tool developed in C/C++ (Purcell et al., 2007); it makes multiple GWAS methods available in one software package. ASREML is also a useful package for MLM analysis (Gilmour et al., 2002).

Multiple Testing Adjustments

Multiple hypothesis testing is an important factor influencing the reliability of the GWAS result, as it may increase the type I error rate (α) and induce more false positive associations than individual hypotheses testing. The number of individual hypothesis testing in GWAS depends on the number of molecular markers (Johnson et al., 2010). Several methods or criterion, including Bonferroni, FDR (false positive rate), and Permutation test, have been used in GWAS to adjust the *p* values or α values in multiple hypothesis testing in order to reduce the frequency of false positives.

In Bonferroni method, the number of individual hypothesis tests is equal to the number of markers, and an adjusted type I error rate (α*) for individual hypothesis test is estimated through

the overall type I error rate (α) divided by the number of individual hypothesis tests (Perneger, 1998). Then, the makers or loci, with a *p* value less than this adjusted α* value, would be significantly associated with the evaluated character. But this method is considered to be too conservative by 'overcorrecting' the cutoff *p* value or α value, therefore it may increase the false negative rate or type II error rate and miss some markers truly associated with the characters (Perneger, 1998). Duggal et al. (2008) tried to optimize the Bonferroni method by using the effective number of independent markers per LD block instead of the total number of markers in GWAS. Gao et al. (2008) also adjusted the original Bonferroni method by using the effective number of independent association tests estimated through principle component analysis.

In order to eliminate the influence from multiple hypothesis testing, Benjamini and Hochberg (1995) pointed out that original *p* values could be transformed to false positive rate (FDR). The original *p* values are first sorted from the smallest to the largest. Then, the largest *p* value is kept to be unchanged, and the other *p* values are divided by their own coefficient, calculated from the total number of markers divided by their own rank after sorting. The marker, with an adjusted *p* value or FDR smaller than the critical value of significance, is significantly associated with the tested character (Benjamini et al., 2001; Sebat et al., 2004). This method is less stringent than Bonferroni, and reduces the type II error rate, so it was widely used in GWAS. The Permutation test is also used to adjust *p* values, but it is more complicated and time-consuming than other methods (van der Laan et al., 2005). None of the above methods can completely avoid the false positive associations. The only solution to reduce the false positive associations is to use replications in GWAS and validations of the findings from initial GWAS (Chanock et al., 2007).

Application of GWAS in Wheat

At the beginning of the 21st century, association analysis was initially used to investigate the association of flowering time with gene *Dwarf8* sequence polymorphisms in maize (Thornsberry et al., 2001). Since then, association analysis has been successful in identifying genomic regions or loci associated with economically important traits in potato (Gebhardt et al., 2004), rice (Zhang et al., 2005), and soybean (Wang et al., 2008). In wheat, Breseghello and Sorrells (2006) used 95 winter wheat cultivars from North America to conduct the first association mapping study to analyze kernel size and milling quality. They used SSR markers based on the MLM correcting population structures and identified 14 loci and 6 loci associated with kernel morphology and milling quality, respectively. They concluded that association analysis could verify and complement information from previous QTL studies. At the same time, Ravel et al. (2006) used 113 bread wheat lines and GLM methods to analyze the association between six SNPs located in two candidate genes and the quantity of high-molecular-weight glutenin subunits. Only SNPs in one of the two candidate genes were associated with the evaluated phenotypes.

Since its successful use in wheat by Breseghello and Sorrells (2006) and Ravel et al. (2006), association analysis has been extensively used in wheat for detecting polymorphisms associated with disease resistance, yield, and other characteristics. Crossa et al. (2007) used GWAS to examine 170 elite spring wheat lines for grain yield and resistance to stem rust, leaf rust, yellow rust, and powdery mildew. Using DArT markers across whole genomes based on MLMs, they identified a number of significant markers for the phenotypes that were evaluated. Some of the significant markers associated with grain yield located at the positions of previously mapped QTL. Yao et al. (2009) used the unified MLM (Yu et al., 2006) in association analysis

of several agronomic traits in 108 winter wheat germplasm accessions from China, which were genotyped by 125 SSR markers. They identified 14 markers associated with six agronomic traits. Maccaferri et al. (2011) evaluated 189 elite durum wheat accessions with grain yield and drought-adaptive traits in 15 environments using 186 SSR markers. They detected several novel loci associated with drought-adaptive traits and grain yield, and confirmed previously known loci. They also found that the numbers of significant markers for grain yield varied significantly in different environments.

Opportunities and Challenges

Advances that have come from next-generation sequencing offer new challenges and opportunities for association analysis. Due to new strategies for genome sequencing, the information of Copy Number Variation (CNV) and Presence-Absence Variation (PAV) of genes and markers between distinguished genomes (Springer et al., 2009) can be taken into account during association analysis. Various continuously optimized resequencing technologies, such as, genotyping-by-sequencing, exome sequencing, and RNA-seq, supply enormous genomic information for association analysis (Huang et al., 2009; Elshire et al., 2011; Ng et al., 2009; Wang et al., 2009). Whole genome sequencing achievements, especially for complex polyploid plants like wheat (Brenchley et al., 2012; Jia et al., 2013; Ling et al., 2013), will accelerate the identification of new genes by association analysis. Availability of a number of new high-throughput phenotyping methods, such as near infrared (NIR) spectroscopy, CT-SCAN, global positioning system, single kernel characterization system for grain quality, and image analysis (Sukumaran and Yu, 2014), could match the level of next-generation genotyping capacity. The large volumes of data that come from high-throughput genotyping and phenotyping will improve the resolution of association mapping. Having to deal with these large volumes of data will

require bigger storage, high computational power, and improved bioinformatics tools and statistical methods.

There are several challenges in utilizing association analysis for precise and efficient detection of loci associated with targeted traits. The first challenge is the difficulty associated with detection of loci having numerous variants with small effects on a complex quantitative trait, for example, the loci in maize controlling flowering time, oil content, and drought tolerance (Buckler et al., 2009; Laurie et al., 2004; Messmer et al., 2009). The phenotypic variation explained by the target allele, together with the population size and LD levels between the target alleles and molecular markers, are all factors that influence efficiency of association analysis (Gordon and Finch, 2005). An allele with small effect is hard to detect by association analysis in a population with a small size. Clearly increasing population size is more important than improving the number or resolution of markers to increase the efficiency of association analysis (Long and Langley, 1999). This means that populations with a large size are necessary to identify QTL with small effects (Rostoks et al., 2006; Atwell et al., 2010).

A second challenge in association analysis is the identification of rare alleles. Although some rare alleles have large effects on phenotypic variation, their low frequencies in a population make them difficult to detect (Ott et al., 2011). To solve this problem, Zhu et al. (2011) introduced a new strategy termed as Composite Resequencing-based Genome-Wide Association Studies (CR-GWAS), which combined the sum test, weighted sum test, and function-aided sum test to deal with association analysis in rare variants.

A third challenge is that most of the detected loci significantly associated with a trait only explain a small proportion of phenotypic variation. This issue is termed as “missing heritability” in humans (Manolio et al., 2009), and it is more common in human GWAS than in plants (Atwell

et al., 2010). The missing heritability could be caused by a number of factors such as rare alleles, numerous small-effect alleles, environment, gene-gene interactions, statistical and multiple testing issues, and copy number variation (CNV) (Sukumaran and Yu, 2014).

In addition to the three challenges reviewed above, there are other challenges for association analysis. These include recognizing the causal genes in follow-up studies in organisms without complete sequence information, and the efficient implementation of new sequencing and genotyping technologies (Sukumaran and Yu, 2014).

In summary, association analysis has been an important method that can be used in plants to identify genetic variants controlling the expression of complex traits. Many significant achievements have been achieved via association analysis in the past decade. Although several challenges exist, new solutions are continuously proposed. This means that association analysis is constantly improving. In the near future, it is expected that association analysis will become a more user-friendly approach for identification of new genes and linked markers for modern crop improvement.

Literature Cited

Abeysekara, N.S., T.L. Friesen, B. Keller and J.D. Faris. 2009. Identification and characterization of a novel host–toxin interaction in the wheat-*Stagonospora nodorum* pathosystem. Theoretical and Applied Genetics 120: 117-126.

Akhunov, E., C. Nicolet and J. Dvorak. 2009. Single nucleotide polymorphism genotyping in polyploid wheat with the Illumina GoldenGate assay. Theoretical and Applied Genetics 119: 507-517.

Ali, S. and L.J. Francl. 2002. A new race of *Pyrenophora tritici-repentis* from Brazil. Plant Disease 86: 1050-1050.

Ali, S., H. Ling, S. Meinhardt and L.J. Francl. 2002. A new race of *Pyrenophora tritici-repentis* that produces a putative host-selective toxin. Phytopathology 92: S3.

Anderson, J.A., R.J. Effertz, J.D. Faris, L.J. Francl, S.W. Meinhardt and B.S. Gill. 1999. Genetic analysis of sensitivity to a *Pyrenophora tritici-repentis* necrosis-inducing toxin in durum and common wheat. Phytopathology 89: 293-297.

Anikster, Y. 1984. The formae speciales. In: W. R. Bushnell and A. P. Roelfs, editors, The cereal rust. Academic Press, Orlando, Florida. p. 115–130.

Anikster, Y., W. R. Bushnell, A. P. Roelfs, T. Eilam and J. Manisterski. 1997. Puccinia recondita causing leaf rust on cultivated wheats, wild wheats, and rye. Canadian Journal of Botany 75: 2082-2096.

Anugrahwati, D.R., K.W. Shepherd, D.C. Verlin, P. Zhang, G. Mirzaghaderi, E. Walker, et al. 2008. Isolation of wheat-rye 1RS recombinants that break the linkage between the stem rust resistance gene *SrR* and secalin. Genome / National Research Council Canada 51: 341-349.

Arseniuk, E., P. Czembor, A. Czaplicki, Q. Song, P. Cregan, et al. 2004. QTL controlling partial resistance to *Stagonospora nodorum* leaf blotch in winter wheat cultivar Alba. Euphytica 137: 225-231.

Atwell, S., Y.S. Huang, B.J. Vilhjalmsson, G. Willems, M. Horton, Y. Li, et al. 2010. Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. Nature 465: 627-631.

Aulchenko, Y.S., D.-J. de Koning and C. Haley. 2007. Genome-wide rapid association using mixed model and regression: a fast and simple method for genome-wide pedigree-based quantitative trait loci association analysis. Genetics 177: 577-585.

Ausemus, E.R., J.B. Harrington, L.P. Reitz and W.W. Worzella. 1946. A summary of genetic studies in hexaploid and tetraploid wheats. Agronomy Journal 38: 1082-1099.

Ballance, G.M., L. Lamari, R. Kowatsch and C.C. Bernier. 1996. Cloning, expression and occurrence of the gene encoding the Ptr necrosis toxin from *Pyrenophora tritici-repentis*. Molecular Plant Pathology. On-line. Publication no. 1996/1209ballance.

Bansal, U.K., E. Bossolini, H. Miah, B. Keller, R.F. Park and H.S. Bariana. 2008. Genetic mapping of seedling and adult plant stem rust resistance in two European winter wheat cultivars. Euphytica. 164:821-828.

Bansal, U.K., M.J. Hayden, B. Keller, C.R. Wellings, R.F. Park and H.S. Bariana. 2009. Relationship between wheat rust resistance genes *Yr1* and *Sr48* and a microsatellite marker. Plant Pathology 58: 1039-1043.

Benjamini, Y., D. Drai, G. Elmer, N. Kafkafi and I. Golani. 2001. Controlling the false discovery rate in behavior genetics research. Behavioural Brain Research 125: 279-284.

Benjamini, Y. and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological): 289-300.

Bennett, F.G.A. 1984. Resistance to powdery mildew in wheat: a review of its use in agriculture and breeding programmes. Plant Pathology 33: 279-300.

Bennett, R.S., S.-H. Yun, T.Y. Lee, B.G. Turgeon, E. Arseniuk, B.M. Cunfer, et al. 2003. Identity and conservation of mating type genes in geographically diverse isolates of *Phaeosphaeria nodorum*. Fungal Genetics and Biology 40: 25-37.

Beteselassie, N., C. Fininsa and A. Badebo. 2007. Sources of resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) in Ethiopian tetraploid wheat accessions. Genetic Resources and Crop Evolution 54: 337-343.

Bhuvaneshwari, G., N.B. Yenagi, R.R. Hanchinal and P.A. Katarki. 2001. Physico-Chemical Characterstics and Milling Quality of Dicoccum Wheat Varieties. Karnataka Journal of Agricultural Sciences 14: 736-742.

Boehm, E.W.A., J.C. Wenstrom, D.J. McLaughlin, L.J. Szabo, A.P. Roelfs and W.R. Bushnell. 1992. An ultrastructural pachytene karyotype for *Puccinia graminis* f. sp. *tritici*. Canadian Journal of Botany 70: 401-413.

Boguslavskij, R.L., O.V. Golik and T.T. Tkachenko. 2000. Cultivated emmer is valuable germplasm for durum wheat breeding. In: C. Royo, M. M. Nachit, D. F. N. and J. L. Araus, editors, Durum wheat improvement in the Mediterranean region: new challenges. Options Mediterranéennes. p. 125-127.

Bowden, R.L. 2013. Waste not, want not: The importance of being earnest about gene stewardship. BGRI Technical Workshop. New Delhi, India. p. 9.

Bradbury, P.J., Z. Zhang, D.E. Kroon, T.M. Casstevens, Y. Ramdoss and E.S. Buckler. 2007. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23: 2633-2635.

Brenchley, R., M. Spannagl, M. Pfeifer, G.L.A. Barker, R. D/'Amore, A.M. Allen, et al. 2012. Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature 491: 705-710.

Breseghello, F. and M.E. Sorrells. 2006. Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172: 1165-1177.

Buckler, E.S., B.S. Gaut and M.D. McMullen. 2006. Molecular and functional diversity of maize. Current Opinion in Plant Biology 9: 172-176.

Buckler, E.S., J.B. Holland, P.J. Bradbury, C.B. Acharya, P.J. Brown, C. Browne, et al. 2009. The genetic architecture of maize flowering time. Science 325: 714-718.

Caldwell, R.M. 1968. Breeding for general and/or specific plant disease resistance. In: K. W. Finaly and K. W. Shepard, editors, International wheat genetic symposium, 3rd. Academy of Science, Canberra, Australia. Butterworth & Co. p. 263-272.

Cavanagh, C.R., S. Chao, S. Wang, B.E. Huang, S. Stephen, S. Kiani, et al. 2013. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proceedings of the National Academy of Sciences 110: 8057.

Chanock, S.J., T. Manolio, M. Boehnke, E. Boerwinkle, D.J. Hunter, G. Thomas, et al. 2007. Replicating genotype–phenotype associations. Nature 447: 655-660.

Chao, S., J. Dubcovsky, J. Dvorak, M.-C. Luo, S.P. Baenziger, R. Matnyazov, et al. 2010. Population- and genome-specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (*Triticum aestivum* L.). BMC Genomics 11: 727.

Chu, C.-G., T.L. Friesen, J.D. Faris and S.S. Xu. 2008a. Evaluation of seedling resistance to tan spot and *Stagonospora nodorum* blotch in tetraploid wheat. Crop Science 48: 1107-1116.

Ciuffetti, L.M., V.A. Manning, I. Pandelova, M.F. Betts and J.P. Martinez. 2010. Host-selective toxins, Ptr ToxA and Ptr ToxB, as necrotrophic effectors in the *Pyrenophora tritici-repentis*–wheat interaction. New Phytologist 187: 911-919.

Ciuffetti, L.M., R.P. Tuori and J.M. Gaventa. 1997. A single gene encodes a selective toxin causal to the development of tan spot of wheat. Plant Cell 9: 135-144.

Cloutier, S., B.D. McCallum, C. Loutre, T.W. Banks, T. Wicker, C. Feuillet, et al. 2007. Leaf rust resistance gene *Lr1*, isolated from bread wheat (*Triticum aestivum* L.) is a member of the large psr567 gene family. Plant Molecular Biology 65: 93-106.

Collins, N.C., F. Tardieu and R. Tuberosa. 2008. Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiology 147: 469-486.

Corazza, L., M. Pasquini and P. Perrino. 1986. Resistance to rusts and powdery mildew in some strains of *Triticum monococcum* L. and *Triticum dicoccum* Schubler cultivated in Italy. Genetica Agraria 40: 243-254.

Cox, T.S., W.J. Raupp, D.L. Wilson, B.S. Gill, S. Leath, W.W. Bockus, et al. 1992. Resistance to foliar diseases in a collection of *Triticum tauschii* germ plasm. Plant Disease 76: 1061-1064.

Crossa, J., J. Burgueno, S. Dreisigacker, M. Vargas, S.A. Herrera-Foessel, M. Lillemo, et al. 2007. Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177: 1889-1913.

Cubadda, R. and E. Marconi. 1996. Technological and nutritional aspects in emmer and spelt. In: S. Padulosi, K. Hammer and J. Heller, editors, The first international workshop on hulled Wheats. IPGRI, Rome. p. 203-211.

Damania, A.B., S. Hakim and M.Y. Moualla. 1992. Evaluation of variation in *Triticum dicoccum* for wheat improvement in stress environments. Hereditas 116: 163-166.

De Wolf, E.D., R.J. Effertz, S. Ali and L.J. Francl. 1998. Vistas of tan spot research. Canadian Journal of Plant Pathology 20: 349-370.

Devlin, B. and K. Roeder. 1999. Genomic control for association studies. Biometrics 55: 997-1004.

Devlin, B., K. Roeder and L. Wasserman. 2001. Genomic control, a new approach to genetic-based association studies. Theoretical Population Biology 60: 155-166.

Dorofeev, V.F., A.A. Filatenko, E.F. Migushova, R.A. Udaczin and M.M. Jakubziner. 1979. Wheat. In: V. F. Dorofeev and O. N. Korovina, editors, Flora of cultivated plants. Kolos, Leningrad. p. 346.

Duggal, P., E.M. Gillanders, T.N. Holmes and J.E. Bailey-Wilson. 2008. Establishing an adjusted p-value threshold to control the family-wide type 1 error in genome wide association studies. BMC Genomics 9: 516.

Dundas, I.S., D.R. Anugrahwati, D.C. Verlin, R.F. Park, H.S. Bariana, R. Mago, and A.K.M.R. Islam. 2007. New sources of rust resistance from alien species: meliorating linked defects and discovery. Australian Journal of Agricultural Research 58:545-549.

Duplessis, S., C.A. Cuomo, Y.-C. Lin, A. Aerts, E. Tisserant, C. Veneault-Fourrey, et al. 2011. Obligate biotrophy features unraveled by the genomic analysis of rust fungi. Proceedings of the National Academy of Sciences 108: 9166-9171.

Dvořák, J., P.d. Terlizzi, H.-B. Zhang and P. Resta. 1993. The evolution of polyploid wheats: identification of the A genome donor species. Genome / National Research Council Canada 36: 21-31.

Dvorak, J. and H.-B. Zhang. 1990. Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. Proceedings of the National Academy of Sciences 87: 9640-9644.

Dyck, P.L. 1992. Transfer of a gene for stem rust resistance from *Triticum araraticum* to hexaploid wheat. Genome / National Research Council Canada 35: 788-792.

Dyck, P.L. and E.R. Kerber. 1977. Chromosome location of gene *Sr29* for reaction to stem rust. Canadian Journal of Genetics and Cytology 19: 371-373.

Dyck, P.L. and E.R. Kerber. 1985. Resistance of the race-specific type. In: A. P. Roelfs and W. R. Bushnell, editors, The cereal rusts. Academic Press, Orlando, Florida, USA. p. 469-500.

Effertz, R.J., S.W. Meinhardt, J.A. Anderson, J.G. Jordahl and L.J. Francl. 2002. Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. Phytopathology 92: 527-533.

Elias, E.M., R.G. Cantrell and R.M.J. Hosford. 1989. Heritability of resistance to tan spot in durum wheat and its association with other agronomic traits. Crop Science 29: 299-304.

Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler, et al. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS One 6: e19379.

Endelman, J.B. and J.-L. Jannink. 2012. Shrinkage estimation of the realized relationship matrix. G3: Genes|Genomes|Genetics 2: 1405-1413.

Eyal, Z. 1999. Breeding for resistance to *Septoria* and *Stagonospora* diseases of wheat. In: J. A. Lucas, P. Bowyer and A. M. Anderson, editors, *Septoria* on cereals: a study of pathosystems IACR Long Ashton Research Station, Bristol, UK. p. 332-344.

Fan, J.-B., A. Oliphant, R. Shen, B.G. Kermani, F. Garcia, K.L. Gunderson, et al. 2003. Highly parallel SNP genotyping. Cold Spring Harbor Symposia on Quantitative Biology 68: 69-78.

Faris, J.D. 2014. Wheat domestication: Key to agricultural revolutions past and future. In: R. Tuberosa, A. Graner and E. Frison, editors, Genomics of plant genetic resources. Springer Netherlands. p. 439-464.

Faris, J.D., N.S. Abeysekara, P.E. McClean, S.S. Xu and T.L. Friesen. 2012. Tan spot susceptibility governed by the *Tsn1* locus and race-nonspecific resistance quantitative trait loci in a population derived from the wheat lines Salamouni and Katepwa. Molecular Breeding 30: 1669-1678.

Faris, J.D., J.A. Anderson, L.J. Francl and J.G. Jordahl. 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. Phytopathology 86: 459-463.

Faris, J.D., J.A. Anderson, L.J. Francl and J.G. Jordahl. 1997. RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theoretical and Applied Genetics 94: 98-103.

Faris, J.D. and T.L. Friesen. 2005. Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theoretical and Applied Genetics 111: 386-392.

Faris, J.D., Z. Liu and S.S. Xu. 2013. Genetics of tan spot resistance in wheat. Theoretical and Applied Genetics 126: 2197-2217.

Faris, J.D., Z. Zhang, H. Lu, S. Lu, L. Reddy, S. Cloutier, et al. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proceedings of the National Academy of Sciences 107: 13544-13549.

Feldman, M., F.G.H. Lupton and T.E. Miller. 1976. Wheats: *Triticum* spp.(*Gramineae-Triticinae*). In: J. Smartt and N. W. Simmonds, editors, Evolution of crop plants. Longman Scientific & Technical Press, London, UK. p. 184-192.

Feuillet, C., S. Travella, N. Stein, L. Albar, A. Nublat and B. Keller. 2003. Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proceedings of the National Academy of Sciences 100: 15253-15258.

Flint-Garcia, S.A., J.M. Thornsberry and E.S. Buckler IV. 2003. Structure of linkage disequilibrium in plants. Annual Review of Plant Biology 54: 357-374.

Flor, H.H. 1971. Current status of the gene-for-gene concept. Annual Review of Phytopathology 9: 275-296.

Francki, M.G., M. Shankar, E. Walker, R. Loughman, H. Golzar and H. Ohm. 2011. New quantitative trait loci in wheat for flag leaf resistance to Stagonospora nodorum blotch. Phytopathology 101: 1278-1284.

Friesen, T.L., C. Chu, S.S. Xu and J.D. Faris. 2012. SnTox5–*Snn5*: a novel *Stagonospora nodorum* effector–wheat gene interaction and its relationship with the SnToxA–*Tsn1* and SnTox3–*Snn3–B1* interactions. Molecular Plant Pathology 13: 1101-1109.

Friesen, T.L. and J.D. Faris. 2004. Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. Theoretical and Applied Genetics 109: 464-471.

Friesen, T.L. and J.D. Faris. 2010. Characterization of the wheat-*Stagonospora nodorum* disease system: what is the molecular basis of this quantitative necrotrophic disease interaction? Canadian Journal of Plant Pathology 32: 20-28.

Friesen, T.L., S.W. Meinhardt and J.D. Faris. 2007. The *Stagonospora nodorum*-wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. The Plant Journal 51: 681-692.

Friesen, T.L., E.H. Stukenbrock, Z. Liu, S. Meinhardt, H. Ling, J.D. Faris, et al. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. Nature Genetics 38: 953-956.

Friesen, T.L., Z. Zhang, P.S. Solomon, R.P. Oliver and J.D. Faris. 2008. Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. Plant Physiology 146: 682-693.

Galterio, G., D. Cardarilli, P. Codianni and R. Acquistucci. 2001. Evaluation of chemical and technological characteristics of new lines of *Triticum turgidum* ssp *dicoccum*. Food/Nahrung 45: 263-266.

Galterio, G., P. Codianni, A.M. Giusti, B. Pezzarossa and C. Cannella. 2003. Assessment of the agronomic and technological characteristics of *Triticum turgidum* ssp. *dicoccum* Schrank and *T. spelta* L. Food/Nahrung 47: 54-59.

Gamba, F.M. and L. Lamari. 1998. Mendelian inheritance of resistance to tan spot (*Pyrenophora tritici-repentis*) in selected genotypes of durum wheat (*Triticum turgidum*). Canadian Journal of Plant Pathology 20: 408-414.

Ganal, M.W., G. Durstewitz, A. Polley, A. Bérard, E.S. Buckler, A. Charcosset, et al. 2011. A large maize (*Zea mays* L.) SNP genotyping array: development and germplasm genotyping, and genetic mapping to compare with the B73 reference genome. PloS One 6: e28334.

Ganal, M.W., A. Polley, E.-M. Graner, J. Plieske, R. Wieseke, H. Luerssen, et al. 2012. Large SNP arrays for genotyping in crop plants. Journal of Biosciences 37: 821-828.

Gao, X., J. Starmer and E. R. Martin. 2008. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. Genetic Epidemiology 32: 361-369.

Gao, Y., J.D. Faris, Z. Liu, Y. Kim, R.A. Syme, et al. 2015. Identification and characterization of the SnTox6-*Snn6* interaction in the *Parastagonospora nodorum* - wheat pathosystem. Molecular Plant-Microbe Interactions. DOI: 10.1094/MPMI-12-14-0396-R.

Gaut, B.S. and A.D. Long. 2003. The lowdown on linkage disequilibrium. The Plant Cell Online 15: 1502-1506.

Gebhardt, C., A. Ballvora, B. Walkemeier, P. Oberhagemann and K. Schüler. 2004. Assessing genetic potential in germplasm collections of crop plants by marker-trait association: a case study for potatoes with quantitative variation of resistance to late blight and maturity type. Molecular Breeding 13: 93-102.

Gill, B.S., R. Appels, A.-M. Botha-Oberholster, C.R. Buell, J.L. Bennetzen, B. Chalhoub, et al. 2004. A workshop report on wheat genome sequencing international genome research on wheat consortium. Genetics 168: 1087-1096.

Gilmour, A.R., B.J. Gogel, B.R. Cullis, S.J. Welham and R. Thompson. 2002. ASReml users guide release 1.0 VSN Int Ltd., Hemel Hempstead, UK.

Gordon, D. and S.J. Finch. 2005. Factors affecting statistical power in the detection of genetic association. The Journal of Clinical Investigation 115: 1408-1418.

Gunderson, K.L. 2009. Whole-genome genotyping on bead arrays. In: M. Dufva, editor DNA microarrays for biomedical research. Humana Press. p. 197-213.

Gupta, P.K., S. Rustgi and P.L. Kulwal. 2005. Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Molecular Biology 57: 461-485.

Gurung, S., S. Mamidi, J.M. Bonman, M. Xiong, G. Brown-Guedira, et al. 2014. Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. PLoS One 9: e108179.

Hane, J.K., R.G.T. Lowe, P.S. Solomon, K.-C. Tan, C.L. Schoch, J.W. Spatafora, et al. 2007. Dothideomycete-plant interactions illuminated by genome sequencing and EST analysis of the wheat pathogen *Stagonospora nodorum*. Plant Cell 19: 3347-3368.

Hardy, O.J. and X. Vekemans. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2: 618-620.

Harris, D., V. Masson, Y. Berezkin, M. Charles, C. Gosden, G. Hillman, et al. 1993. Investigating early agriculture in Central Asia: new research at Jeitun, Turkmenistan. Antiquity 67: 324-338.

Herrera-Foessel, S.A., R.P. Singh, M. Lillemo, J. Huerta-Espino, S. Bhavani, et al. 2014. *Lr67/Yr46* confers adult plant resistance to stem rust and powdery mildew in wheat. Theoretical and Applied Genetics 127: 781-789.

Hiebert, C.W., T.G. Fetch, T. Zegeye, J.B. Thomas, D.J. Somers, D.G. Humphreys, et al. 2011. Genetics and mapping of seedling resistance to Ug99 stem rust in Canadian wheat cultivars 'Peace'and 'AC Cadillac'. Theoretical and Applied Genetics 122: 143-149.

Hill, W.G. and A. Robertson. 1968. The effects of inbreeding at loci with heterozygote advantage. Genetics 60: 615-628.

Hodson, D.P., J. Grønbech-Hansen, P. Lassen, Y. Alemayehu, J. Arista, K. Sonder, et al. 2012. Tracking the wheat rust pathogens. In: R. McIntosh, editor Borlaug Global Rust Initiative 2012 Technical Workshop. Beijing, China.

Huang, L., S.A. Brooks, W. Li, J.P. Fellers, H.N. Trick and B.S. Gill. 2003. Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164: 655-664.

Huang, X., Q. Feng, Q. Qian, Q. Zhao, L. Wang, A. Wang, et al. 2009. High-throughput genotyping by whole-genome resequencing. Genome Research 19: 1068-1076.

Huerta-Espino, J. 1992. Analysis of wheat leaf and stem rust virulence on a worldwide basis. University of Minnesota.

Huerta-Espino, J., R.P. Singh, S. Germán, B.D. McCallum, R.F. Park, W.Q. Chen, et al. 2011. Global status of wheat leaf rust caused by *Puccinia triticina*. Euphytica 179: 143-160.

Hyten, D.L., Q. Song, I.-Y. Choi, M.-S. Yoon, J.E. Specht, L.K. Matukumalli, et al. 2008. High-throughput genotyping with the GoldenGate assay in the complex genome of soybean. Theoretical and Applied Genetics 116: 945-952.

Jia, J., S. Zhao, X. Kong, Y. Li, G. Zhao, W. He, et al. 2013. *Aegilops tauschii* draft genome sequence reveals a gene repertoire for wheat adaptation. Nature 496: 91-95.

Jin, Y., M. Rouse, P.D. Olivera and B.J. Steffenson. 2009. Progress and prospects in discovery and use of novel sources of stem rust resistance. In: R. McIntosh, editor Borlaug Global Rust Initiative Technical Workshop. Cd. Obregón, Sonora, Mexico. p. 89-91.

Jin, Y. and R.P. Singh. 2006. Resistance in US wheat to recent eastern African isolates of *Puccinia graminis* f. sp. *tritici* with virulence to resistance gene *Sr31*. Plant Disease 90: 476-480.

Jin, Y., L.J. Szabo and Z.A. Pretorius. 2008a. Virulence variation within the Ug99 lineage. In: R. Appels, R. Eastwood, E. Lagudah, P. Langridge, M. Mackay, L. McIntye and P. Sharp, editors, 11th International Wheat Genet Symposium, Sydney, Australia. Sydney University Press. p. 4-6.

Johnson, R.C., G.W. Nelson, J.L. Troyer, J.A. Lautenberger, B.D. Kessing, C.A. Winkler, et al. 2010. Accounting for multiple comparisons in a genome-wide association study (GWAS). BMC Genomics 11: 724.

Johnson, T. 1954. Selfing studies with physiologic races of wheat stem rust, *Puccinia graminis* var. *tritici*. Canadian Journal of Botany 32: 506-522.

Johnson, T. and M. Newton. 1940. Mendelian inheritance of certain pathogenic characters of *Puccinia graminis tritici*. Canadian Journal of Research 18: 599-611.

Jones, E., W.-C. Chu, M. Ayele, J. Ho, E. Bruggeman, K. Yourstone, et al. 2009. Development of single nucleotide polymorphism (SNP) markers for use in commercial maize (*Zea mays* L.) germplasm. Molecular Breeding 24: 165-176.

Jones, S.S., J. Dvořák, D.R. Knott and C.O. Qualset. 1991. Use of double-ditelosomic and normal chromosome 1D recombinant substitution lines to map *Sr33* on chromosome arm 1DS in wheat. Genome / National Research Council Canada 34: 505-508.

Kang, H.M., J.H. Sul, S.K. Service, N.A. Zaitlen, S.-y. Kong, N.B. Freimer, et al. 2010. Variance component model to account for sample structure in genome-wide association studies. Nature Genetics 42: 348-354.

Kang, H.M., N.A. Zaitlen, C.M. Wade, A. Kirby, D. Heckerman, M.J. Daly, et al. 2008. Efficient control of population structure in model organism association mapping. Genetics 178: 1709-1723.

Keller, S.M., J.M. McDermott, R.E. Pettway, M.S. Wolfe and B.A. McDonald. 1997a. Gene flow and sexual reproduction in the wheat glume blotch pathogen *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*). Phytopathology 87: 353-358.

Keller, S.M., M.S. Wolfe, J.M. McDermott and B.A. McDonald. 1997b. High genetic similarity among populations of *Phaeosphaeria nodorum* across wheat cultivars and regions in Switzerland. Phytopathology 87: 1134-1139.

Klindworth, D.L., Z. Niu, S. Chao, T.L. Friesen, Y. Jin, J.D. Faris, et al. 2012. Introgression and characterization of a goatgrass gene for a high level of resistance to Ug99 stem rust in tetraploid wheat. G3: Genes|Genomes|Genetics 2: 665-673.

Knott, D.R. 1962. The inheritance of rust resistance: IX. The inheritance of resistance to races 15B and 56 of stem rust in the wheat variety Khapstein. Canadian Journal of Plant Science 42: 415-419.

Knott, D.R. 1968. The inheritance of resistance to stem rust races 56 and 15B-1L (Can.) in the wheat varieties Hope and H-44. Canadian Journal of Genetics and Cytology 10: 311-320.

Kolmer, J.A. 2001. Molecular polymorphism and virulence phenotypes of the wheat leaf rust fungus *Puccinia triticina* in Canada. Canadian Journal of Botany 79: 917-926.

Kolmer, J.A. 2013. Leaf rust of wheat: pathogen biology, variation and host resistance. Forests 4: 70-84. doi:10.3390/f4010070.

Kolmer, J.A., Y. Jin and D.L. Long. 2007. Wheat leaf and stem rust in the United States. Crop and Pasture Science 58: 631-638.

Kolmer, J.A., J.Q. Liu and M. Sies. 1995. Virulence and molecular polymorphism in *Puccinia recondita* f. sp. *tritici* in Canada. Phytopathology 85: 276-285.

Korte, A., B.J. Vilhjálmsson, V. Segura, A. Platt, Q. Long and M. Nordborg. 2012. A mixed-model approach for genome-wide association studies of correlated traits in structured populations. Nature Genetics 44: 1066-1071.

Kraakman, A.T.W., F. Martinez, B. Mussiraliev, F.A. Van Eeuwijk and R.E. Niks. 2006. Linkage disequilibrium mapping of morphological, resistance, and other agronomically relevant traits in modern spring barley cultivars. Molecular Breeding 17: 41-58.

Krattinger, S.G., E.S. Lagudah, W. Spielmeyer, R.P. Singh, J. Huerta-Espino, H. McFadden, et al. 2009. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323: 1360-1363.

Krupinsky, J.M. 1982. Observations on the host range of isolates of *Pyrenophora trichostoma Triticum aestivum* and seven grasses: Elymus, North America. Canadian Journal of Plant Pathology 4: 42-46.

Lage, J., B. Skovmand and S.B. Andersen. 2004. Field evaluation of emmer wheat-derived synthetic hexaploid wheat for resistance to Russian wheat aphid (*Homoptera: Aphididae*). Journal of Economic Entomology 97: 1065-1070.

Lamari, L. and C.C. Bernier. 1989a. Evaluation of wheat lines and cultivars to tan spot *Pyrenophora tritici-repentis* based on lesion type. Canadian Journal of Plant Pathology 11: 49-56.

Lamari, L. and C.C. Bernier. 1989b. Toxin of *Pyrenophora tritici-repentis*: host-specificity, significance in disease, and inheritance of host reaction. Phytopathology 79: 740-744.

Lamari, L., B.D. McCallum and R.M. dePauw. 2005. Forensic pathology of Canadian bread wheat: The case of tan spot. Phytopathology 95: 144-152.

Lamari, L., R. Sayoud, M. Boulif and C.C. Bernier. 1995. Identification of a new race in *Pyrenophora tritici-repentis*: implications for the current pathotype classification system. Canadian Journal of Plant Pathology 17: 312-318.

Lamari, L., S.E. Strelkov, A. Yahyaoui, J. Orabi and R.B. Smith. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. Phytopathology 93: 391-396.

Laurie, C.C., S.D. Chasalow, J.R. LeDeaux, R. McCarroll, D. Bush, B. Hauge, et al. 2004. The genetic architecture of response to long-term artificial selection for oil concentration in the maize kernel. Genetics 168: 2141-2155.

Leonard, K.J. and L.J. Szabo. 2005. Stem rust of small grains and grasses caused by *Puccinia graminis*. Molecular Plant Pathology 6: 99-111.

Lewontin, R.C. 1964. The interaction of selection and linkage. I. General considerations; heterotic models. Genetics 49: 49-67.

Lewontin, R.C. and K.-i. Kojima. 1960. The evolutionary dynamics of complex polymorphisms. Evolution 14: 458-472.

Ling, H.-Q., S. Zhao, D. Liu, J. Wang, H. Sun, C. Zhang, et al. 2013. Draft genome of the wheat A-genome progenitor *Triticum urartu*. Nature 496: 87-90.

Lipka, A.E., F. Tian, Q. Wang, J. Peiffer, M. Li, P.J. Bradbury, et al. 2012. GAPIT: genome association and prediction integrated tool. Bioinformatics 28: 2397-2399.

Lippert, C., J. Listgarten, Y. Liu, C.M. Kadie, R.I. Davidson and D. Heckerman. 2011. FaST linear mixed models for genome-wide association studies. Nature Methods 8: 833-835.

Liu, W., Y. Jin, M. Rouse, B. Friebe, B. Gill and M.O. Pumphrey. 2011a. Development and characterization of wheat-*Ae. searsii* Robertsonian translocations and a recombinant chromosome conferring resistance to stem rust. Theoretical and Applied Genetics 122:1537-1545.

Liu, W., M. Rouse, B. Friebe, Y. Jin, B. Gill and M. Pumphrey. 2011b. Discovery and molecular mapping of a new gene conferring resistance to stem rust, *Sr53*, derived from *Aegilops geniculata* and characterization of spontaneous translocation stocks with reduced alien chromatin. Chromosome Research 19:669-682.

Liu, W., T.V. Danilova, M.N. Rouse, R.L. Bowden, B. Friebe, B.S. Gill and M.O. Pumphrey. 2013. Development and characterization of a compensating wheat-*Thinopyrum intermedium* Robertsonian translocation with *Sr44* resistance to stem rust (Ug99). Theoretical and Applied Genetics 126:1167-1177.

Liu, X.M., G.L. Brown-Guedira, J.H. Hatchett, J.O. Owuoche and M.S. Chen. 2005. Genetic characterization and molecular mapping of a Hessian fly-resistance gene transferred from *T. turgidum* ssp. *dicoccum* to common wheat. Theoretical and Applied Genetics 111: 1308-1315.

Liu, Z., J.D. Faris, R.P. Oliver, K.-C. Tan, P.S. Solomon, M.C. McDonald, et al. 2009. SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. PLoS Pathogens 5: e1000581.

Liu, Z., Z. Zhang, J.D. Faris, R.P. Oliver, R. Syme, M.C. McDonald, et al. 2012. The cysteine rich necrotrophic effector SnTox1 produced by *Stagonospora nodorum* triggers susceptibility of wheat lines harboring *Snn1*. PLoS Pathogens 8: e1002467.

Liu, Z.H., J.D. Faris, S.W. Meinhardt, S. Ali, J.B. Rasmussen and T.L. Friesen. 2004a. Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially purified host-selective toxin produced by *Stagonospora nodorum*. Phytopathology 94: 1056-1060.

Liu, Z.H., T.L. Friesen, J.B. Rasmussen, S. Ali, S.W. Meinhardt and J.D. Faris. 2004b. Quantitative trait loci analysis and mapping of seedling resistance to *Stagonospora nodorum* leaf blotch in wheat. Phytopathology 94: 1061-1067.

Lobell, D.B., W. Schlenker and J. Costa-Roberts. 2011. Climate trends and global crop production since 1980. Science 333: 616-620.

Loegering, W.Q. and H.R. Powers. 1962. Inheritance of pathogenicity in a cross of physiological races 111 and 36 of *Puccinia graminis* f. sp. *tritici*. Phytopathology 52: 547-554.

Loiselle, B.A., V.L. Sork, J. Nason and C. Graham. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). American Journal of Botany 82: 1420-1425.

Long, A.D. and C.H. Langley. 1999. The power of association studies to detect the contribution of candidate genetic loci to variation in complex traits. Genome Research 9: 720-731.

Long, D.L. and J.A. Kolmer. 1989. A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology 79: 525-529.

Luo, M.C., Z.L. Yang, F.M. You, T. Kawahara, J.G. Waines and J. Dvorak. 2007. The structure of wild and domesticated emmer wheat populations, gene flow between them, and the site of emmer domestication. Theoretical and Applied Genetics 114: 947-959.

Lynch, M. and K. Ritland. 1999. Estimation of pairwise relatedness with molecular markers. Genetics 152: 1753-1766.

Maccaferri, M., M.C. Sanguineti, A. Demontis, A. El-Ahmed, L. Garcia del Moral, F. Maalouf, et al. 2011. Association mapping in durum wheat grown across a broad range of water regimes. Journal of Experimental Botany 62: 409-438.

Machacek, J.E. 1945. The prevalence of *Septoria* on cereal seed in Canada. Phytopathology 35: 51-53.

Mackay, I.J., P. Bansept-Basler, T. Barber, A.R. Bentley, J. Cockram, et al. 2014. An eight-parent multiparent advanced generation inter-cross population for winter-sown wheat: creation, properties, and validation. G3: Genes|Genomes|Genetics 4: 1603-1610.

Mago, R., D. Verlin, P. Zhang, U. Bansal, H. Bariana, et al. 2013. Development of wheat–*Aegilops speltoides* recombinants and simple PCR-based markers for *Sr32* and a new stem rust resistance gene on the 2S#1 chromosome. Theoretical and Applied Genetics 126: 2943-2955.

Mamidi, S., S. Chikara, R.J. Goos, D.L. Hyten, D. Annam, S.M. Moghaddam, et al. 2011. Genome-wide association analysis identifies candidate genes associated with iron deficiency chlorosis in soybean. The Plant Genome 4: 154-164.

Manning, V.A., I. Pandelova and L.M. Ciuffetti. 2002. A race for a novel host-selective toxin. Phytopathology 92: S51.

Manolio, T.A., F.S. Collins, N.J. Cox, D.B. Goldstein, L.A. Hindorff, D.J. Hunter, et al. 2009. Finding the missing heritability of complex diseases. Nature 461: 747-753.

Marais, G.F. and A.S. Marais. 1994. The derivation of compensating translocations involving homoeologous group 3 chromosomes of wheat and rye. Euphytica 79: 75-80.

Marais, G.F., W.G. Wessels, M. Horn and F. Du Toit. 1998. Association of a stem rust resistance gene (*Sr45*) and two Russian wheat aphid resistance genes (*Dn5* and *Dn7*) with mapped structural loci in common wheat. South African Journal of Plant and Soil 15: 67-71.

Martinez, J.P., S.A. Ottum, S. Ali, L.J. Francl and L.M. Ciuffetti. 2001. Characterization of the *ToxB* gene from *Pyrenophora tritici-repentis*. Molecular Plant-Microbe Interactions 14: 675-677.

Matsuzaki, H., H. Loi, S. Dong, Y.-Y. Tsai, J. Fang, J. Law, et al. 2004. Parallel genotyping of over 10,000 SNPs using a one-primer assay on a high-density oligonucleotide array. Genome Research 14: 414-425.

McDonald, B.A., J. Miles, L.R. Nelson and R.E. Pettway. 1994. Genetic variability in nuclear DNA in field populations of *Stagonospora nodorum*. Phytopathology 84: 250-255.

McFadden, E.S. 1930. A successful transfer of emmer characters to *vulgare* wheat. Journal of the American Society of Agronomy 22: 1020-1034.

McIntosh, R.A., P.L. Dyck and G.J. Green. 1976. Inheritance of leaf rust and stem rust resistances in wheat cultivars Agent and Agatha. Crop and Pasture Science 28: 37-45.

McIntosh, R.A., N.H. Luig and E.P. Baker. 1967. Genetic and cytogenetic studies of stem rust, leaf rust and powdery midew resistances in Hope and related wheat cultivars. Australian Journal of Biological Sciences 20:1181-1192.

McIntosh, R.A., C.R. Wellings and R.F. Park. 1995. Wheat rusts: an atlas of resistance genes. CSIRO Publications, East Melbourne, Australia.

McIntosh, R.A., Y. Yamazaki, J. Dubcovsky, J. Rogers, C. Morris, R. Appels, et al. 2013. Catalogue of gene symbols for wheat. 12th International Wheat Genetics Symposium, Yokohama, Japan

Mendgen, K. and M. Hahn. 2002. Plant infection and the establishment of fungal biotrophy. Trends in Plant Science 7: 352-356.

Mergoum, M., P.K. Singh, S. Ali, E.M. Elias, J.A. Anderson, K.D. Glover, et al. 2007. Reaction of elite wheat genotypes from the northern Great Plains of North America to *Septoria* diseases. Plant Disease 91: 1310-1315.

Messmer, R., Y. Fracheboud, M. Bänziger, M. Vargas, P. Stamp and J.-M. Ribaut. 2009. Drought stress and tropical maize: QTL-by-environment interactions and stability of QTLs across environments for yield components and secondary traits. Theoretical and Applied Genetics 119: 913-930.

Mithal, S.K. and M.N. Kopper. 1990. Evaluation and conservation of wheat genetic resources in India. In: J. P. Srivastava and A. B. Damania, editors, Wheat genetic resources: meeting diverse needs. John Wiley & Sons, Chichester. p. 201-209.

Moreno, M.V., S.A. Stenglein, P.A. Balatti and A.E. Perelló. 2008. Pathogenic and molecular variability among isolates of *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat in Argentina. European Journal of Plant Pathology 122: 239-252.

Morris, J.F., B.F. Carver, R.M. Hunger and A.R. Klatt. 2010. Greenhouse assessment of seedling reaction to tan spot in synthetic hexaploid wheat. Crop Science 50: 952-959.

Murray, G.M. and J.P. Brennan. 2009. Estimating disease losses to the Australian wheat industry. Australasian Plant Pathology 38: 558-570.

Nagle, B.J., R.C. Frohberg and R.M.J. Hosford. 1982. Inheritance of resistance to tan spot of wheat. In: R. M. J. Hosford, editor Tan spot of wheat and related diseases workshop. North Dakota State University, Fargo.

Nazari, K., M. Mafi, A. Yahyaoui, R.P. Singh and R.F. Park. 2009. Detection of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) race TTKSK (Ug99) in Iran. Plant Disease 93: 317-317.

Neale, D.B. and O. Savolainen. 2004. Association genetics of complex traits in conifers. Trends in Plant Science 9: 325-330.

Nelson, L.R., D.D. Morey and A.R. Brown. 1974. Wheat cultivar responses to severe glume blotch in Georgia. Plant Disease Reporter 58: 21-23.

Ng, S.B., E.H. Turner, P.D. Robertson, S.D. Flygare, A.W. Bigham, C. Lee, et al. 2009. Targeted capture and massively parallel sequencing of 12 human exomes. Nature 461: 272-276.

Nijman, I.J., S. Kuipers, M. Verheul, V. Guryev and E. Cuppen. 2008. A genome-wide SNP panel for mapping and association studies in the rat. BMC Genomics 9: 95.

Niu, Z., D.L. Klindworth, T.L. Friesen, S. Chao, Y. Jin, X. Cai, et al. 2011. Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. Genetics 187: 1011-1021.

Niu, Z., D.L. Klindworth, G. Yu, T.L. Friesen, S. Chao, Y. Jin, et al. 2014. Development and characterization of wheat lines carrying stem rust resistance gene *Sr43* derived from *Thinopyrum ponticum*. Theoretical and Applied Genetics 127: 969-980.

Njau, P.N., S. Bhavani, J. Huerta-Espino, R. Wanyera and R.P. Singh. 2013. Breeding for rust resistance in Kenya: Successes, setbacks and future approaches. BGRI Technical Workshop. New Delhi, India. p. 13.

O'connor, B.P. 2000. SPSS and SAS programs for determining the number of components using parallel analysis and Velicer's MAP test. Behavior Research Methods, Instruments, & Computers 32: 396-402.

Oerke, E.-C. 2006. Crop losses to pests. The Journal of Agricultural Science 144: 31-43.

Oliver, R.E., X. Cai, T.L. Friesen, S. Halley, R.W. Stack and S.S. Xu. 2008b. Evaluation of Fusarium head blight resistance in tetraploid wheat (*Triticum turgidum* L.). Crop Science 48: 213-222.

Oliver, R.E., X. Cai, R.-C. Wang, S.S. Xu and T.L. Friesen. 2008. Resistance to tan spot and *Stagonospora nodorum* blotch in wheat-alien species derivatives. Plant Disease 92: 150-157.

Oliver, R.P., T.L. Friesen, J.D. Faris and P.S. Solomon. 2012. *Stagonospora nodorum*: from pathology to genomics and host resistance. Annual Review of Phytopathology 50: 23-43.

Oliver, R.P. and S.V.S. Ipcho. 2004. Arabidopsis pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens. Molecular Plant Pathology 5: 347-352.

Oliver, R.P. and P.S. Solomon. 2010. New developments in pathogenicity and virulence of necrotrophs. Current Opinion in Olant Biology 13: 415-419.

Olivera, P.D., A. Badebo, S.S. Xu, D.L. Klindworth and Y. Jin. 2012. Resistance to Race TTKSK of *Puccinia graminis* f. sp. *tritici* in Emmer Wheat. Crop Science 52: 2234-2242.

Olivier, M. 2005. The Invader® assay for SNP genotyping. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 573: 103-110.

Orolaza, N.P., L. Lamari and G.M. Ballance. 1995. Evidence of a host-specific chlorosis toxin from *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat. Phytopathology 85: 1282-1287.

Ott, J., Y. Kamatani and M. Lathrop. 2011. Family-based designs for genome-wide association studies. Nature Reviews Genetics 12: 465-474.

Ozkan, H., A. Brandolini, C. Pozzi, S. Effgen, J. Wunder and F. Salamini. 2005. A reconsideration of the domestication geography of tetraploid wheat. Theoretical and Applied Genetics 110: 1052-1060.

Ozkan, H., A. Brandolini, R. Schäfer-Pregl and F. Salamini. 2002. AFLP analysis of a collection of tetraploid wheats indicates the origin of emmer and hard wheat domestication in southeast Turkey. Molecular Biology and Evolution 19: 1797-1801.

Perneger, T.V. 1998. What's wrong with Bonferroni adjustments. BMJ (Clinical research ed.) 316: 1236-1238.

Piergiovanni, A.R., R. Rizzi, E. Pannacciulli and C.D. Gatta. 1997. Mineral composition in hulled wheat grains: a comparison between emmer (*Triticum dicoccon* Schrank) and spelt (*T. spelta* L.) accessions. International Journal of Food Sciences and Nutrition 48: 381-386.

Pretorius, Z.A., R.P. Singh, W.W. Wagoire and T.S. Payne. 2000. Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis*. f. sp. *tritici* in Uganda. Plant Disease 84: 203-203.

Price, A.L., N.J. Patterson, R.M. Plenge, M.E. Weinblatt, N.A. Shadick and D. Reich. 2006. Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics 38: 904-909.

Pritchard, J.K. and M. Przeworski. 2001. Linkage disequilibrium in humans: models and data. The American Journal of Human Genetics 69: 1-14.

Pritchard, J.K. and N.A. Rosenberg. 1999. Use of unlinked genetic markers to detect population stratification in association studies. The American Journal of Human Genetics 65: 220-228.

Pritchard, J.K., M. Stephens and P. Donnelly. 2000a. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Pritchard, J.K., M. Stephens, N.A. Rosenberg and P. Donnelly. 2000b. Association mapping in structured populations. The American Journal of Human Genetics 67: 170-181.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M.A.R. Ferreira, D. Bender, et al. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics 81: 559-575.

Qi, L.L., M.O. Pumphrey, B. Friebe, P. Zhang, C. Qian, R.L. Bowden, M.N. Rouse, Y. Jin and B.S. Gill. 2011. A novel Robertsonian translocation event leads to transfer of a stem rust resistance gene (*Sr52*) effective against race Ug99 from *Dasypyrum villosum* into bread wheat. Theoretical and Applied Genetics 123:159-167.

Quaedvlieg, W., G.J.M. Verkley, H.-D. Shin, R.W. Barreto, A.C. Alfenas, et al. 2013. Sizing up Septoria. Studies in Mycology 75: 307-390.

R Development Core Team. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ravel, C., S. Praud, A. Murigneux, L. Linossier, M. Dardevet, F. Balfourier, et al. 2006. Identification of *Glu-B1-1* as a candidate gene for the quantity of high-molecular-weight glutenin in bread wheat (*Triticum aestivum* L.) by means of an association study. Theoretical and Applied Genetics 112: 738-743.

Rees, R.G. and G.J. Platz. 1990. Sources of resistance to *Pyrenophora tritici-repentis* in bread wheats. Euphytica 45: 59-69.

Reif, J.C., P. Zhang, S. Dreisigacker, M.L. Warburton, M. van Ginkel, D. Hoisington, et al. 2005. Wheat genetic diversity trends during domestication and breeding. Theoretical and Applied Genetics 110: 859-864.

Remington, D.L., J.M. Thornsberry, Y. Matsuoka, L.M. Wilson, S.R. Whitt, J. Doebley, et al. 2001. Structure of linkage disequilibrium and phenotypic associations in the maize genome. Proceedings of the National Academy of Sciences 98: 11479-11484.

Riede, C.R., N.D. Williams and J.D. Miller. 1995a. Wheat lines monogenic for resistance to stem rust from the wheat cultivar 'Waldron'. Theoretical and Applied Genetics 90: 1164-1168.

Riedelsheimer, C., J. Lisec, A. Czedik-Eysenberg, R. Sulpice, A. Flis, C. Grieder, et al. 2012. Genome-wide association mapping of leaf metabolic profiles for dissecting complex traits in maize. Proceedings of the National Academy of Sciences 109: 8872-8877.

Ritland, K. 1996. A marker-based method for inferences about quantitative inheritance in natural populations. Evolution 50: 1062-1073.

Rodriguez-Quijano, M., R. Lucas and J.M. Carrillo. 2003. Waxy proteins and amylose content in tetraploid wheats *Triticum dicoccum* Schulb, *Triticum durum* L. and *Triticum polonicum* L. Euphytica 134: 97-101.

Roelfs, A.P. 1985. Wheat and rye stem rust. In: W. R. Bushnell and A. P. Roelfs, editors, The cereal rusts. Academic Press, Orlando, Florida, USA. p. 3-37.

Roelfs, A.P. and J.W. Martens. 1988. An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. Phytopathology 78: 526-533.

Roelfs, A.P., R.P. Singh and E.E. Saari. 1992. Rust diseases of wheat: concepts and methods of disease management, CIMMYT, Mexico, D.F.

Rostoks, N., L. Ramsay, K. MacKenzie, L. Cardle, P.R. Bhat, M.L. Roose, et al. 2006. Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. Proceedings of the National Academy of Sciences 103: 18656-18661.

Rouse, M., J. Nirmala, Y. Jin, S. Chao, T. Fetch, Jr., et al. 2014. Characterization of *Sr9h*, a wheat stem rust resistance allele effective to Ug99. Theoretical and Applied Genetics 127: 1681-1688.

Rouse, M.N., I.C. Nava, S. Chao, J.A. Anderson and Y. Jin. 2012. Identification of markers linked to the race Ug99 effective stem rust resistance gene *Sr28* in wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 125: 877-885.

Rouse, M.N., E.L. Olson, B.S. Gill, M.O. Pumphrey and Y. Jin. 2011b. Stem rust resistance in *Aegilops tauschii* germplasm. Crop Science 51: 2074-2078.

Saintenac, C., W. Zhang, A. Salcedo, M.N. Rouse, H.N. Trick, E. Akhunov, et al. 2013. Identification of wheat gene *Sr35* that confers resistance to Ug99 stem rust race group. Science 341: 783-786.

Salvi, S., R. Tuberosa and R. Phillips. 2001. Development of PCR-based assays for allelic discrimination in maize by using the 5′-nuclease procedure. Molecular Breeding 8: 169-176.

Sayed, H.I. 1985. Diversity of salt tolerance in a germplasm collection of wheat (*Triticum* spp.). Theoretical and Applied Genetics 69: 651-657.

Schafer, J.F., A.P. Roelfs and W.R. Bushnell. 1984. Contributions of early scientists to knowledge of cereal rusts. In: W. R. Bushnell and A. P. Roelfs, editors, The cereal rust. Academic Press, Orlando, Florida, USA.

Scharen, A.L. and J.M. Krupinsky. 1969. Effect of *Septoria nodorum* infection on CO_2 absorption and yield of wheat. Phytopathology 59: 1228-1301.

Schilder, A.M.C. and G.C. Bergstorm. 1992. The dispersal of conidia and ascospores of *Pyrenophora rritici-repentis*. In: L. J. Francl, J. M. Krupinsky and M. P. McMullen, editors, the 2nd Int. Tan Spot Workshop. North Dakota State University, Fargo. p. 96-99.

Schnurbusch, T., S. Paillard, D. Fossati, M. Messmer, G. Schachermayr, et al. 2003. Detection of QTLs for Stagonospora glume blotch resistance in Swiss winter wheat. Theoretical and Applied Genetics 107: 1226-1234.

Schwarz, G. 1978. Estimating the dimension of a model. Annals of Statistics 6: 461-464.

Sebat, J., B. Lakshmi, J. Troge, J. Alexander, J. Young, P. Lundin, et al. 2004. Large-scale copy number polymorphism in the human genome. Science 305: 525-528.

Segura, V., B.J. Vilhjálmsson, A. Platt, A. Korte, Ü. Seren, Q. Long, et al. 2012. An efficient multi-locus mixed-model approach for genome-wide association studies in structured populations. Nature Genetics 44: 825-830.

Shabeer, A. and W.W. Bockus. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. Plant Disease 72:599–602.

Siedler, H., A. Obst, S.L.K. Hsam and F.J. Zeller. 1994. Evaluation for resistance to *Pyrenophora tritici-repentis* in *Aegilops tauschii* Coss. and synthetic hexaploid wheat amphiploids. Genetic Resources and Crop Evolution 41: 27-34.

Singh, P.K., J.L. Gonzalez-Hernandez, M. Mergoum, S. Ali, T.B. Adhikari, S.F. Kianian, et al. 2006b. Identification and molecular mapping of a gene conferring resistance to *Pyrenophora tritici-repentis* race 3 in tetraploid wheat. Phytopathology 96: 885-889.

Singh, P.K., M. Mergoum, S. Ali, T.B. Adhikari, E.M. Elias, J.A. Anderson, et al. 2006c. Evaluation of elite wheat germ plasm for resistance to tan spot. Plant Disease 90: 1320-1325.

Singh, P.K., M. Mergoum, S. Ali, T.B. Adhikari, E.M. Elias and G.R. Hughes. 2006d. Identification of new sources of resistance to tan spot, *Stagonospora nodorum* blotch, and *Septoria tritici* blotch of wheat. Crop Science 46: 2047-2053.

Singh, P.K., R.P. Singh, E. Duveiller, M. Mergoum, T.B. Adhikari and E.M. Elias. 2010. Genetics of wheat-*Pyrenophora tritici-repentis* interactions. Euphytica 171: 1-13.

Singh, R.P. et al. *Lr34/Yr18/Pm38/Ltn1* confers slow rusting, adult plant resistance to *Puccinia graminis* f. sp. *tritici*. Manuscript in preparation.

Singh, R.P., D.P. Hodson, J. Huerta-Espino, Y. Jin, S. Bhavani, P. Njau, et al. 2011a. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annual Review of Phytopathology 49: 465-481.

Singh, R.P., J. Huerta-Espino, S. Bhavani, S.A. Herrera-Foessel, D. Singh, P.K. Singh, et al. 2011b. Race non-specific resistance to rust diseases in CIMMYT spring wheats. Euphytica 179: 175-186.

Singh, R.P., J. Huerta-Espino, W. Pfeiffer and P. Figueroa-Lopez. 2004a. Occurrence and impact of a new leaf rust race on durum wheat in northwestern Mexico from 2001 to 2003. Plant Disease 88: 703-708.

Springer, N.M., K. Ying, Y. Fu, T. Ji, C.-T. Yeh, Y. Jia, et al. 2009. Maize inbreds exhibit high levels of copy number variation (CNV) and presence/absence variation (PAV) in genome content. PLoS Genetics 5: e1000734.

Stallknecht, G.F., K.M. Gilbertson and J.E. Ranney. 1996. Alternative wheat cereals as food grains: Einkorn, emmer, spelt, kamut, and triticale. In: J. Janick, editor Progress in new crops. ASHS Press, Alexandria. p. 156-170.

Steffenson, B.J., P. Olivera, J.K. Roy, Y. Jin, K.P. Smith and G.J. Muehlbauer. 2007. A walk on the wild side: mining wild wheat and barley collections for rust resistance genes. Crop and Pasture Science 58: 532-544.

Stehno, Z. 2007. Emmer wheat Rudico can extend the spectra of cultivated plants. Czech Journal of Genetics and Plant Breeding 43: 113-115.

Stich, B., J. Möhring, H.-P. Piepho, M. Heckenberger, E.S. Buckler and A.E. Melchinger. 2008. Comparison of mixed-model approaches for association mapping. Genetics 178: 1745-1754.

Strelkov, S.E. and L. Lamari. 2003. Host–parasite interactions in tan spot (*Pyrenophora tritici-repentis)* of wheat. Canadian Journal of Plant Pathology 25: 339-349.

Strelkov, S.E., L. Lamari and G.M. Ballance. 1999. Characterization of a host-specific protein toxin (Ptr ToxB) from *Pyrenophora tritici-repentis*. Molecular Plant-Microbe Interactions 12: 728-732.

Sukumaran, S. and J. Yu. 2014. Association mapping of genetic resources: achievements and future perspectives. In: R. Tuberosa, A. Graner and E. Frison, editors, Genomics of Plant Genetic Resources. Springer Netherlands. p. 207-235.

Sunderwirth, S.D. and A.P. Roelfs. 1980. Greenhouse evaluation of the adult plant resistance of *Sr2* to wheat stem rust. Phytopathology 70: 634-637.

Svishcheva, G.R., T.I. Axenovich, N.M. Belonogova, C.M. van Duijn and Y.S. Aulchenko. 2012. Rapid variance components-based method for whole-genome association analysis. Nature Genetics 44: 1166-1170.

Szabo, L.J. and J.A. Kolmer. 2007. Development of simple sequence repeat markers for the plant pathogenic rust fungus *Puccinia triticina*. Molecular Ecology Notes 7: 708-710.

Tadesse, W., S.L.K. Hsam, G. Wenzel and F.J. Zeller. 2006a. Identification and monosomic analysis of tan spot resistance genes in synthetic wheat lines (*Triticum turgidum* L.× *Aegilops tauschii* Coss.). Crop Science 46: 1212-1217.

Tadesse, W., S.L.K. Hsam and F.J. Zeller. 2006b. Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'. Plant Breeding 125: 318-322.

Tadesse, W., M. Schmolke, S.L.K. Hsam, V. Mohler, G. Wenzel and F.J. Zeller. 2007. Molecular mapping of resistance genes to tan spot [*Pyrenophora tritici-repentis* race 1] in synthetic wheat lines. Theoretical and Applied Genetics 114: 855-862.

The, T.T. 1973. Chromosome location of genes conditioning stem rust resistance transferred from diploid to hexaploid wheat. Nature 241: 256-256.

Thomas, S.C. and W.G. Hill. 2000. Estimating quantitative genetic parameters using sibships reconstructed from marker data. Genetics 155: 1961-1972.

Thornsberry, J.M., M.M. Goodman, J. Doebley, S. Kresovich, D. Nielsen and E.S. Buckler. 2001. *Dwarf8* polymorphisms associate with variation in flowering time. Nature Genetics 28: 286-289.

Tomás, A., G.H. Feng, G.R. Reeck, W.W. Bockus and J.E. Leach. 1990. Purification of a cultivar-specific toxin from *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. Molecular Plant-Microbe Interactions 3: 221-224.

Tommasini, L., T. Schnurbusch, D. Fossati, F. Mascher and B. Keller. 2007. Association mapping of *Stagonospora nodorum* blotch resistance in modern European winter wheat varieties. Theoretical and Applied Genetics 115: 697-708.

van der Laan, M.J., M.D. Birkner and A.E. Hubbard. 2005. Empirical bayes and resampling based multiple testing procedure controlling tail probability of the proportion of false positives. Statistical Applications in Genetics and Molecular Biology 4: 1-32.

van Slageren, M.W. 1994. Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (Poaceae). Wageningen Agricultural University Papers. 1994(7). 513 pp.

VanRaden, P.M. 2008. Efficient methods to compute genomic predictions. Journal of Dairy Science 91: 4414-4423.

Velicer, W.F. 1976. Determining the number of components from the matrix of partial correlations. Psychometrika 41: 321-327.

Wang, J. 2002. An estimator for pairwise relatedness using molecular markers. Genetics 160: 1203-1215.

Wang, Ju, P.E. McClean, R. Lee, R.J. Goos, T. Helms. 2008. Association mapping of iron deficiency chlorosis loci in soybean (*Glycine max* L. Merr.) advanced breeding lines. Theoretical and Applied Genetics 116: 777-787.

Wang, S., D. Wong, K. Forrest, A. Allen, S. Chao, B.E. Huang, et al. 2014. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnology Journal: 1-10. doi:10.1111/pbi.12183.

Wang, W.Y.S., B.J. Barratt, D.G. Clayton and J.A. Todd. 2005. Genome-wide association studies: theoretical and practical concerns. Nature Reviews Genetics 6: 109-118.

Wang, X., G. Bakkeren and B. McCallum. 2010. Virulence and molecular polymorphisms of the wheat leaf rust fungus *Puccinia triticina* in Canada from 1997 to 2007. Botany 88: 575-589.

Wang, Z., M. Gerstein and M. Snyder. 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nature Reviews Genetics 10: 57-63.

Weber, A.L., W.H. Briggs, J. Rucker, B.M. Baltazar, J. de Jesús Sánchez-Gonzalez, P. Feng, et al. 2008. The genetic architecture of complex traits in Teosinte (*Zea mays* ssp. *parviglumis*): New evidence from association mapping. Genetics 180: 1221-1232.

Weber, G.F. 1922. *Septoria* diseases of cereals. II. *Septoria* diseases of Wheat. Phytopathology 12: 537-585.

Willcox, G. 1998. Archaeobotanical evidence for the beginnings of agriculture in Southwest Asia. In: A.B. Damania, J. Valkoun, G. Willcox and C. O. Qualset, editors, The Origins of agriculture and crop domestication. ICARDA, Aleppo. p. 25-38.

Wright, W.T., S.V. Heggarty, I.S. Young, D.P. Nicholls, R. Whittall, S.E. Humphries, et al. 2008. Multiplex MassARRAY spectrometry (iPLEX) produces a fast and economical test for 56 familial hypercholesterolaemia-causing mutations. Clinical Genetics 74: 463-468.

Xiong, Y.-C., F.-M. Li and T. Zhang. 2006. Performance of wheat crops with different chromosome ploidy: root-sourced signals, drought tolerance, and yield performance. Planta 224: 710-718.

Xu, S.S., T.L. Friesen and A. Mujeeb-Kazi. 2004. Seedling resistance to tan spot and *Stagonospora nodorum* blotch in synthetic hexaploid wheats. Crop Science 44: 2238-2245.

Xu, S.S., Y. Jin, D.L. Klindworth, R.R.C. Wang and X. Cai. 2009. Evaluation and Characterization of Seedling Resistances to Stem Rust Ug99 Races in Wheat-Alien Species Derivatives. Crop Science 49: 2167-2175.

Yao, J., L. Wang, L. Liu, C. Zhao and Y. Zheng. 2009. Association mapping of agronomic traits on chromosome 2A of wheat. Genetica 137: 67-75.

Yu, G., Q. Zhang, T.L. Friesen, M.N. Rouse, Y. Jin et al. 2015. Identification and mapping of *Sr46* from *Aegilops tauschii* accession CIae 25 conferring resistance to race TTKSK (Ug99) of wheat stem rust pathogen. Theoretical and Applied Genetics 128: 431-443.

Yu, J., G. Pressoir, W.H. Briggs, I. Vroh Bi, M. Yamasaki, J.F. Doebley, et al. 2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nature Genetics 38: 203-208.

Zaharieva, M., N.G. Ayana, A. Al Hakimi, S.C. Misra and P. Monneveux. 2010. Cultivated emmer wheat (*Triticum dicoccon* Schrank), an old crop with promising future: a review. Genetic Resources and Crop Evolution 57: 937-962.

Zambino, P.J., A.R. Kubelik and L.J. Szabo. 2000. Gene action and linkage of avirulence genes to DNA markers in the rust fungus *Puccinia graminis*. Phytopathology 90: 819-826.

Zhang, D., G. Bai, C. Zhu, J. Yu and B.F. Carver. 2010b. Genetic diversity, population structure, and linkage disequilibrium in U.S. elite winter wheat. Plant Genome 3: 117-127.

Zhang, J.X., R.P. Singh, J.A. Kolmer, J. Huerta-Espino, Y. Jin, et al. 2008. Genetics of leaf rust resistance in Brambling wheat. Plant Disease 92: 1111-1118.

Zhang, N., Y. Xu, M. Akash, S. McCouch and J.H. Oard. 2005. Identification of candidate markers associated with agronomic traits in rice using discriminant analysis. Theoretical and Applied Genetics 110: 721-729.

Zhang, Z., E. Ersoz, C.-Q. Lai, R.J. Todhunter, H.K. Tiwari, M.A. Gore, et al. 2010c. Mixed linear model approach adapted for genome-wide association studies. Nature Genetics 42: 355-360.

Zhang, Z., T.L. Friesen, S.S. Xu, G. Shi, Z. Liu, J.B. Rasmussen, et al. 2011. Two putatively homoeologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to *Stagonospora nodorum*. The Plant Journal 65: 27-38.

Zhao, K., M.J. Aranzana, S. Kim, C. Lister, C. Shindo, C. Tang, et al. 2007. An Arabidopsis example of association mapping in structured samples. PLoS Genetics 3: e4.

Zheng, Q., D.L. Klindworth, T.L. Friesen, Y. Jin, A.-F. Liu, Z.-S. Li, et al. 2014. Characterization of *Thinopyrum* species for wheat stem rust resistance and ploidy level. Crop Science 54: 2663-2672.

Zhong, S., Y. Leng, T.L. Friesen, J.D. Faris and L.J. Szabo. 2009. Development and characterization of expressed sequence tag-derived microsatellite markers for the wheat stem rust fungus *Puccinia graminis* f. sp. *tritici*. Phytopathology 99: 282-289.

Zhou, X. and M. Stephens. 2012. Genome-wide efficient mixed-model analysis for association studies. Nature Genetics 44: 821-824.

Zhou, X. and M. Stephens. 2014. Efficient multivariate linear mixed model algorithms for genome-wide association studies. Nature Methods 11: 407-409.

Zhu, C., M. Gore, E.S. Buckler and J. Yu. 2008. Status and prospects of association mapping in plants. The Plant Genome 1: 5-20.

Zhu, C., X. Li and J. Yu. 2011. Integrating rare-variant testing, function prediction, and gene network in composite resequencing-based genome-wide association studies (CR-GWAS). G3: Genes, Genomes, Genetics 1: 233-243.

Zondervan, K.T. and L.R. Cardon. 2004. The complex interplay among factors that influence allelic association. Nature Reviews Genetics 5: 89-100.

CHAPTER 3. ASSOCIATION ANALYSIS OF STEM RUST RESISTANCE IN CULTIVATED EMMER WHEAT

Abstract

Cultivated emmer wheat (*Triticum turgidum* subsp. *dicoccum*) is known to be a good source of resistance to wheat stem rust (caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & Henn., abbreviated as *Pgt*). Several major genes/alleles conferring resistance to stem rust (e.g. *Sr2*, *Sr9d*, *Sr9e*, *Sr13*, *Sr14*, and *Sr17*) that are currently deployed in modern wheat cultivars and germplasm were derived from cultivated emmer. As part of our effort to find novel *Sr* genes, we previously evaluated 359 cultivated emmer accessions for reactions to seven *Pgt* races, including Ug99 (TTKSK), and identified 107 accessions with resistance to multiple races. The objective of this study was to detect *Sr* genes in these resistant accessions using association mapping. For the analysis, a panel of 180 cultivated emmer accessions was assembled based on stem rust reactions and geographic origins, and then was genotyped using the wheat 9K SNP Infinium array. After filtering for missing data points and minor allele frequencies, 4,134 SNPs were chosen for association analysis on 178 emmer wheat accessions. Genome-wide association analysis revealed 222 SNP markers that were significantly associated with the stem rust resistance at the seedling stage. Among the 36 significant markers at the significant level of 0.1 percentile, 10 co-located with several previously known genes or QTL conferring resistance to stem rust, including *Sr12*, *Sr14*, *Sr22*, *Sr58*, and *QSr.sun-7A*. The remaining 26 markers, located on chromosomes 1A, 2A, 3A, 4A, 4B, 5A, 5B, 6A, 6B, and 7A, were found in the genomic regions where no known *Sr* genes were previously identified, suggesting that some of the emmer wheat accessions carry novel *Sr* genes. Significant markers with major effects on stem rust resistance were selected based on stepwise regression. Their allelic combinations may be useful

for marker-assisted selection in wheat breeding programs. This study provided preliminary evidence for discovering novel stem rust resistance genes in cultivated emmer wheat germplasm.

Introduction

Stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & Henn., is a historically, economically, and globally important disease of wheat, including durum wheat (*Triticum turgidum* subsp. *durum*) and common wheat (*T. aestivum*). In the 20th century there are numerous examples of stem rust epidemics in Europe and Australia. In the U.S., losses of more than 20% occurred several times between 1917 and 1935. In eight of the 40 years spanning 1920 to 1960, losses reached 10% in the spring wheat region of Minnesota, North Dakota, and South Dakota (Leonard and Szabo, 2005). Starting in 1960, the heavy losses caused by stem rust were reduced due to the worldwide deployment of stem rust resistance (*Sr*) genes (Singh et al., 2006; Xu et al., 2009). One of the most important and widely deployed genes was *Sr31*, derived from rye, *Secale cereale* L. (Singh et al., 2006). In 1999, a new threat from stem rust was detected in Uganda, a race that is virulent to *Sr31* and subsequently called Ug99 (*pgt*-TTKSK) (Pretorius et al., 2000; Jin et al., 2008). Ug99 spread rapidly from the highlands of East Africa to adjacent wheat-growing regions, such as Yemen (Jin et al., 2008) and Iran (Nazari et al., 2009).

Ug99 is evolving rapidly. Several new variants of TTKSK have been detected in the past 15 years, such as TTKST (virulent to *Sr24*), TTTSK (virulent to *Sr36*), TTKSF (virulent to *Sr21*), TTKSP (virulent to *Sr31*, *Sr21*, and *Sr24*), PTKSK, PTKST, and TTKSF+ (Jin et al., 2008, 2009; Hodson et al., 2012). Clearly the Ug99 group of races and populations has the ability to evolve broad virulence against the *Sr* genes that are currently deployed in wheat cultivars around the world (Singh et al., 2011; Jin and Singh, 2006). So far, only a small number of wheat

cultivars have been found to be resistant to Ug99 (Singh et al., 2011; Jin and Singh, 2006). Therefore it is crucial to identify novel Ug99 resistance genes among various wheat gene pools.

Cultivated emmer wheat (*T. turgidum* ssp. *dicoccom,* $2n = 4x = 28$, AABB) is a hulled tetraploid wheat subspecies. It was major cultivated wheat in ancient times. Today it is rarely an important crop, except in regions that suffer from drought, e.g., India, Ethiopia, and Yemen (Zaharieva et al., 2010). On the other hand, emmer constitutes an important genetic resource for the improvement of the two most important wheats, common wheat and durum wheat. Several wheat breeding programs have developed cultivars with good disease resistance derived from cultivated emmer (Zaharieva et al., 2010).

For resistance to stem rust, six *Sr* genes/alleles (i.e. *Sr2*, *Sr9d*, *Sr9e*, *Sr13*, *Sr14*, and *Sr17*) were derived from cultivated emmer (McIntosh et al., 2013). Among these genes/alleles, *Sr2*, *Sr9d*, and *Sr17* were initially transferred from Yaroslav emmer to common wheat variety Marquis (McFadden, 1930; McIntosh et al., 1967). *Sr2* is located on chromosome arm 3BS and confers adult plant resistance (APR) to TTKSK, as well as many other *Pgt* races. This gene has been extensively used in wheat breeding and it now exists in many wheat cultivars around the world (Roelfs, 1985). *Sr2* is currently an important *Sr* gene for developing Ug99-resistant cultivars in many wheat breeding programs worldwide. Both *Sr9d* and *Sr17* are not resistant to TTKSK and they have not been extensively used in durum and common wheat breeding (Jin et al., 2007; McIntosh et al., 2013). *Sr17* was deployed in some common wheat cultivars while *Sr9d* is present in only a few of durum and common wheat cultivars (McIntosh et al., 2013). The genes *Sr13* and *Sr14* were originally transfered from Khapli emmer to common wheat variety Marquis (Knott, 1962) and they are located on chromosome arms 6AL and 1BL, respectively (McIntosh, 1972; 1980). *Sr13* is a major gene used in modern durum cultivars to control various

stem rust races, including TTKSK. Because *Sr13* is susceptible to race TRTTF (Singh et al., 2011), it is necessary to pyramid *Sr13* with other Ug99-resistance genes in durum. *Sr14* has not been deployed in durum and common wheat cultivars and its resistance to TTKSK has not been confirmed yet (Jin et al., 2007). The *Sr9* allele *Sr9e* on chromosome arm 2BL was originally transferred from Vernal emmer to durum wheat (Smith, 1957). This gene is present in many durum cultivars but it is not resistant to TTKSK (Jin et al., 2007).

The six *Sr* genes/alleles described above were derived from just three cultivated emmer accessions. Clearly, most of the cultivated emmer accessions maintained in various genetic resource centers worldwide have not been explored for stem rust resistance. Beteselassie et al. (2007) reported that 18 (44%) of 41 cultivated emmer accessions were resistant at the seedling stage when exposed to a mixture of six *Pgt* isolates. In a recent study, Olivera et al. (2012a) screened 359 emmer wheat accessions for reactions to multiple *Pgt* races, including TTKSK, and found that 31.8% of the accessions were resistant to most races. There is a strong possibility that some of the resistant accessions identified by Beteselassie et al. (2007) and Olivera et al. (2012a) possess new *Sr* genes for resistance to various stem rust races, including races in the Ug99 lineage.

In recent years, association mapping, also named as linkage disequilibrium mapping or association analysis, has become an important strategy for identifying major genes and quantitative trait loci (QTL) in various crop species such as rice, maize, and soybean (Salvi and Tuberosa, 2005; Thornsberry et al., 2001; Gupta et al., 2005; Zondervan and Cardon, 2004). Association mapping utilizes linkage disequilibrium among alleles at different loci to analyze the association between markers and characters. It identifies functional alleles, DNA sequences, or genotypes that are strongly associated with mutations of the genes that contribute to the

phenotypic trait of interest (Gupta et al., 2005). There are two types of association analysis. One is based on genome scan or genome-wide association study (GWAS). The other is based on candidate-gene testing (Zhu et al., 2008). For both strategies, procedures include: 1) assembling, phenotyping and genotyping a natural population, 2) analyzing the population structure and kinship of the population, 3) choosing an appropriate statistical model to estimate the significance level of associations between molecular markers and the evaluated characters, and 4) discovering the targeted genes and QTL (Breseghello and Sorrells, 2006).

Genome-wide association study has been used to identify molecular markers that are significantly associated with resistance to Ug99. Through association analysis, Yu et al. (2010) identified four DArT markers near the location of *Sr2* associated with the *Sr2* haplotype, and one significant DArT marker associated with the *Sr25* haplotype. Yu et al. (2011, 2012) conducted association analysis on 276 spring and 232 winter wheat breeding lines that were scored for adult resistance to Ug99 in field tests in Kenya. They identified 15 and 12 markers significantly associated with resistance to stem rust in spring wheat and winter wheat, respectively. In a recent study, Letta et al. (2013) conducted GWAS on 183 durum accessions genotyped with 1,253 molecular markers. They identified 12 markers significantly associated with Ug99 resistance, which was evaluated in the field during three to four seasons in Ethiopia. Most of the significant markers identified in these studies (Yu et al., 2011, 2012; Letta et al., 2013) were reported to be at, or near, the genomic regions harboring the known *Sr* genes or QTL. Several other markers were located at the genomic regions where no known *Sr* genes were previously reported.

So far, association mapping has not been used to find resistance to any wheat diseases in cultivated emmer wheat germplasm. The objective of the present study is to detect genomic

regions associated with stem rust resistance in a cultivated emmer population consisting 180 accessions through GWAS using wheat 9K SNP markers.

Materials and Methods

Plant Material

Plant materials used in this study included 180 cultivated emmer wheat accessions (Table A1), a sub set of 359 accessions evaluated for stem rust resistance by Olivera et al. (2012a). The original seeds of 180 cultivated emmer accessions were kindly provided by Dr. Harold E. Bockelman at the USDA-ARS National Small Grains Collection (Aberdeen, ID). Except four accessions with unknown origins, all other accessions originated from 36 countries in northern Africa, southwest Asia, Europe, and North or South America (Table A1).

In addition to cultivated emmer wheat, 10 durum lines carrying known or unknown *Sr* genes, including KL-B (*Sr13*), KL-D (*Sr14*), 8155-B1, 8155-B2, 8155-C1, Vn-B1 (*Sr9e*), Im-C2, Im-B7, ST-464-A1 (*Sr8*), and ST-464-C1 (*Sr13*), were used as resistant controls and durum line Rusty (Klindworth et al., 2006) was used as susceptible control in stem rust testing and genotyping.

Stem Rust Resistance Evaluation

Stem rust resistance at seedling stage was evaluated by Olivera et al. (2012a) in USDA-ARS Cereal Disease Laboratory, St. Paul, MN. All accessions at seedling stage were evaluated in the greenhouse for the stem rust resistance against seven races including QTHJC, MCCFC, RKQQC, TPMKC, TRTTF, TTKSK, and TTTTF. The race designation is based on the nomenclature system described by Jin et al. (2008). The virulence and avirulence phenotypic responses of the seven races to major *Sr* genes were listed in Table 3.5. Except for two races TTKSK and TRTTF originating from Kenya and Yemen, respectively, the other five races

originated from the U.S. The detailed inoculation and evaluation procedure has been described by Olivera et al. (2012a). All of the assessments were performed with one replicate, in which five seedlings per accession were inoculated and each accession was scored once based on the reactions of all five seedlings (Olivera et al., 2012a).

The stem rust infection types (ITs) were scored based on the scoring system introduced by Stakman et al. (1962). According to Stakman's rating system, the IT scores were represented with five basic levels (0, ;, 1, 2, 3, and 4) and two signs "-" and "+" to indicate smaller and larger pustules, respectively, for each of 1, 2, or 3 basic level (Roelfs and Martens, 1988). A level less than 3 was considered as resistant, and a level of 3 or larger was considered as susceptible. A combination of ITs was used for representing the disease reactions of single genotype and the predominant type was ordered first. When there was a mixture of resistant and susceptible ITs on the same leaf of single plant, the predominant IT would be used for deciding whether the plant was resistant or susceptible.

For association analysis, ITs of each accession were converted to a single value using the method described by Zhang et al. (2014). Briefly, Stakman's ITs of 0, 1-, 1, 1+, 2-, 2, 2+, 3-, 3, and 3+ are converted to 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9, respectively. The middle number in a combination of ITs and the letter C and N were ignored, and the predominant IT was weighted double than the last score in the combination of ITs for each accession. Flecks (;) was coded as 0, and 4 was coded as 9. Double minus and double plus were converted to single minus and single plus, respectively. For example, if one accession is scored as ;21++, the ITs would be first collapsed to ;1+, and then converted to a single value of 1 based on the above method. These linearized 0-to-9 scale values for all emmer accessions would be utilized for further statistical analysis. Among all of the linearized IT scores for seven races, the values of Spearman

correlation coefficients (r_s) were calculated through CORR procedure in SAS v. 9.3 (SAS Institute, 2011).

Genotyping

The 180 cultivated emmer wheat accessions and 11 durum wheat lines were genotyped with 9K SNP array using the Infinium Assay developed by Illumina (Cavanagh et al., 2013). Raw SNP marker data set was filtered using several criteria. First, the accessions and the markers with missing data points larger than 20% of total SNPs were deleted. Second, the remaining missing data was imputed using the FastPHASE version 2.0 with default settings (Scheet and Stephens, 2006). Third, the SNP markers which have minor allele frequency (MAF) values less than 0.05 were removed. The filtered data set with 4,134 SNPs was used for association mapping.

Pairwise Linkage Disequilibrium and Linkage Disequilibrium Decay

The pairwise LD values (r^2) between any two SNPs were calculated using Plink version 1.07 (Purcell et al., 2007). Of the 4,134 SNPs, only the SNPs mapped to the 9K consensus map were used for the LD decay study (Cavanagh et al., 2013). After all the duplicated markers were removed, 3,230 markers with MAF larger than 0.05 and 2,933 markers with MAF larger than 0.1 were used for LD decay analysis. The LD values (r^2) between intra-chromosomal pairs of mapped SNPs were plotted against the genetic distances (cM) between the pairs of the SNPs. The LD decay line was drawn on this graph using the nonlinear regression model introduced by Remington et al. (2001) and Pyhajarvi et al. (2007). The nonlinear regression analysis was performed in SAS PROC NLIN. The LD decay analysis was performed at both levels of chromosomes and whole genome. The results using two different SNP marker sets with different

MAF levels (0.05 and 0.1) were compared as well. The LD extent levels in each chromosome and whole genome were estimated according to the LD decay plots.

Population Structure and Kinship

Of the 4,134 SNPs, the SNP markers with MAF value less than 0.1 and a pair-wise LD value with other markers larger than 0.5 were deleted, and the remaining 159 markers were used for the analysis of population structure and kinship. Principle component analysis (PCA) was performed using this sub-set of markers in SAS PROC PRINCOMP. And the PC matrix obtained from PCA was used for population structure analysis in GWAS (Price et al., 2006). For the first three PCs, the PC1 was plotted against PC2, and PC2 was plotted against PC3. Therefore, one dot represented an accession in the plot, which resulted in a three-dimensional view of accession clustering. The cultivated emmer population was grouped into several sub-populations based on those clustering results.

LD decay analysis was applied as well in different sub-populations at genome level, using the marker sets with 3,230 markers with MAF larger than 0.05 and 2,933 markers with MAF larger than 0.1. The Fst values (Li and Nelson, 2001) and Nei's genetic distances (Nei, 1978) among those sub-populations and the 11 durum lines were calculated using the 159 SNP markers in SPAGeDi version 1.4 (Hardy and Vekemans, 2002), and the differences among the Fst values or among the Nei's genetic distances values were tested in *F* tests with 20,000 permutations for each test. Based on the genotypes of the 159 markers, a phylogenetic tree showing the evolutionary relationships among the emmer accessions and the durum lines was drawn in TASSEL version 4.0 based on UPGMA algorithm (Un-weighted Pair Group Method with Arithmetic Mean) (Bradbury et al., 2007). A simple identity-by-state allele-sharing matrix (kinship matrix or K matrix) indicating the kinship relationships among the emmer accessions

were calculated using the data set with 159 markers in GAPIT (Genome Association and Prediction Integrated Tool) (Lipka et al., 2012), based on the EMMA algorithm (Kang et al., 2008).

Association Analysis and LD Block Analysis

Association analysis was performed using GAPIT, and marker-trait association was estimated using three regression models, including mixed-effects linear model (MLM), general linear models (GLM) and the naïve model (Table A2). In MLM, the vector of markers was fitted as a fixed-effect factor, PC matrix as covariates, and kinship matrix (K matrix) as a random-effect factor while kinship matrix was ignored in GLM and both PC matrix and kinship matrix were ignored in the naïve model. In this study, 'PC25', 'PC50', and 'PC-BIC' are GLM models and they specify the PCs in PC matrix that could explain 25% (PC25) and 50% (PC50) of the total variance of the marker data and the number of PCs suggested by the BIC (Bayesian Information Criterion) method (PC-BIC) (Schwarz, 1978), respectively. The designations 'PC25+K', 'PC50+K', and 'PC-BIC+K' are MLM models in which the effects from both PCs and kinship (K) were considered. The designation 'K' is also a MLM model in which PC matrix was not considered. We compared different models in reducing false positives according to the Q-Q plot and the value of rank-based mean squared difference (MSD) (Mamidi et al., 2011). The model with the lowest MSD and having a line nearest to the expected line with a slope equal to one in Q-Q plot is considered as the best one. The significance of associations between one marker and phenotypic values was based on an *F* test. The cutoff *p* value for significance was calculated based on the method introduced by Mamidi et al. (2014), and it was defined by choosing a predefined percentile tail from an empirical *p* value distribution. In this study, two

levels of significance were defined, within 0.1 percentile and 1 percentile tails of the empirical *p* value distribution of 10,000 bootstraps.

For each of the identified significant markers (0.1 percentile), their explained phenotypic variation (R^2) was estimated in SAS PROC GLM using a simple regression method. The genomic positions of the significant markers were referred to the adjusted 9K SNP consensus map (Cavanagh et al., 2013). In addition, two integrated genetic maps based on SynOpDH (Synthetic W7984/'OpataM85') (Saintenac et al., 2013) and GP ('Grandin'/PI 277012) (Chu et al., 2011) populations in hexaploid wheat and a SNP consensus map in tetraploid wheat (Maccaferri et al., 2014) were used to detect the significant SNPs that are near to or at the same genomic positions of known QTL or genes related to stem rust resistance.

A group of linked SNPs having high LD values (0.7-1.0) was considered as a single LD block, i.e. the SNPs in the same LD block tend to have a very low recombination rate. In this study, I surveyed all the genomic regions harboring one or several significant markers (0.1 percentile) for identifying possible LD blocks.

Stepwise Regression Analysis

To identify the minimum numbers of SNPs independently associated to stem rust resistance, stepwise regression analysis was applied to all detected significant markers (1 percentile) using SAS PROC REG (Mamidi et al., 2011). The *p* value of 0.05 was set as the threshold of significance for both of the markers and model in the stepwise inclusion procedure. This stepwise regression method has several advantages, for example, identifying the significant markers in major putative QTL, masking the influences from the minor putative QTL, and excluding the significant markers in the same LD with major putative QTL (Mamidi et al., 2014). Additionally, than the entire set of significant markers (1 percentile), the small subset of

stepwise included SNPs is more suitable for marker-assisted selection (MAS) in breeding. The combination of the significant marker alleles included in stepwise regression model is referred to as allelic combination. In this study, the mean values and standard deviations of the stem rust reactions were estimated for all the allelic combinations present in the population. The specific allelic combinations associated to resistant or susceptible responses were identified according to the cutoff values for resistance and susceptibility and were further used to evaluate sources of stem rust resistance based on genotypes.

Results

A total of 180 emmer accessions and 11 durum lines were previously evaluated for seedling reactions to seven *Pgt* races in greenhouses (Olivera et al., 2012a). In this study, these emmer accessions and durum lines were genotyped using 9K SNP array. The stem rust evaluation in greenhouse and the 4,134 polymorphic SNP markers were used for genome-wide association analysis to identify stem rust resistant loci in cultivated emmer wheat.

Reactions of Emmer Wheat Accessions to Stem Rust at Seedling Stage

The stem rust evaluation at seedling stage showed that among the 180 emmer accessions, 61 (33.9%), 66 (36.7%), 66 (36.7%), 73 (40.6%), 110 (61.1%), 112 (62.2%), and 92 (51.1%) were resistant to races TTKSK, TRTTF, TTTTF, TPMKC, RKQQC, QTHJC, and MCCFC, respectively, with 50 (27.8%) and 43 (23.9%) accessions being resistant and susceptible, respectively, to all the seven races (Table 3.1). The Spearman correlation analysis among all the linearized ITs for seven races (Table 3.6) indicated that the reactions to all seven races were highly correlated to each other ($p < 0.0001$). The races TTKSK and TRTTF had the highest correlation ($r_s = 0.82$), while TRTTF and QTHJC had the lowest correlation ($r_s = 0.56$).

Table 3.1. Disease reactions of 180 cultivated emmer wheat accessions to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at seedling stage.

Accession[a]	Seedling reaction to *Pgt* races in greenhouse[b]						
	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
CItr 7687-1	2+N	X	;2-	X-	X	X	X-
CItr 12213-1	2N	22-	;2-N	2+	2-	2-N	2-N
CItr 14133-1	;2+	3+	3+3	3	3+	;N	0;
CItr 14621-1	2+	2=1;2	2+	2+	22+	22+;C	22-
CItr 14637-1	2+1;	2	2+	2+1	2	2-2	21;
CItr 14916-1	2+2-;	;C1-	22+	22+	;C2-	;C1-	;C2-
CItr 14917-1	2+	2	2-	2-	2-	2+	2+
CItr 14919-1	2-N	2-	;2-	2-2N	2-;N	2-N	2-;
PI 41024-1	2-N	2-	2-N	2-N	2-	2-	2-;N
PI 74108-1	1;2=	2	1;2=	2-;C	1;C	2-;N	2-;C
PI 94616-1	2	22+	;C	3-2+;	;C	3-2+;	;C
PI 94621-1	2-;	2-;	22+;	2-;	2-;	2+;	2++;
PI 94625-1	2;	X	;3-	X-	X-	;C	X-
PI 94626-1	22+	22-;	;C	3-;	;C1-	2+	2-;C
PI 94627-1	2	2+	;	2+2	;CN1-	2-	;C
PI 94631-1	X-	22-;	X-	2-	2-;	2+	2+
PI 94634-1	2	2-2;	2-;	2+	22+	22+	2
PI 94635-1	;C2-	2=1;	2-;	;C2-	;C2-	2-;	;C
PI 94638-1	2+3-	3	;C	2-	;C2-	21;	;C
PI 94648-1	22+	33+	2	2	2-	2-C	2-
PI 94656-1	2-N	2-	;2-	2-N	2-	2-	;N2-
PI 94664-1	2-2	2-	2-	2-	2-	2+2;	22+;
PI 94666-1	22-	22-	3	22-	;C2=	2+2	2++
PI 94673-1	2+3-	1;	2-;	2-;	2-;	2+3-	32;
PI 94674-1	;2-N	;1	;N1-	2-;	;CN	2-;	;CN1-
PI 94675-1	2-	2	;2-	2-;	;C2-	2-;	;C1-
PI 94676-1	2-N	;C	3	2-N	2-	2-N	2-N
PI 94738-1	2-2;	2-	;N2-	2-	;C1-	2-	;C
PI 94747-1	2-N	2-;	2-;	2-N	2-	2-N	;N2-
PI 101971-1	2-N	2-;	;2-	2-N	2-	2-;N	2-N
PI 133134-1	22-	2-;	;2-	2-N	2-;	2-;N	2-;N
PI 154582-1	2-	22+	2-	2-N	2-;	;2-	22-
PI 164578-1	2-N	2-2	2-;	2-N	2-;	2-;N	2-;
PI 168673-1	2-N	2-;	2-;	22-	2-	2-N	2-;N
PI 193641-1	2+	2+	2-	22+	2-	2-	;2-
PI 193873-1	;12-N	22-	2-	2-	2-;	2-;	;C2-
PI 193879-1	2-N	2	;N	2-N	2-;N	2-;	;1
PI 193880-1	;12-	2-2=	;2-	;12-	;12-	;2-1	;1
PI 193882-1	;2-	2-	;2-	2-	22-	2-;	;1
PI 193883-1	;2-	2-2	2-	2-2	2-;	;2-	;2-
PI 194042-1	X	2-	2-	2-	2-	X	2+2
PI 194375-1	22+	2-	2-	2-2	2-	2+2	2
PI 197483-1	X-	2+	2+	22+	;2-	22-	;N2=
PI 197485-1	;2-	;C	2-;	2-;	2-;	2-;	;C
PI 217637-1	2	22-	2-	2-N	2-	2-;	2-;N
PI 217639-1	2-N	2	2-;N	2-;	2-;N	;2-	2-N;
PI 217640-1	2-N	2-;	2-;N	2-;N	2-;	2-;	;N2-
PI 221400-1	2+	2+3-	2-;	2	2-	;2-	;2-N
PI 225332-1	3-2;	32;	;32	32;	;23	;32	;23-
PI 244341-1	2++	2+	2+	2+	2-2	2+	2;
PI 254165-1	32;	3+2;	3-2;	3-2;	3-2;	3-2;	32;

Table 3.1. Disease reactions of 180 cultivated emmer wheat accessions to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at seedling stage (Continued).

Accession[a]	Seedling reaction to *Pgt* races in greenhouse[b]						
	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
PI 254167-1	31;	31;	3;	3;	3-;	32;	3-2;
PI 254189-1	3-2;	3-2	;2-	2-;	2-;	2+2;	;2-
PI 254190-1	32+;	3-2;	3-2;	3-2;	2-;	32;	3;
PI 272533-1	2+2	22-	2+3-	32;	22-;	2;	;1-
PI 273981-1	3-;	3-;	3-2+;	2-2	22-	2	3-;
PI 275996-1	2+3-	2-	;3-	2+3-	2-	;3-	2-2
PI 298582-1	22+	22+	2	22+	22+	22+	2-;
PI 310471-1	2	22-	2-	2	2-	2-	2N
PI 319869-1	22-	2	2-	2-2	2-	2-	2-;
PI 322232-1	22+	22+	2-;	2N	2-	2-N	2-;
PI 324076-1	2+	22+	;2-	2-2N	2-;	2-N	2-N
PI 349043-1	31;	32+	31;	22-	2-	3-1;	3
PI 349046-1	3-2;	32;	3-2;	22-	2-	3-2;	2+
PI 352548-1	22-	2	2	2-N	2-	2-2N	2-N
PI 355477-1	2	2	;C	2+2	2	2N	2-N
PI 355507-1	3-2;	32;	;3	3-2;	;2-N	2-;	;12-
PI 377655-1	32;	3;	32;	3;	22+	22-	3;
PI 377657-1	2+	2+	3-2	2+3-	2	2	2-;
PI 384332-1	22+	2	NA	2	2-	2+	NA
PI 434992-1	23-;	3-2	3-2;	3-2;	3-2;	2+;	22-N
PI 480460-1	2	2	2+	2	2-	2-	2-;
PI 532305-1	2	2	22+	22+	22+	22+	22+
CItr 3686	3+	2	3	3-3	2=	;1	;1-
CItr 4013	33+	4	3+	3-3	2-;	2-;	;1-
CItr 7685	3	3+	33+	3-	33-	3-	NA
CItr 7686	33+	4	33+	3	3	33-	3
CItr 7779	3-3	2-;	33-	X-	2-;N	;N2=	;N2-
CItr 7962	3+	3+	4	3-	3-	3-	3
CItr 14085	3	3	3-;	3	3-	33-	33-
CItr 14086	3+	3+	X-	3-2+	33-	33-	2+
CItr 14098	3-3	2+2	22+	2	2-;N	2-2	2-
CItr 14639	3-	2+	3-	2-2	;2=	2-	2-
CItr 14751	NA	3+	3+	3-	3-	22+	3
CItr 14822	4	3+3	3+	3	22+	3-2+	3-2+
CItr 14834	3+	3+	4	3-3	2+	2+3-	3-3
CItr 14866	3+	3+	3-3	33-	2+	2	3-
CItr 14971	3+	3+	33+	3	3-3	3-	3-3
PI 41025	3+	4	4	3	3-3	3	33-
PI 58788	4	4	3+	3-	2+	3	33-
PI 60704	3	3+	33-	3-2+	2+3-	3	3-
PI 74106	3+	4	;N2=	3-;	2-;N	2+;	X-
PI 94617	3+	3+	3+	3-	3-	3-3	3-3
PI 94630	3	33+	4	3-	2+	22+	3-3
PI 94654	3+3	3+	3	3	2-;	2-	2-;
PI 94663	3-	3-	NA	3-2+	3-2+	3-2+	3-2+
PI 94665	33+	33+	3+	2-	2=;	3-	3-3
PI 94668	3+	3+	2-	3-;	3-2+	3-;	3-
PI 94680	3+3	4	3+	3	3	3-2+	2
PI 113961	3+3	33+	1-	3-	2+	3-	3-
PI 168675	3+	3+	4	3-3	3	3-2+	3-3
PI 190920	3+3	33+	3-2+	3-2+	3-	3-	3-

Table 3.1. Disease reactions of 180 cultivated emmer wheat accessions to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at seedling stage (Continued).

Accession[a]	Seedling reaction to *Pgt* races in greenhouse[b]						
	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
PI 190926	33+	33+	3	3-2+	3	3-2+	3-3
PI 191091	3+	3+	3+	3-	3-	3-3	3
PI 193643	3+	4	3+	3-2+	3	2+2	3
PI 193878	4	3+	3+	3-	3	2	3+
PI 195721	3+	33+	3+	3-	3	22+	33-
PI 196100	3+3	2+	33+	3-2+	2++	2	3-
PI 196905	3+	2+3-;	3-2+	3-3	3-	2	3+3
PI 197482	3+	3;	3+	3-	3+	2+2	3-
PI 197490	33+	3-2+	22+	22+	;N2=	22+	;N2-
PI 221401	3-2+	3-2	2+2;3	2+3-	2-	2	22-
PI 226951	3	33-	33-	2-2	2-	2-	2-
PI 248991	3-	3-;	3-	;N2-	2-	2-	2-;N
PI 254146	3+	33+	33+	3-3	3-2+	NA	3-
PI 254163	33-	3-;	NA	3-;	2;N	3-;N	3-;
PI 254188	3+	33+	33+	33-	3-	3-2+	3-3
PI 254193	3+	3+	3	3	3	3	3-
PI 272527	33+	3+3;	3+;	3-	3-2+	3-	3-
PI 273982	3-	2;3-	2+3-;	2-	2-	2-	2-
PI 275997	3+	3+	3	33-	3-2+	2+	3-3
PI 275998	33+	3+	33+	33+	3-3	33-	3
PI 275999	3+	3+	3+	3	33-	3-2+	3
PI 276000	3+	3+	3+	3	33-	3-3	33-
PI 276005	3+	33+	3+	33-	3-	3-	3
PI 276006	33+	33+	3	3-2+	2++	2	3
PI 276007	3-2	3	NA	33-	3-	3-	3-3
PI 276012	33+	33+	33+	2+	2+	2	3-
PI 276014	3+	3+	3+	3-	3	3-	33-
PI 277670	33+	33+	3	33+	33+	3	33-
PI 277671	3+	33+	33+	33-	3-	3-3	3-
PI 277677	3+	3+	3+	3-	3-2+	3	3
PI 286061	3+	33+	3	3-	3-	3	3
PI 289603	3	3;	3;	3+	3	33+	2-
PI 295065	33+	3	3+	3-	X-	X-	2-
PI 297830	3	3	3-	2	2-	2	2-
PI 298543	3+	33+	3+	3-	3	2+	3-2+
PI 298548	33+	3	3+	3-3	3	3-	3-
PI 306536	3	3	33+	3-2+	3-2+	3-2+	3-3
PI 330544	3	3	3	3-	3-2+	3-2+	2+
PI 349045	32+	32	3;	3-	33-	X-	33-
PI 350001	3-2	2;N	3-2;	33-	22-	2-2	2-
PI 352335	3	3	3	X-	2-N	2=;N	2-;N
PI 352337	3+	3	33+	3-3	3-	33-	33-
PI 352338	33-	32;	3-2	3-	3-	3-	33-
PI 352341	33-	33-	3-	3-2+	3-	3-2+	3-
PI 352342	33-	33-	3-	3-2+	3-2+	3-2+	3-2+
PI 352358	3+	3+	3+	3-2+	2+3-	3	22+
PI 352365	3+	4	33+	3-	3-	2+	3-
PI 355460	3+	3+	3	3-3	3-	3-2+	3-
PI 355461	3+	3+	3+	3-	3-	3	2
PI 355470	3+	3+	33+	2+3-	3	2-;N	33-
PI 355475	3+	4	3+	3-3	3-2+	3+	NA

Table 3.1. Disease reactions of 180 cultivated emmer wheat accessions to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at seedling stage (Continued).

Accession[a]	Seedling reaction to *Pgt* races in greenhouse[b]						
	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
PI 355483	3+	4	3	3-	3	3-	33-
PI 355485	3	3	3	3-2+	2+	2+2	3-
PI 355486	3+	3	3	4	3	3	33+
PI 355489	33-	3	33-	3-	3-	3-	3-3
PI 355497	3+	33+	3+	3	3-	3-	3-
PI 355505	3+	33+	3	3-	3-2+	3-2+	2-
PI 361833	3	3	3	3-2+	3-2+	3-	3
PI 362438	33-	33-	3-;	3-2+	22-	22-	2-N
PI 362500	3-	3	3	3-	2-	2-	2-
PI 362697	3-	3-	3	3-	2-	2-	2-
PI 374685	3+	3+	3+	3-	2-	2-	2-
PI 377650	32	32;	32	3-	2-	2-	2-N
PI 377672	3	3-2	3	3	3	2+	2-
PI 384297	3+	3	3	3	2	2	3
PI 384302	3+	3+	3+	3	2	2+	3
PI 384318	3+	33+	3+	3-	3	2-	3-3
PI 384331	3+	3+	33-	3-	3	2-	3
PI 434996	3-	32	3-2;	3-3	2-	2-	2-
PI 470737	32;	;2-2	32;	2-;N	2-;	3	3-
PI 479957	3	3	33+	3-	2+	3	33-
PI 479965	33-	3	NA	2-	2+3-	22+	3-
PI 480307	3+	NA	3+	3-	22+	2+	3-
PI 480312	3+	3+	3+	3-2+	2+	2+	3-
PI 480313	3	3-	3	3-2+	3	3	3-3
PI 480461	3+	3+	3+	3-2+	3	3-2+	3-
PI 480462	3+	3+	3+	3-	2+	3	3-3
PI 532304	3+	3+	3+3	3-	3	2+	33-

[a]PI and CItr are accession number in the USDA National Small Grains Collection (Aberdeen, ID). The accessions with dash sign represented the selected lines from the original accessions based on their reactions to stem rust pathogen.

[b]C, N, and X represented "more chlorosis than normal for the infection type", "more necrosis than normal for the infection type", and "random distribution of variable-sized uredia on single leaf with a pure culture", respectively (Roelfs and Martens, 1988). NA, Not available.

Marker Analysis and Linkage Disequilibrium

A total of 5,911 polymorphic SNPs were initially identified among the 180 emmer wheat accessions. After removing the markers and the accessions with the missing values larger than 20% and the markers with MAF less than 0.05, 178 emmer accessions and 4,134 SNP markers were used for association analysis. Of the 4,134 SNPs, 3,897 markers were previously mapped to the consensus 9K SNP map (Cavanagh et al., 2013) and they distributed on 14 A- and B-genome chromosomes ranging from 112 SNPs on 4B to 483 SNPs on 2B (Figure 3.1).

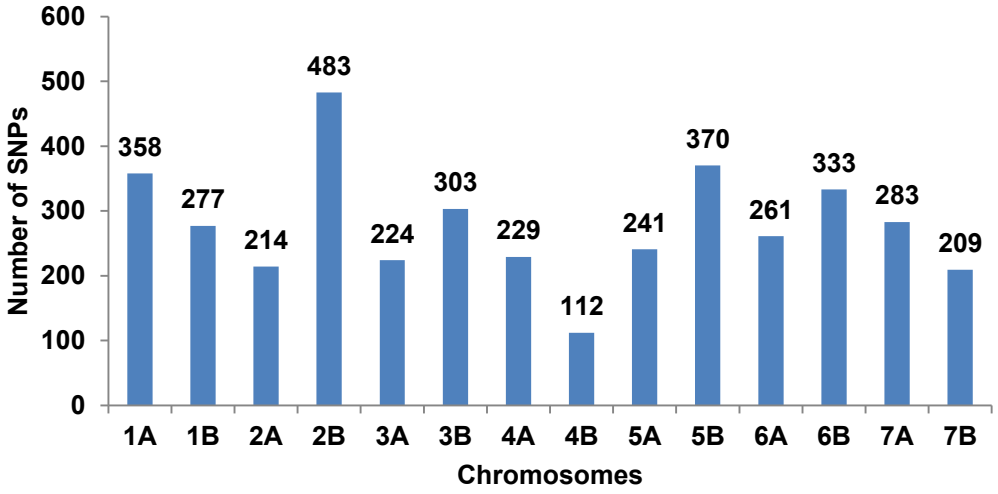

Figure 3.1. Distributions of the mapped polymorphism markers with a MAF (minor allele frequency) cutoff value of 0.05 in the genome after imputation. The number at the top of each column represented the number of polymorphic markers on each chromosome.

The LD extents were analyzed at both whole genome and individual chromosome levels when LD decays to 0.1 (r^2). For the whole genome, the LD extents of the 178 emmer accessions were estimated to be about 6 cM and 12 cM based on the marker sets with MAF larger than 0.05 and 0.1, respectively (Figure 3.5, Table 3.7). At the chromosome level with a MAF threshold of 0.05, 6A had the largest LD extent of 20 cM and 5A had the smallest LD extent of 1 cM (Table 3.7). For the MAF threshold of 0.1, 5B had the largest LD extent of 31 cM, and 5A had the

smallest LD extent of 1 cM. The rates of LD decay are different when MAF threshold changed. For the whole genome, the LD extent was estimated to be smaller with a smaller MAF threshold. For most chromosomes, the LD extents were also smaller with a smaller MAF threshold, excluding the chromosomes 5A and 6B which had the same LD extents with different MAF cutoff values, and the chromosomes 6A and 7A which had larger LD extents with smaller MAF cutoff value. These results indicated that the LD extent could be influenced by the existence of rare alleles. The LD extent would be larger without rare alleles (MAF = 0.1) than with rare alleles (MAF = 0.05).

Principle Component Analysis (PCA) and Population Structure

For principle component (PC) analysis, the 159 markers with pair-wise LD values smaller than 0.5 were used for population structure and kinship analysis. The elbow in the scree plot located at the 8th PC, after which the changes of eigenvalues were much smaller than those before the 8th PCs (Figure 3.6). For the first eight PCs, eigenvalues changed from about 18 (PC1) to about 4 (PC8) while for the PCs after PC8, the eigenvalues are between 4 and 0. The first three PCs and the first eight PCs could explain up to 28.14% and 50.86% of the variances of the 159 SNPs used respevtively. The scatter plots of PC1 versus PC2 (Figure 3.2A) and PC2 versus PC3 (Figure 3.2B) were drawn for all the 178 cultivated emmer accessions. The planes of the two scatter plots are perpendicular to each other and formed a three dimensional view of clustering of the cultivated emmer accessions. Based on the clustering result, most of the 178 accessions could be grouped to five sub-populations except for three accessions (PI 193641-1, CItr 14971, and PI 94665). The sub-population 3 could be separated in both of the plots of PC1 versus PC2 and PC2 versus PC3. The sub-populations 1 and 2 were overlapped in the plot of PC2 versus PC3, and could be separated with other sub-populations in plot of PC1 versus PC2.

The sub-populations 4 and 5 were overlapped in the plot of PC1 versus PC2, and could be separated with other sub-populations in plot of PC2 versus PC3.

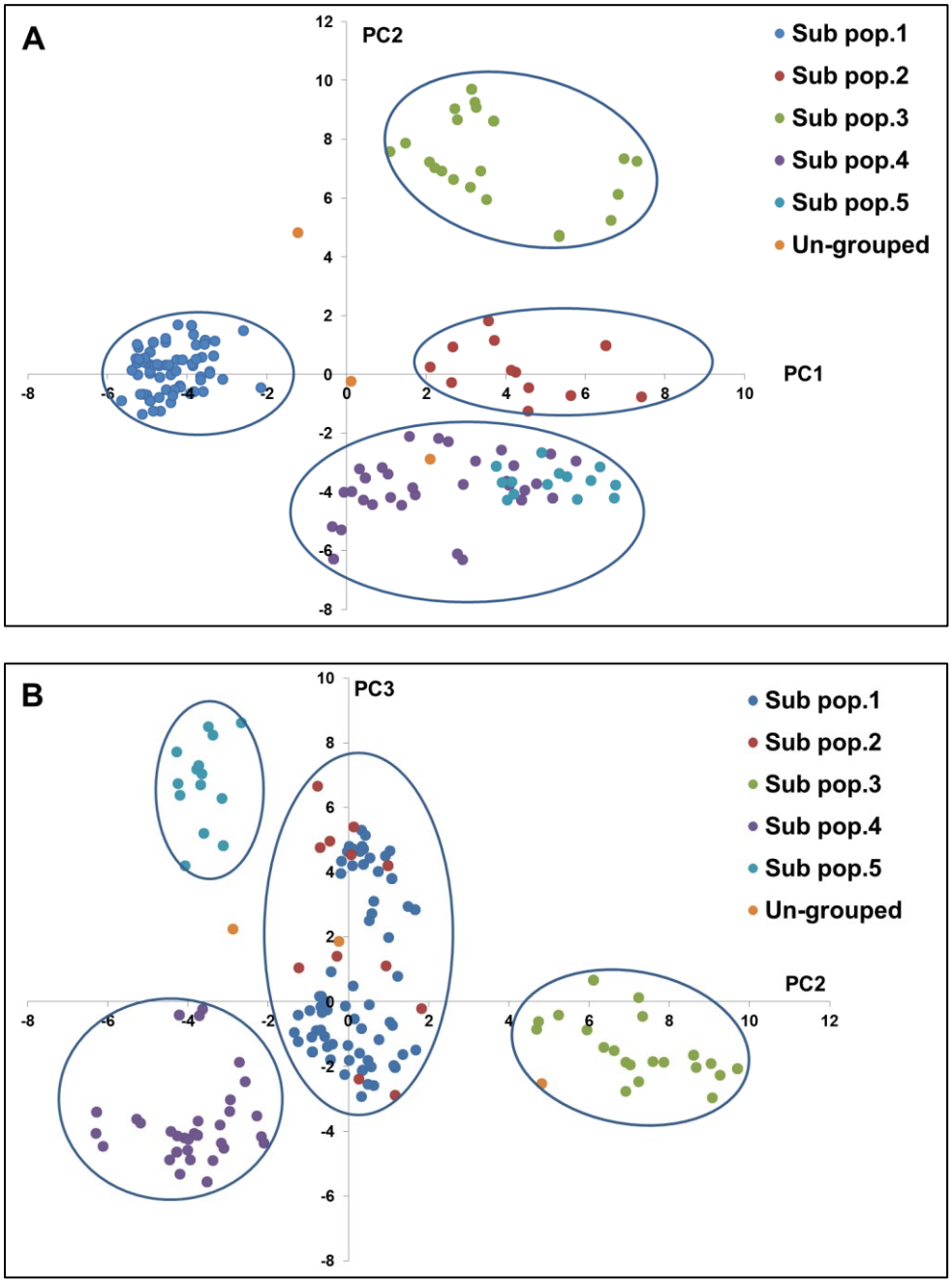

Figure 3.2. Population stratification based on the principle component analysis (PCA) of 178 emmer accessions. Figure A was produced by PC1 versus PC2, and Figure B was produced by PC2 versus PC3. One dot represented one accession. Of the 178 accessions, 175 accessions were clustered into five sub-populations and three accessions cannot be clustered to any sub-populations.

To find the relationship between the sub-populations and their geographic locations, two scatter plots (Figure 3.7) of PC1 versus latitude and PC2 versus longitude were developed for the accessions having available information of geographic locations (Table A1). The two scatter plots showed that the accessions in the same sub-populations generally originated from the similar geographic locations. For example, most accessions in the sub-population 1 have the geographic origins centered at around latitude 10° N and longitude 40° E, with a few accessions scattering at other locations (Figure 3.7). Therefore, the genetic similarity of the accessions revealed by PC analysis is highly related to the origins of the accessions. Sub-population 1 includes 78 accessions, with 52 (66.7%), 10 (12.8%) and 16 accessions originating from Ethiopia, India, and other 14 countries or regions, respectively. Sub-population 2 and 3 has 14 and 26 accessions, respectively, which mainly originated from Russian, Iran, Georgia, and other eight countries or regions. Sub-population 4 has 42 accessions with 39 (92.9%) originating from Europe (Spain, Germany, and 11 other European countries or regions), two accessions with unknown origin, and one accession from ancient Palestine. Sub-population 5 has 15 accessions, which were mainly from European countries or regions at the southeast of Europe or Balkan area, including Serbia, Former Yugoslavia, Montenegro, Bulgaria, Bosnia and Herzegovina, and Italy.

A phylogenetic tree revealed the evolutionary relationship among the cultivated emmer sub-populations and the durum lines (Figure 3.3). The sub-populations 1, 3, and durum each formed a single branch. The sub-population 4 formed two single branches. The sub-populations 2 and 5 formed a single branch together. Seven of 14 accessions in sub-population 2 were not clustered together with the other accessions and made the sub-population 2 the worst for clustering among the five sub-populations. Additionally, two accessions (PI 355497 and PI

330544) of sub-population 4 and one durum line Vn-B1 (*Sr9e*) were clustered with other sub-populations. The phylogenetic tree clearly showed that the durum lines were separated from emmer accessions. The sub-population 1 was most closely related to sub-population 4 and least related to sub-populations 5 and 2.

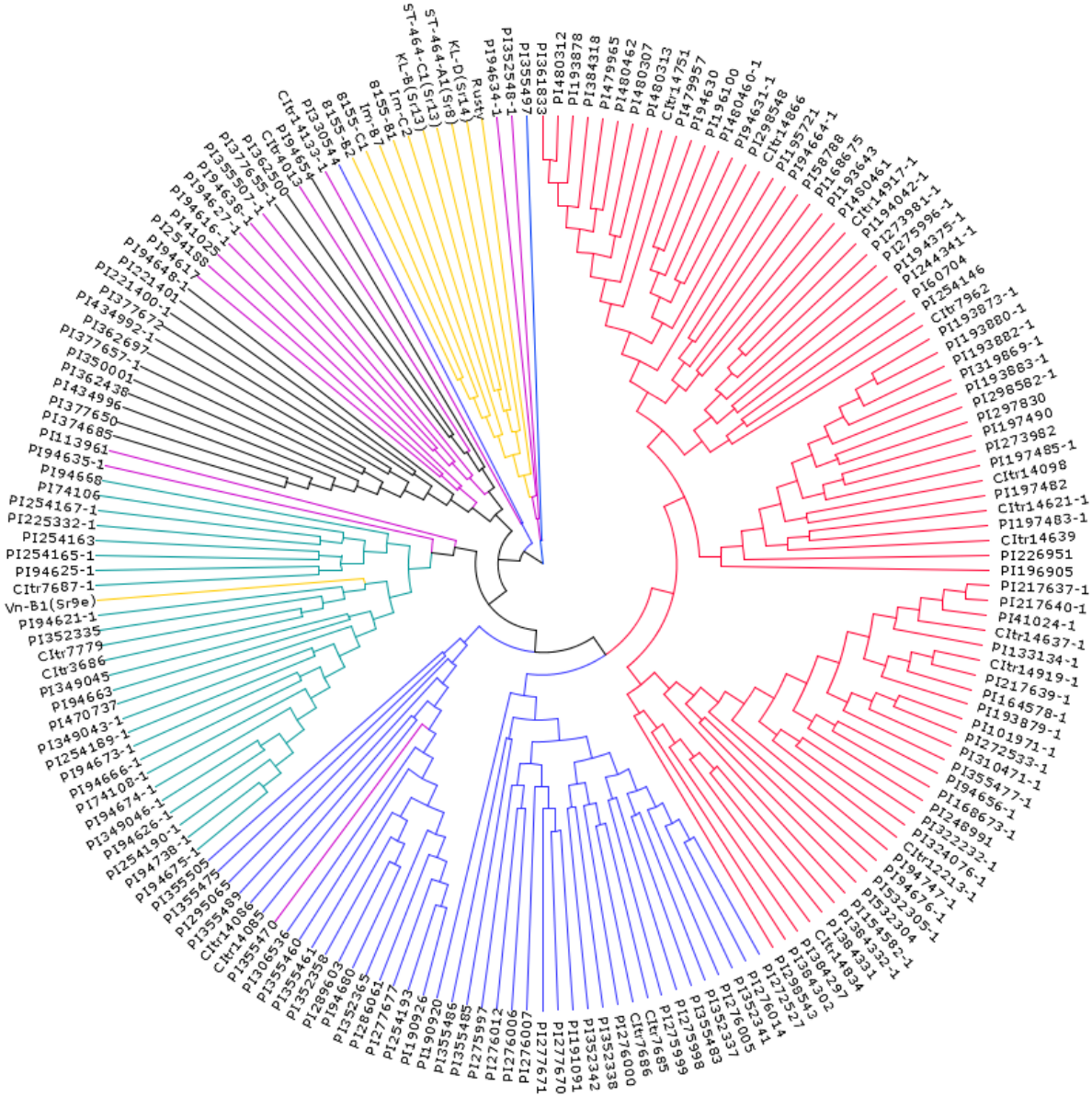

Figure 3.3. Phylogenetic tree representing the relationships among 175 emmer accessions in five subpopulations and 11 durum lines based on UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) algorithm. Red, blue, green, purple, black, and yellow colors stands for the sub-population 1, 4, 3, 2, 5, and 11 durum lines, respectively.

After removing the accessions admixed with other populations in phylogenetic tree, the Fst values and Nei's genetic distance were estimated among the five sub-populations and durum lines (Table 3.2). The values of Nei's genetic distance ranged from 0.1433 between the sub-populations 1 and 4 to 0.4536 between the sub-population 5 and durum lines. The values of Fst had a range from 0.2936 between the sub-populations 1 and 4 to 0.5763 between the sub-population 5 and durum lines. The relationship among those sub-populations revealed by the values of Fst was in agreement with that revealed by Nei's genetic distances, which was also consistent with the phylogenetic tree. Each of the emmer sub-populations had the most distant relationship with the durum lines than with other sub-populations. The durum lines had the shortest genetic distance with sub-population 4 and the longest genetic distance with sub-population 5.

Table 3.2. Pairwise estimates of Fst and Nei's genetic distance among five cultivated emmer sub-populations and 11 durum lines based on 159 SNPs.

Cluster	Sub pop.1	Sub pop.2	Sub pop.3	Sub pop.4	Sub pop.5	Durum
Sub pop.1		0.2395[a]	0.2015	0.1433	0.2426	0.2975
Sub pop.2	0.4305		0.2546	0.2807	0.1658	0.4102
Sub pop.3	0.3929	0.4154		0.2445	0.3512	0.3646
Sub pop.4	0.2936	0.3737	0.3686		0.2579	0.2955
Sub pop.5	0.4349	0.3217	0.4920	0.3709		0.4536
Durum	0.4937	0.5525	0.5247	0.4111	0.5763	

[a]The estimations of Nei's genetic distance appear above the diagonal, and Fst values appear below the diagonal. All Fst values and Nei's genetic distances are significantly different based on a permutation test ($p < 0.0001$).

The LD extent level in each sub-population when r^2 equaled to 0.1 was estimated based on the plot of LD decay (Table 3.8). When MAF was set to 0.05, LD extents ranged from 6 cM in sub-population 2 to 34 cM in sub-population 5, and when MAF was set to 0.1, LD extents ranged from 7 cM in sub-population 2 to 64 cM in sub-population 5. Therefore, for different

levels of MAF, the rankings of LD extent in the five sub-populations were the same. When MAF was larger, for the same sub-population the LD extent level would be larger as well, except the sub-populations 3 and 4. For the 11 durum lines, the LD extent was larger than any of the sub-populations and it was 70 cM and 72 cM with MAF equal to 0.05 and 0.1 respectively. These results indicated that the LD extent of a population was negatively associated with the number of polymorphic markers.

Association Analysis

For analyzing the linearized seedling ITs for seven *Pgt* races, a best linear model was first chosen for each of the seven races based on MSD values (Table 3.9) and Q-Q plots (Figure 3.8) of eight different models. The mixed linear model "PC25+K" is the best model for analyzing seedling ITs of all the races, except RKQQC for which the general linear model "PC-BIC" with the first seven PCs is the best model.

At the significant level of 0.1 percentile, the cutoff *p* values were estimated at 5.69×10^{-4}, 1.80×10^{-4}, 2.93×10^{-4}, 2.03×10^{-4}, 3.34×10^{-4}, 8.19×10^{-5}, and 3.19×10^{-4}, for *Pgt* races TTKSK, TRTTF, TTTTF, TPMKC, RKQQC, QTHJC, and MCCFC, respectively. At the significant level of 1 percentile, the cutoff *p* values were estimated at 3.12×10^{-3}, 5.96×10^{-3}, 1.75×10^{-3}, 3.83×10^{-3}, 4.10×10^{-3}, 5.15×10^{-3}, and 4.18×10^{-3}, for the seven *Pgt* races, respectively. The SNP with a *p* value smaller than the cutoff *p* value identified by the best model for each phenotype was considered to be significantly associated to the phenotype. A total of 222 significant markers (1 percentile) were detected, 165, 45, 11, and 1 of which associated with one, two, three, and five races resistance, respectively (Table 3.12). These markers were located on 14 A- and B-genome chromsomes. Their genomic positions and significant levels were shown in the Manhattan plots (Figure 3.4).

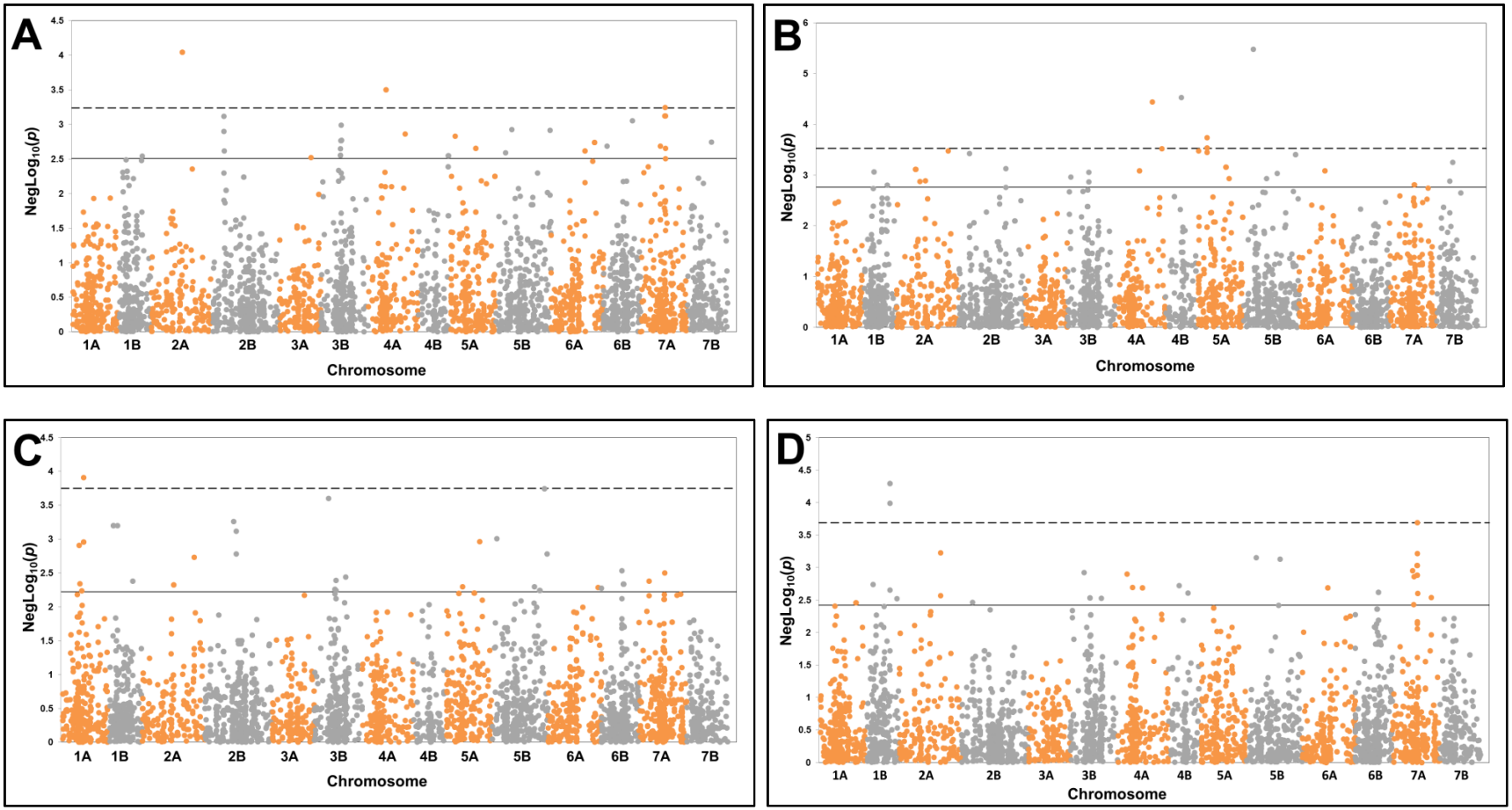

Figure 3.4. Manhattan plots of -$\log_{10}$(p-value) of all SNP markers used in association analysis with 178 cultivated emmer accessions for seven *P. graminis* f. sp. *tritici* (*Pgt*) races. Figures A, B, C, D, E, F, and G represented the plots for race TTKSK, TTTTF, TRTTF, TPMKC, RKQQC, QTHJC, and MCCFC, respectively. In each figure, the black solid and dashed horizontal lines indicate the significant levels of 1 percentile and 0.1 percentile, respectively. The dots above different horizontal lines represent the identified significant markers associated with disease resistance at different significant levels. Y axis indicates –$\log_{10}$ (p-value) and X axis indicates the chromosomes. The position for each marker was based on the wheat consensus SNP map (Cavanagh et al., 2013).

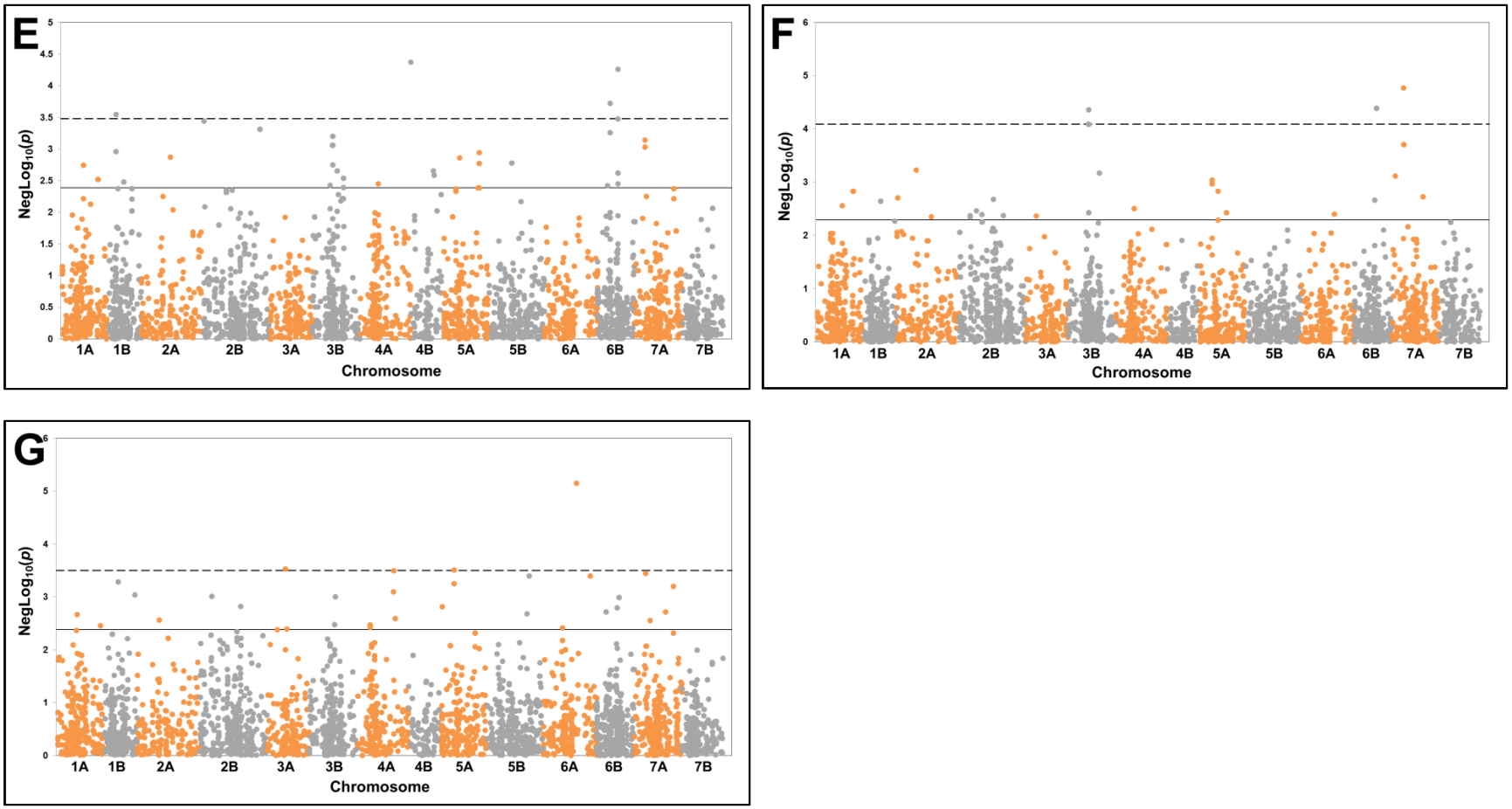

Figure 3.4. Manhattan plots of -$\log_{10}$(*p*-value) of all SNP markers used in association analysis with 178 cultivated emmer accessions for seven *P. graminis* f. sp. *tritici* (*Pgt*) races (Continued). Figures A, B, C, D, E, F, and G represented the plots for race TTKSK, TTTTF, TRTTF, TPMKC, RKQQC, QTHJC, and MCCFC, respectively. In each figure, the black solid and dashed horizontal lines indicate the significant levels of 1 percentile and 0.1 percentile, respectively. The dots above different horizontal lines represent the identified significant markers associated with disease resistance at different significant levels. Y axis indicates –$\log_{10}$ (*p*-value) and X axis indicates the chromosomes. The position for each marker was based on the wheat consensus SNP map (Cavanagh et al., 2013).

At the significant level of 0.1 percentile, five, four, six, five, five, seven, and five SNPs were significantly associated with resistance to *Pgt* races TTKSK, TRTTF, TTTTF, TPMKC, RKQQC, QTHJC, and MCCFC, respectively. The phenotypic variation (R^2) explained by each marker ranged from 0.05% to 33.31% (Table 3.3). Among those 36 significant markers, 10 were at or near to genomic regions containing known *Sr* genes or QTL on chromosomes 1B (*Sr14* and *Sr58*), 3B (*Sr12*), and 7A (*QSr.sun-7A* and *Sr22*) (Table 3.3). The remaining significant markers on chromosomes 1A, 2A, 3A, 4A, 4B, 5A, 5B, 6A, 6B, and 7A did not cover the genomic regions with known *Sr* genes or QTL based on the three genetic maps that were used. The genomic regions with those markers likely harbor novel *Sr* genes.

Fourteen LD blocks with significant SNPs (0.1 percentile) were identified on nine chromosomes (Table 3.4). Among the 14 LD blocks, 10 had their markers at the same map position and four had markers at different positions with a length of the block ranging from 0.26 cM to 3.26 cM. Based on the mapping positions of the significant markers in LD blocks, four blocks were found to be at or close to genomic regions containing previously identified QTL or *Sr* genes (Table 3.4).

Table 3.3. Significant markers at the significant level of 0.1 percentile associated with reactions of 178 cultivated emmer accessions to seven *P. graminis* f. sp. *tritici* (*Pgt*) races.

Race	SNP	Chr[a]	Pos[b]	MAF[c]	$NegLog_{10}$ (*p*)	R^2 (%)	SRI[d]	Near gene/QTL	Ref[e]
MCCFC	*IWA3024*	6A	135.94	0.22	5.15	11.24	Yes		
MCCFC	*IWA6108*	3A	74.98	0.13	3.53	0.16			
MCCFC	*IWA5538*	5A	59.97	0.28	3.51	17.38			
MCCFC	*IWA5539*	5A	59.97	0.28	3.51	17.38			
MCCFC	*IWA3068*	4A	148.63	0.05	3.50	3.54			
QTHJC	*IWA1759*	7A	46.57	0.18	4.77	0.89			
QTHJC	*IWA4382*	6B	90.36	0.08	4.39	9.82	Yes		
QTHJC	*IWA8462*	6B	90.36	0.08	4.39	9.82			
QTHJC	*IWA1898*	3B	84.19	0.49	4.36	0.05		*Sr12*	1
QTHJC	*IWA1607*	3B	84.19	0.45	4.09	1.97	Yes	*Sr12*	1
QTHJC	*IWA2800*	3B	84.19	0.45	4.09	1.97		*Sr12*	1
QTHJC	*IWA3997*	3B	82.66	0.45	4.09	1.97		*Sr12*	1
RKQQC	*IWA6457*	4B	0.00	0.15	4.37	15.93	Yes		
RKQQC	*IWA5345*	6B	82.68	0.28	4.26	28.90			
RKQQC	*IWA3424*	6B	51.39	0.18	3.72	0.29			
RKQQC	*IWA43*	1B	38.15	0.39	3.54	0.87	Yes	*Sr14*	2
RKQQC	*IWA5346*	6B	82.68	0.32	3.48	33.31	Yes		
TPMKC	*IWA4031*	1B	102.74	0.29	4.29	2.86		*Sr58/Lr46/Yr29*	3, 4
TPMKC	*IWA7141*	1B	102.74	0.29	4.29	2.86		*Sr58/Lr46/Yr29*	3, 4
TPMKC	*IWA6663*	1B	102.74	0.30	3.99	2.32		*Sr58/Lr46/Yr29*	3, 4
TPMKC	*IWA18*	NA[f]	NA	0.35	3.77	33.12			
TPMKC	*IWA7749*	7A	102.48	0.25	3.69	7.42		*QSr.sun-7A, Sr22*	5, 6
TRTTF	*IWA18*	NA	NA	0.35	4.39	29.73	Yes		
TRTTF	*IWA5174*	1A	91.27	0.33	3.91	16.53	Yes		
TRTTF	*IWA1061*	5B	212.94	0.22	3.75	7.97			
TRTTF	*IWA3360*	5B	212.38	0.22	3.75	7.97			
TTKSK	*IWA240*	2A	127.40	0.18	4.04	8.60			
TTKSK	*IWA241*	2A	127.40	0.18	4.04	8.60			
TTKSK	*IWA1793*	4A	73.84	0.05	3.50	1.67	Yes		
TTKSK	*IWA8341*	4A	73.84	0.05	3.50	1.67			
TTKSK	*IWA3639*	7A	101.83	0.36	3.24	4.70		*QSr.sun-7A, Sr22*	5, 6
TTTTF	*IWA2610*	5B	39.37	0.25	5.48	32.17			
TTTTF	*IWA3400*	4B	64.50	0.20	4.53	18.82	Yes		
TTTTF	*IWA6563*	4A	153.72	0.47	4.45	0.41			
TTTTF	*IWA1988*	5A	45.95	0.32	3.74	17.72	Yes		
TTTTF	*IWA291*	5A	45.95	0.32	3.53	17.01			
TTTTF	*IWA1253*	5A	45.95	0.32	3.53	17.01			

[a]Chr, Chromosome; [b]Pos, Positions; [c]MAF, Minor allele frequency; [d]SRI, Stepwise regression included. [e]Ref, References: 1, Yu et al., 2014; 2, McIntosh, 1980; 3, Rosewarne et al., 2006; 4, Singh et al., 2013; 5, Kaur et al., 2009; 6, Khan et al., 2005. [f]NA, Not available.

Table 3.4. Linkage disequilibrium (LD) blocks including one or several significant markers associated with stem rust resistance.

LD block	Chr[a]	Pos[b](cM)	SNP	Extented length(cM)[c]	Associated phenotype	Near gene/QTL	Ref[d]
1	1B	38.15	***IWA43****, *IWA44*	0	RKQQC	*Sr14*	1
2	1B	102.74	*IWA4031**, *IWA7141**, *IWA6663**	0	TPMKC	*Sr58/Lr46/Yr29*	2, 3
3	2A	127.40	*IWA240**, *IWA241**	0	TTKSK		
4	3A	74.98	*IWA6108**	0.26	MCCFC		
		75.24	*IWA8283*				
5	3B	80.93	*IWA4452*	3.26	QTHJC	*Sr12*	4
		82.66	*IWA3997**				
		84.19	***IWA1607****, *IWA1898**, *IWA2800**, *IWA7247*, *IWA7333*				
6	4A	73.84	***IWA1793****, *IWA8341**	0	TTKSK		
7	5A	45.95	*IWA114*, *IWA291**, *IWA1253**, ***IWA1988****	0	TTTTF		
8	5A	59.97	*IWA5538**, *IWA5539**, *IWA3975*	0	MCCFC		
9	5B	212.38	*IWA3360**	0.56	TRTTF		
		212.94	*IWA1061**				
10	6B	51.39	*IWA2307*, *IWA3424**, *IWA7896*	0	RKQQC		
11	6B	82.68	*IWA1553*, *IWA5345**, ***IWA5346****, *IWA7901*	0	RKQQC		
12	6B	90.36	***IWA4382****, *IWA8462**	0	QTHJC		
13	7A	46.57	*IWA1759**	2.33	QTHJC		
		48.00	*IWA3831*, *IWA3832*				
		48.90	*IWA2535*				
14	7A	102.48	*IWA7749**, *IWA7660*	0	TPMKC	*QSr.sun-7A, Sr22*	5, 6

*Significant markers. The bold significant markers are stepwise regression model included markers.
[a]Chr, Chromosome. [b]Pos, Positions. [c]0 means that the SNPs in this LD block are at the same position.
[d]Ref, Reference: 1, McIntosh, 1980; 2, Rosewarne et al., 2006; 3, Singh et al., 2013; 4, Yu et al., 2014; 5, Kaur et al., 2009; 6, Khan et al., 2005.

At the significant level of 1 percentile, 42, 41, 42, 42, 42, 42, and 42 SNPs were significantly associated with resistance to *Pgt* races TTKSK, TRTTF, TTTTF, TPMKC, RKQQC, QTHJC, and MCCFC, respectively (Table 3.12). Stepwise regression analysis showed that six, eight, nine, six, five, eight, and nine markers had major effects on the seedling resistance to the seven races, respectively, and they explained phenotypic variation ranging from 40.84% (TTKSK) to 60.61% (QTHJC) (Table 3.10). Based on the result of stepwise regression analysis, among the 14 identified LD blocks, six harboring significant SNPs with major effects were putative major QTL, and the remaining eight were putative minor QTL. Among the six LD blocks with major effects, two were near to known *Sr* genes and four at genomic regions with no *Sr* genes or QTL that were previously reported (Table 3.4).

A single SNP cannot be always significantly associated with a disease resistance in different populations because of changing effects. But a combination of several significant makers would be more consistent than a single marker. The stepwise regression helped finding the significant markers with major effects and removing the significant markers with small or redundant effects. Based on the number of markers fitted into the stepwise regression, I identified 16 to 44 allelic combinations of the significant markers with major effects on all the seven phenotypes (Table 3.11). The average reaction of the accessions with specific allelic combination could be used for inferring the resistance or susceptible of an accession (Table 3.11). The combinations of significant markers associated with resistance could be used for marker-assisted selection in breeding programs.

Discussion

Linkage Disequilibrium of Cultivated Emmer Wheat Population

Association analysis is based on the linkage disequilibrium present in a natural population. The cultivated emmer wheat collections maintained in USDA-ARS National Small Grains Collection (Aberdeen, ID) were collected from different geographic regions from its origin and major production areas and thus form a natural population that maintains an optimal level of linkage disequilibrium suitable for association analysis of the desirable genes for wheat improvement. The present study was the first attampt to estimate LD decay present in a population of 180 of the cultivated emmer accessions at both whole genome and chromosome level.

The estimates of LD decay of the 180 of the cultivated emmer accessions at the whole genome level were 12 cM ($r^2 = 0.1$), which agreed with the results of several previous studies on in durum and common wheat. Maccaferri et al. (2006) analyzed 189 durum wheat accessions with broad genetic diversity and discovered that the LD decayed to approximately 10 cM with threshold of r^2 equal to 0.1. Zhang et al. (2010) reported that the average genome-wide LD also declined to 10 cM with threshold of r^2 equal to 0.1 in a population of 205 elite bread wheat breeding lines from the U.S. However, several different estimates of whole genome LD extent in certain wheat populations were also reported. For example, Crossa et al. (2007) identified that whole genome LD extent was 40 cM with an r^2 critical value of 0.115 in a population of 170 CIMMYT elite spring wheat lines. The LD decay changes in different studies are largely attributed to the differences in the types and quantity of experiment materials, population structures, and thresholds of r^2 values.

This study revealed that the difference between the average LD extents of A and B genome was less than 2 cM. For the seven homoeologous groups, the LD extents in four A-genome chromosomes (1A, 3A, 4A, and 6A) were longer than those in four B-genome chromosomes (1B, 3B, 4B, and 6B), while the LD extents in other three A-genome chromosomes (2A, 5A, and 7A) were shorter than those in three B-genome chromosomes (2B, 5B, and 7B). Chao et al. (2010) also reported that the A and B genomes had slight difference in the LD extents. However, some other studies indicated that the A genome had higher LD levels and lower genetic diversity than B genome because of the longer breeding history of A genome and more genes for adaptive traits than B genome (Ren et al., 2013). The different results might be explained by the fact that most of cultivated emmer accessions (69%) used in this study are landraces instead of cultivars (3%) and less selective pressures were applied to different genomes.

This study showed that the LD extents varied from chromosome to chromosome ranging from 1 cM on 5A to 20 cM on 6A when MAF equals to 0.05 (Table 3.7). Zhang et al. (2010) reported the LD extents for different genomic regions were highly variable. Using a population of 95 soft winter wheat cultivars, Breseghello and Sorrells (2006) estimated the LD extent of chromosome 2D and part of 5A as 1 cM and 5 cM, respectively, with an r^2 critical value of 0.065. This divergence of LD decay at different genomic regions might be related to the genomic locations of some conservative genes controlling important adaptive traits. Selective pressure and breeding activity applied on those genes make the genomic regions harboring those genes with higher LD levels. It is thought that the complicated evolutionary and breeding history have large influence on the LD decay at different genomic regions (Dubcovsky and Dvorak, 2007).

The LD decay analysis was important to GWAS as the marker density needed was highly related to the LD extent (Gurung et al., 2014). In general, when LD extent was short, more markers would be needed for identify putative QTL or genes in GWAS. If the LD extent was long, fewer markers would be needed. However, target genes would be hard to find in a long LD extent although several significant markers could be identified (Gurung et al., 2014). In present study, the LD extent was estimated as 6 cM (MAF = 0.05) for whole genome level, and the total length of 14 chromosomes based on the 9K consensus map is about 2,700 cM. That indicates at least about 450 markers (2700/6) are needed for association analysis using this emmer panel, based on that one single LD needs at least one marker. The number of markers (4,134) used here is much larger than 450. There are different LD levels for different chromosomes ranging from 1 to 20 cM (MAF = 0.05), indicating that the different marker densities were needed for different chromosomes. For all the 14 chromosomes, the number of markers used in this study exceeded the number of marker necessarily needed, based on that one single LD needs at least one marker. Only three gaps between markers were larger than 15 cM on chromosomes 2A, 3B, and 6A, and 19 gaps with size between 10 and 15 cM. Therefore, except for a few of gaps on the map, the markers in this study were evenly distributed and had enough density on most of the genomic regions for effective GWAS.

Population Structure

The first three PCs from principle component analysis suggested five sub-populations for the 178 emmer accessions. This result is very similar to the report of the population structure analysis of 186 cultivated emmer accessions described by Luo et al. (2007). According to their report, the cultivated emmer wheat could be divided into two principle populations, northern population and southern population, originating from the northern and southern Levant of Fertile

Crescent, respectively. Then based on their diffusion paths, northern population was subdivided to northeast population located at northeast Turkey, Iran, and Transcaucasia, and northwest population located at northern Balkans, Yaroslav region in northern Russia, and northwest Turkey. Southern population was subdivided to southeast population located at Ethiopia, Oman, southern India, and Levant, and southwest population located at the Levant and Mediterranean (Luo et al., 2007).

In present study, the sub-populations are also highly associated with specific geographic regions (Figure 3.7). Based on the geographic regions of the sub-populations as well as their relationships revealed by phylogenetic tree and genetic distances, the sub-population 1, 3, 4, 5 were corresponding to the southeast population, northeast population, southwest population, northwest population described by Luo et al. (2007), respectively. Although sub-population 2 had diverse origins including Iran, Georgia, and northwest Turkey, it was also considered to be a part of northwest population as well as sub-population 5, because it shared a single branch with sub-population 5 in phylogenetic tree and also had the shortest genetic distance with it than with any other sub-population. I found that 19 accessions used by Luo et al. (2007) were in common with the present study. Among the 19 accessions, only one accession was clustered into different sub-populations in those two different studies. Excluding the sub-population 2, the southern population (sub-populations 1 and 4) had shorter LD extent and corresponded higher genetic diversity than northern populations (sub-populations 3 and 5). This result was in agreement with the evolutionary model of cultivated emmer wheat. Luo et al. (2007) assumed that the gene flow from northern cultivated emmer population to southern wild emmer population generated southern cultivated emmer population with a higher level of genetic diversity than northern population.

Based on the population structure, the origin of the accessions from Europe, Asia, and America could be deduced. The accessions from West Europe were clustered into the southwest population while those from northern Balkans and Russia were grouped to northern population. Therefore, the cultivated emmer in West Europe evolved from the southern Levant area while that at northern Balkans and Russia evolved from northern Levant area. In our cultivated emmer panel, only one accession was from East Asia (Taiwan) and it was clustered to the southeast population. So, the emmer wheat at East Asia might be spread from India through the diffusion route of the southeast population. There were six accessions from South or North America, which were grouped into three different sub-populations. Three of the six accessions, including one each from Canada, Peru, and the U.S., were grouped into southeast population. Another three accessions from U.S. were clustered to northeast or northwest populations. Therefore, the cultivated emmer in South or North America had more complex genetic background than that of other continents probably because of the geographic separation with any other continents. The durum lines had shorter genetic distances with the southern populations, sub-population 1 (D = 0.2975) and sub-population 4 (D = 0.2955), than with the northern population (Table 3.2). Thus, durum wheat more likely evolved from southern Levant area than northern Levant area. This inference was consistent with the archeological record that durum wheat first appeared in Egypt which was on the route of the diffusion of southern cultivated emmer wheat (Nesbitt and Samuel, 1996).

For the five sub-populations, the variation among their LD decays at whole genome level was highly associated with their genetic diversities which were reflected by the number of polymorphic markers in each of the sub-populations (Hamrick and Godt, 1996) (Table 3.8). It could be inferred that a high level of genetic diversity leads to a short LD extent level for a

specific population. Except for sub-population 2, other four sub-populations had higher LD extent levels than the whole population. The higher genetic diversity in the sub-population 2 is likely caused by the diverse origins of the accessions in this sub-population. The different genetic diversities among the sub-populations could be the result of different selection pressure from distinguishing breeding programs. For the 11 durum lines, the LD extent was longer than all the five emmer sub-populations and also longer than those reported in most other studies on durum wheat. That might be due to the small sample size and close phylogenetic relationships among the 11 lines (Figure 3.3).

Association Analysis of the Stem Rust Resistance

In the primary gene pool of cultivated wheat, cultivated emmer wheat is a good source for stem rust resistance. In the emmer panel used in this study, 50 (28%) accessions showed seedling resistance to all the seven *Pgt* races. Through GWAS, 222 SNPs were identified to significantly associate with the resistance at the significant level of 1 percentile.

Based on the genomic position of the significant markers at the significant level of 0.1 percentile, I found 10 significant markers and four LD blocks located at the same or near to the genomic regions with known stem rust resistance genes or QTL, including *Sr12*, *Sr14*, *Sr22*, *Sr58*, and *QSr.sun-7A* (Tables 3.3, 3.4). Among these known genes, *Sr14* was originally derived from cultivated emmer and conferred seedling resistance to specific *Pgt* races (Knott, 1962; Smith, 1957). *IWA43* on 1B significantly associated with RKQQC resistance was identified to be near to the genomic region with *Sr14* (Table 3.3). It is possible that the genomic regions with *IWA43* may carry the locus *Sr14* or any unknown *Sr* genes that are closely linked to this gene. Because other known genes and QTL such as *Sr12*, *Sr22*, *Sr58*, and *QSr.sun-7A* were not originally derived from cultivated emmer, the genomic regions containing significant markers

and LD blocks near or at regions of these genes unlikely harbors these genes. It is possible that the genomic regions may contain unknown genes at or near the regions of these known genes.

Except for the 10 SNPs associated with genomic regions with known genes or QTL, the remaining SNPs located to the genomic regions where no known stem rust resistance genes were identified on chromosomes 1A, 2A, 3A, 4A, 4B, 5A, 5B, 6A, 6B, and 7A. Most of these regions on 4AL, 4BS, 5AS, 5BS, 6AL, 6BS, and 6BL were also identified to associate with stem rust resistance in other studies on association mapping for stem rust resistance in wheat (See Yu et al., 2014). These genomic regions most likely harbor the novel stem rust resistance genes. The cultivated emmer accessions with high levels of broad-spectrum resistance and favorable alleles in the marker loci at these regions will be useful materials for new *Sr* gene identification through linkage mapping analysis using bi-parental mapping population.

In summary, the results from GWAS provide a preliminary evidence of the genomic regions associated with stem rust resistance in cultivated emmer wheat. Detailed detection of stem rust resistance genes still needs linkage analysis using bi-parental mapping populations. However, GWAS provided guidance for searching for genomic regions harboring resistance genes. The identified LD blocks especially for the LD blocks with major effects will be the candidate regions. The allelic combinations of significant markers selected by stepwise regression could be used for identifying wheat genotypes with *Sr* genes located in targeted genomic regions. Therefore, our genome-wide association study provided the first step towards identifying novel *Sr* genes in emmer wheat and pyramiding resistance loci from emmer wheat for MAS breeding. It will eventually increase the genetic diversity for resistance to stem rust in modern durum and bread wheat germplasm. Furthermore, the linkage disequilibrium and population structure analysis proved that the association mapping panel assembled in this study

is a valuable genetic resource and could be used in GWAS for resistance to leaf rust, stripe rust, tan spot, Septoria nodorum blotch, and other wheat diseases.

Supplementary Tables

Table 3.5. Virulence and avirulence responses to major *Sr* genes for the seven races used in this study.

Race	Isolate	Origin	Avirulence	Virulence
TTKSK	04KEN156/04	Kenya	*24 36 Tmp*	*5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 31 38 McN*
TRTTF	06YEM34-1	Yemen	*8a 24 31*	*5 6 7b 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp*
TTTTF	01MN84A-1-2	USA	*24 31*	*5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp*
TPMKC	74MN1409	USA	*6 9a 9b 24 30 31 38*	*5 7b 8a 9d 9e 9g 10 11 17 21 36 McN Tmp*
RKQQC	99KS76A-1	USA	*9e 10 11 17 24 30 31 38 Tmp*	*5 6 7b 8a 9a 9b 9d 9g 21 36 McN*
QTHJC	75ND717C	USA	*7b 9a 9e 24 30 31 36 38 Tmp*	*5 6 8a 9b 9d 9g 10 11 17 21 McN*
MCCFC	59KS19	USA	*6 8a 9a 9b 9d 9e 11 21 24 30 31 36 38*	*5 7b 9g 10 17 McN Tmp*

Source: Olivera et al., 2013; Jin et al., 2007.

Table 3.6. Spearman correlation coefficient values (lower diagonal) and the p-values (upper diagonal) among the reactions to stem rust in greenhouse.

Diseases	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
TTKSK		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
TRTTF	0.81881		<.0001	<.0001	<.0001	<.0001	<.0001
TTTTF	0.77704	0.74307		<.0001	<.0001	<.0001	<.0001
TPMKC	0.77782	0.73115	0.71419		<.0001	<.0001	<.0001
RKQQC	0.67587	0.67772	0.68013	0.75497		<.0001	<.0001
QTHJC	0.64987	0.56040	0.57583	0.60417	0.66223		<.0001
MCCFC	0.70297	0.62476	0.64640	0.61628	0.74098	0.72686	

Table 3.7. Linkage disequilibrium (LD) extents based on 178 emmer wheat accessions calculated using two marker sets with different minor allele frequency (MAF) values of 0.05 and 0.1 at both of the whole genome level and chromosome levels when LD decays to $r^2 = 0.1$.

Chromosome	LD (cM)	
	0.05[a]	0.1
1A	10	14
1B	7	12
2A	3	5
2B	12	23
3A	11	21
3B	2	3
4A	13	22
4B	3	4
5A	1	1
5B	15	31
6A	20	14
6B	4	4
7A	4	3
7B	6	10
Whole Genome	6	12

[a]0.05 and 0.1 indicate specific LD was calculated based on marker set with minor allele frequency (MAF) equal to 0.05 and 0.1, respectively.

Table 3.8. Linkage disequilibrium (LD) extents in five sub-populations of emmer wheat and eleven durum lines calculated based on marker sets with different minor allele frequency (MAF) values of 0.1 and 0.05 at the genome levels when LD decays to $r^2 = 0.1$.

Populations	LD (cM)		No. of accession	Polymorphic markers
	0.05[a]	0.1		
Sub-pop. 1	32	58	78	2509
Sub-pop. 2	6	7	14	3764
Sub-pop. 3	28	23	26	1391
Sub-pop. 4	17	13	42	3328
Sub-pop. 5	34	64	15	1335
Durum	70	72	11	2625

[a]0.05 and 0.1 indicate specific LD was calculated based on marker set with minor allele frequency (MAF) equal to 0.05 and 0.1, respectively.

Table 3.9. Mean squared difference (MSD) values for different models used for identifying SNPs significantly associated with stem rust resistance.

Model	MCCFC	RKQQC	QTHJC	TPMKC	TRTTF	TTKSK	TTTTF
Naïve	5.22E-02	3.60E-02	2.06E-02	5.46E-02	4.74E-02	3.29E-02	3.74E-02
PC25	2.88E-02	1.84E-02	2.97E-03	8.35E-03	7.28E-03	6.90E-03	2.04E-02
PC50	1.30E-02	4.71E-04	7.82E-03	4.45E-03	2.76E-03	5.00E-03	1.35E-02
PC25+K	5.10E-03	2.22E-03	1.01E-03	1.35E-03	2.11E-03	1.29E-03	5.28E-03
PC50+K	5.99E-03	2.98E-03	2.72E-03	4.22E-03	3.68E-03	2.36E-03	8.31E-03
PC-BIC	1.26E-02	2.64E-04	2.69E-02	4.46E-03	4.74E-02	2.94E-03	1.92E-02
PC-BIC+K	9.63E-03	1.17E-02	1.42E-03	8.66E-03	7.86E-03	2.77E-03	1.31E-02
K	9.63E-03	1.17E-02	1.42E-03	8.66E-03	7.86E-03	2.77E-03	1.31E-02

Table 3.10. The number of stepwise regression included markers and the explained phenotypic variation by them together for each phenotype for stem rust.

Phenotype	No. of markers		Phenotypic variation (%)
	Significant	Included	
TTKSK	42	6	40.84
TRTTF	41	8	56.71
TTTTF	42	9	58.97
TPMKC	42	6	57.86
RKQQC	42	5	53.25
QTHJC	42	8	60.61
MCCFC	42	9	54.98

Table 3.11. Allelic combinations of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile and the sample statistics of each allelic combination's phenotypic data.

Race	Allelic combination[a]	No. of obs[b]	Mean (IT)	Std dev[c]	Min[d]	Max[e]	Reaction[f]
MCCFC	BAAAABBBB	1	0.00	.	0.00	0.00	R
MCCFC	BABABBBBA	1	0.00	.	0.00	0.00	R
MCCFC	BBBAABABB	1	1.33	.	1.33	1.33	R
MCCFC	BABAABBBA	8	1.63	1.84	0.00	5.33	R
MCCFC	BBABABBBB	10	1.83	1.85	0.00	4.67	R
MCCFC	BBBAABAAA	11	2.06	1.47	0.00	4.00	R
MCCFC	BBAAABBBA	19	2.74	1.20	0.33	5.00	R
MCCFC	BABAAABBA	2	2.83	0.24	2.67	3.00	R
MCCFC	BBBAABBBA	2	3.17	1.18	2.33	4.00	R
MCCFC	ABABBBABB	1	4.00	.	4.00	4.00	R
MCCFC	BABBABBBA	1	4.00	.	4.00	4.00	R
MCCFC	BBABAABBA	1	4.00	.	4.00	4.00	R
MCCFC	BBABABBBA	9	4.07	0.70	2.67	5.33	R
MCCFC	BBAAABBAA	4	4.58	2.64	1.33	7.67	R
MCCFC	BBAAABABA	1	4.67	.	4.67	4.67	R
MCCFC	AABBAABAA	1	5.00	.	5.00	5.00	R
MCCFC	ABBBABBBA	5	5.33	0.67	4.67	6.00	R
MCCFC	BBBBABBBA	1	5.33	.	5.33	5.33	R
MCCFC	AABAAABBA	1	6.00	.	6.00	6.00	R
MCCFC	ABABABBBA	1	6.00	.	6.00	6.00	R
MCCFC	BABABABBA	1	6.00	.	6.00	6.00	R
MCCFC	BBBAABBAA	37	6.32	1.79	1.33	9.00	R
MCCFC	ABBAABBBA	2	6.33	0.47	6.00	6.67	R
MCCFC	BABABABAA	13	6.38	1.50	4.00	8.00	R
MCCFC	AABABBBAA	1	7.00	.	7.00	7.00	S
MCCFC	AABBABBBA	1	7.00	.	7.00	7.00	S
MCCFC	AABBBBBAA	1	7.00	.	7.00	7.00	S
MCCFC	ABBBBBBBA	2	7.00	0.00	7.00	7.00	S
MCCFC	BBBBAABBA	1	7.00	.	7.00	7.00	S
MCCFC	BBBABABAA	3	7.11	0.96	6.00	7.67	S
MCCFC	ABBAABBAA	1	7.33	.	7.33	7.33	S
MCCFC	BABBBABAA	1	7.33	.	7.33	7.33	S
MCCFC	BBABABABA	1	7.33	.	7.33	7.33	S
MCCFC	BBBBBABBA	2	7.33	0.00	7.33	7.33	S
MCCFC	BBBAAABAA	4	7.50	0.64	6.67	8.00	S
MCCFC	AABABABAA	17	7.55	0.47	6.67	8.33	S
MCCFC	AABABABBA	2	7.67	0.47	7.33	8.00	S
MCCFC	AABBBBBBA	1	7.67	.	7.67	7.67	S
MCCFC	BBBBBBBBA	1	7.67	.	7.67	7.67	S
MCCFC	ABBAAABBA	1	8.00	.	8.00	8.00	S
MCCFC	BBBBBBBAA	1	8.67	.	8.67	8.67	S
QTHJC	ABABBBBB	1	0.00	.	0.00	0.00	R
QTHJC	BBABBBBA	1	1.33	.	1.33	1.33	R
QTHJC	ABBBBBBA	5	1.80	0.87	0.67	2.67	R
QTHJC	ABBBBBAA	1	2.67	.	2.67	2.67	R

Table 3.11. Allelic combinations of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile and the sample statistics of each allelic combination's phenotypic data (Continued).

Race	Allelic combination[a]	No. of obs[b]	Mean (IT)	Std dev[c]	Min[d]	Max[e]	Reaction[f]
QTHJC	BBABBBBB	1	2.67	.	2.67	2.67	R
QTHJC	BABBBABA	14	3.50	1.38	0.67	5.33	R
QTHJC	BBAABBBB	7	3.57	1.26	1.33	5.00	R
QTHJC	BAABBBBA	23	3.71	1.03	1.33	6.00	R
QTHJC	ABABABBA	1	4.00	.	4.00	4.00	R
QTHJC	BAAABBBA	1	4.00	.	4.00	4.00	R
QTHJC	BABABABB	1	4.00	.	4.00	4.00	R
QTHJC	BBAABBAB	2	4.00	0.00	4.00	4.00	R
QTHJC	BBAAAAAB	1	4.33	.	4.33	4.33	R
QTHJC	BBBBBBBA	13	4.41	1.52	2.33	6.67	R
QTHJC	ABBBABBA	6	4.50	2.29	0.00	6.00	R
QTHJC	BBAABBBA	9	4.52	0.67	4.00	6.00	R
QTHJC	BABBBAAA	1	5.00	.	5.00	5.00	R
QTHJC	BBBBABAA	1	5.33	.	5.33	5.33	R
QTHJC	BBBBABBA	8	5.67	1.04	4.67	7.00	R
QTHJC	BABBBBBA	28	5.95	1.19	4.00	8.00	R
QTHJC	BABBBBAA	6	6.06	1.42	4.00	8.00	R
QTHJC	BBBBBBAA	2	6.83	1.65	5.67	8.00	R
QTHJC	BAAABBAA	1	7.00	.	7.00	7.00	S
QTHJC	BABBABBA	1	7.00	.	7.00	7.00	S
QTHJC	BBBABBBA	9	7.07	0.74	6.00	8.00	S
QTHJC	BBBAABBA	20	7.10	0.58	5.67	8.00	S
QTHJC	BABABBAA	3	7.33	0.67	6.67	8.00	S
QTHJC	BBBABBAA	8	7.38	0.82	6.00	8.33	S
QTHJC	BBBAABAA	2	8.00	1.41	7.00	9.00	S
RKQQC	ABBAB	4	1.08	1.20	0.00	2.67	R
RKQQC	BBBAB	30	3.09	1.46	0.00	5.33	R
RKQQC	ABBBB	20	3.18	1.44	0.33	6.00	R
RKQQC	ABABB	2	5.00	1.41	4.00	6.00	R
RKQQC	BABAB	61	5.17	1.78	2.67	9.00	R
RKQQC	BABBB	1	5.33	.	5.33	5.33	R
RKQQC	BBAAB	5	5.53	2.38	1.33	7.00	R
RKQQC	BBBBB	3	6.00	1.76	4.00	7.33	R
RKQQC	BAAAB	4	6.08	1.64	4.00	8.00	R
RKQQC	BBBAA	2	6.33	2.36	4.67	8.00	R
RKQQC	BAABA	1	6.67	.	6.67	6.67	R
RKQQC	BBABA	3	7.11	0.19	7.00	7.33	S
RKQQC	BBABB	2	7.17	0.71	6.67	7.67	S
RKQQC	BBAAA	38	7.18	0.62	6.00	8.33	S
RKQQC	BAAAA	1	8.00	.	8.00	8.00	S
RKQQC	BAABB	1	9.00	.	9.00	9.00	S
TPMKC	BBABAB	1	1.33	.	1.33	1.33	R
TPMKC	BAABBA	6	3.22	0.89	2.67	4.67	R
TPMKC	AABAAB	1	4.00	.	4.00	4.00	R
TPMKC	ABAAAB	1	4.00	.	4.00	4.00	R

Table 3.11. Allelic combinations of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile and the sample statistics of each allelic combination's phenotypic data (Continued).

Race	Allelic combination[a]	No. of obs[b]	Mean (IT)	Std dev[c]	Min[d]	Max[e]	Reaction[f]
TPMKC	BABAAB	1	4.00	.	4.00	4.00	R
TPMKC	BABBAB	19	4.19	1.10	1.33	6.00	R
TPMKC	ABBAAB	19	4.56	1.27	1.33	7.00	R
TPMKC	BBABBA	16	4.92	1.41	2.67	7.00	R
TPMKC	AAAABB	2	5.00	0.47	4.67	5.33	R
TPMKC	AABBAB	2	5.00	1.41	4.00	6.00	R
TPMKC	ABABAB	1	5.33	.	5.33	5.33	R
TPMKC	BBABBB	1	5.33	.	5.33	5.33	R
TPMKC	AAABBA	4	5.42	1.45	4.00	7.33	R
TPMKC	AABABA	2	5.67	0.94	5.00	6.33	R
TPMKC	AAABBB	2	5.83	1.65	4.67	7.00	R
TPMKC	ABABBA	3	6.22	1.02	5.33	7.33	R
TPMKC	ABBBAB	35	6.47	1.13	4.00	8.00	R
TPMKC	AABBBB	3	6.67	1.53	5.00	8.00	R
TPMKC	ABAABB	1	6.67	.	6.67	6.67	R
TPMKC	ABBABB	4	6.83	1.11	5.33	8.00	R
TPMKC	ABBABA	2	7.00	0.47	6.67	7.33	S
TPMKC	ABBBAA	1	7.00	.	7.00	7.00	S
TPMKC	BBBBBA	1	7.00	.	7.00	7.00	S
TPMKC	ABBBBB	4	7.17	0.69	6.33	8.00	S
TPMKC	ABBBBA	43	7.33	0.68	6.00	9.00	S
TPMKC	BBBBBB	1	7.33	.	7.33	7.33	S
TPMKC	ABABBB	1	7.67	.	7.67	7.67	S
TPMKC	AABABB	1	8.00	.	8.00	8.00	S
TRTTF	AAAAABBB	1	2.67	.	2.67	2.67	R
TRTTF	BBAAABAB	5	2.67	1.94	0.00	4.67	R
TRTTF	BBBAABBB	3	2.89	2.04	0.67	4.67	R
TRTTF	ABBAAABB	1	3.33	.	3.33	3.33	R
TRTTF	AABAABBA	1	4.00	.	4.00	4.00	R
TRTTF	BBBAABAB	2	4.00	1.89	2.67	5.33	R
TRTTF	BAAAAABA	21	4.02	1.33	0.00	5.33	R
TRTTF	BABAAABA	8	5.00	2.09	1.33	8.33	R
TRTTF	BBAAABBB	1	5.00	.	5.00	5.00	R
TRTTF	BBBBABBB	2	5.00	3.30	2.67	7.33	R
TRTTF	BBBAABBA	2	5.17	0.24	5.00	5.33	R
TRTTF	AAAABAAA	1	5.33	.	5.33	5.33	R
TRTTF	ABAAABAB	1	5.33	.	5.33	5.33	R
TRTTF	ABAABBBA	1	5.33	.	5.33	5.33	R
TRTTF	ABBABBBB	1	5.33	.	5.33	5.33	R
TRTTF	BBBBABBA	5	5.33	0.47	4.67	6.00	R
TRTTF	AABAAABA	19	5.67	1.76	3.33	9.00	R
TRTTF	ABAAAABA	1	6.33	.	6.33	6.33	R
TRTTF	ABAAABBA	1	6.33	.	6.33	6.33	R
TRTTF	BBBBABAB	6	6.33	2.26	2.67	9.00	R
TRTTF	ABAABBAA	5	6.40	0.98	5.33	8.00	R

Table 3.11. Allelic combinations of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile and the sample statistics of each allelic combination's phenotypic data (Continued).

Race	Allelic combination[a]	No. of obs[b]	Mean (IT)	Std dev[c]	Min[d]	Max[e]	Reaction[f]
TRTTF	ABBABBBA	4	6.92	0.69	6.00	7.67	R
TRTTF	BBBAAABA	2	7.00	2.83	5.00	9.00	S
TRTTF	BBBAAABB	1	7.00	.	7.00	7.00	S
TRTTF	BBBBABAA	1	7.00	.	7.00	7.00	S
TRTTF	ABBABABA	2	7.50	2.12	6.00	9.00	S
TRTTF	AABAAAAA	4	7.58	1.77	5.00	9.00	S
TRTTF	AABBAAAA	3	7.67	2.31	5.00	9.00	S
TRTTF	ABAABAAA	1	8.00	.	8.00	8.00	S
TRTTF	ABBAABAA	1	8.00	.	8.00	8.00	S
TRTTF	ABBBAAAB	1	8.00	.	8.00	8.00	S
TRTTF	BBBAAAAA	2	8.00	0.00	8.00	8.00	S
TRTTF	ABBAAABA	16	8.06	1.21	5.33	9.00	S
TRTTF	AABABBAB	1	8.33	.	8.33	8.33	S
TRTTF	ABAAAAAA	1	8.33	.	8.33	8.33	S
TRTTF	ABAABAAB	1	8.33	.	8.33	8.33	S
TRTTF	ABBAAAAA	30	8.42	0.86	5.33	9.00	S
TRTTF	ABBBAAAA	4	8.58	0.50	8.00	9.00	S
TRTTF	ABBABAAA	6	8.67	0.37	8.33	9.00	S
TRTTF	ABAABABA	2	9.00	0.00	9.00	9.00	S
TRTTF	ABBABBAA	2	9.00	0.00	9.00	9.00	S
TRTTF	ABBBAABA	2	9.00	0.00	9.00	9.00	S
TRTTF	ABBBBBAA	1	9.00	.	9.00	9.00	S
TRTTF	BBBBAABA	1	9.00	.	9.00	9.00	S
TTKSK	AABBAA	1	2.00	.	2.00	2.00	R
TTKSK	ABBABA	1	4.00	.	4.00	4.00	R
TTKSK	ABBAAA	1	4.67	.	4.67	4.67	R
TTKSK	BBBBBA	3	4.67	3.06	1.33	7.33	R
TTKSK	BABABA	35	4.88	1.19	2.67	9.00	R
TTKSK	BBABAA	1	5.00	.	5.00	5.00	R
TTKSK	BABBBA	10	5.23	2.35	1.33	9.00	R
TTKSK	ABAAAA	4	5.25	1.10	4.00	6.33	R
TTKSK	BABABB	3	5.44	1.90	3.33	7.00	R
TTKSK	BBBABA	22	5.86	2.68	1.33	9.00	R
TTKSK	BBAABA	30	7.58	1.80	1.33	9.00	S
TTKSK	BBBABB	18	7.83	1.23	5.33	9.00	S
TTKSK	BABBBB	1	8.33	.	8.33	8.33	S
TTKSK	BBBBAA	23	8.43	0.67	6.33	9.00	S
TTKSK	BBBBBB	16	8.54	0.56	7.67	9.00	S
TTKSK	ABBBBA	1	8.67	.	8.67	8.67	S
TTKSK	ABBBAB	1	9.00	.	9.00	9.00	S
TTKSK	BBAABB	4	9.00	0.00	9.00	9.00	S
TTKSK	BBABBA	1	9.00	.	9.00	9.00	S
TTKSK	BBBBAB	1	9.00	.	9.00	9.00	S
TTTTF	BAAAABBAA	3	0.00	0.00	0.00	0.00	R
TTTTF	BABAAABBA	1	0.00	.	0.00	0.00	R

Table 3.11. Allelic combinations of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile and the sample statistics of each allelic combination's phenotypic data (Continued).

Race	Allelic combination[a]	No. of obs[b]	Mean (IT)	Std dev[c]	Min[d]	Max[e]	Reaction[f]
TTTTF	BAAAAAAAA	1	1.00	.	1.00	1.00	R
TTTTF	BBAAAABBA	1	1.33	.	1.33	1.33	R
TTTTF	BABAABBAA	1	2.67	.	2.67	2.67	R
TTTTF	BBABAAABA	1	2.67	.	2.67	2.67	R
TTTTF	BBBBABBAA	1	2.67	.	2.67	2.67	R
TTTTF	AAAAABABB	19	2.77	2.02	0.00	7.00	R
TTTTF	BAAAAABBA	6	2.78	1.31	1.33	4.67	R
TTTTF	BAAABABBA	10	3.03	1.89	0.33	5.33	R
TTTTF	AAAAABBAA	2	3.33	0.94	2.67	4.00	R
TTTTF	AAAAABAAB	1	4.00	.	4.00	4.00	R
TTTTF	AAABABBAA	3	4.67	0.00	4.67	4.67	R
TTTTF	BABBAABBA	1	5.00	.	5.00	5.00	R
TTTTF	AABAABBBB	8	5.33	2.59	2.33	8.33	R
TTTTF	ABABABBAA	2	5.33	0.94	4.67	6.00	R
TTTTF	BAAABABBB	1	5.33	.	5.33	5.33	R
TTTTF	BBAABABBA	1	5.33	.	5.33	5.33	R
TTTTF	AAAAABBBB	3	5.56	3.01	2.67	8.67	R
TTTTF	AAAAABBAB	5	6.07	2.17	4.00	9.00	R
TTTTF	AABAABBAA	4	6.58	1.64	5.00	8.00	R
TTTTF	AABBABBBB	2	6.67	1.89	5.33	8.00	R
TTTTF	AABAABBAB	34	6.98	2.33	1.33	9.00	R
TTTTF	AABBABBAA	7	7.43	1.56	4.67	9.00	S
TTTTF	AABBABBAB	4	7.67	1.56	6.00	9.00	S
TTTTF	BBBABABBB	3	7.89	0.19	7.67	8.00	S
TTTTF	ABBBABAAB	1	8.00	.	8.00	8.00	S
TTTTF	BBBABABBA	1	8.00	.	8.00	8.00	S
TTTTF	ABABABBAB	36	8.07	0.90	5.33	9.00	S
TTTTF	ABABABAAB	1	8.33	.	8.33	8.33	S
TTTTF	BBBBBBBAA	1	8.33	.	8.33	8.33	S
TTTTF	ABBBABBAB	1	8.67	.	8.67	8.67	S
TTTTF	ABBBBBBAA	3	8.78	0.38	8.33	9.00	S
TTTTF	AAABABBAB	1	9.00	.	9.00	9.00	S
TTTTF	ABAAABBAB	1	9.00	.	9.00	9.00	S
TTTTF	ABBABBBBA	1	9.00	.	9.00	9.00	S
TTTTF	BBBBBBBAB	1	9.00	.	9.00	9.00	S

[a]A and B refer to the alleles in the 9K SNP wheat chip.
Orders of SNPs associated with MCCFC: *IWA3024*, *IWA5076*, *IWA3975*, *IWA7120*, *IWA5345*, *IWA1721*, *IWA690*, *IWA2042*, *IWA7552*; Orders of SNPs associated with QTHJC: *IWA4382*, *IWA1607*, *IWA3975*, *IWA1889*, *IWA3024*, *IWA1204*, *IWA3935*, *IWA7552*; Orders of SNPs associated with RKQQC: *IWA6457*, *IWA43*, *IWA5346*, *IWA7633*, *IWA7679*; Orders of SNPs associated with TPMKC: *IWA2610, IWA8522, IWA1885, IWA6252, IWA1491, IWA1755*; Orders of SNPs associated with TRTTF: *IWA18, IWA5174, IWA8522, IWA4135, IWA3536, IWA2865, IWA3646, IWA8595*; Orders of SNPs associated with TTKSK: *IWA1793, IWA8064, IWA2099, IWA446, IWA2082, IWA523*; Orders of SNPs associated with TTTTF: *IWA3400, IWA1988, IWA4651, IWA1930, IWA2067, IWA2107, IWA4800, IWA18, IWA4031*. [b]Number of observations; [c]Standard deviation; [d]Min, minimum; [e]Max, Maximum; [f]R, Resistant; S, Susceptible.

Table 3.12. The *p* values of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile.

SNP	Chr[a]	Position	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
IWA3536	1A	73.08		1.23E-03					
IWA2768	1A	76.49		4.56E-03					
IWA8143	1A	84.56		5.78E-03					
IWA6756	1A	86.15							2.15E-03
IWA5174	1A	91.27		**1.24E-04**[b]					
IWA6707	1A	91.61		1.10E-03					
IWA6708	1A	91.61		1.10E-03					
IWA560	1A	92.90					1.80E-03		
IWA7570	1A	107.73						2.75E-03	
IWA6253	1A	152.13				3.50E-03	3.02E-03	1.48E-03	
IWA6252	1A	153.13				3.50E-03	3.02E-03	1.48E-03	
IWA4944	1A	180.92							3.49E-03
IWA2578	1B	23.65		6.33E-04					
IWA3169	1B	23.65		6.33E-04					
IWA1885	1B	33.39				1.82E-03			
IWA43	1B	38.15					**2.85E-04**		
IWA44	1B	38.15					1.10E-03		
IWA1947	1B	40.42		6.33E-04					
IWA5664	1B	48.22			8.63E-04				
IWA5665	1B	48.22			8.63E-04				
IWA5076	1B	64.07							5.21E-04
IWA5635	1B	69.61					3.32E-03		
IWA1889	1B	76.37						2.30E-03	
IWA1890	1B	76.37						2.30E-03	
IWA7422	1B	102.42		4.19E-03		2.22E-03			
IWA4031	1B	102.74			1.58E-03	**5.10E-05**			
IWA7141	1B	102.74			1.58E-03	**5.10E-05**			
IWA6663	1B	102.74	2.86E-03			**1.02E-04**			
IWA3238	1B	131.46				3.01E-03			
IWA1355	1B/1A	132.06/180.78							9.25E-04
IWA4989	2A	3.69						1.97E-03	
IWA6922	2A	3.69						1.97E-03	
IWA2067	2A	76.64			7.68E-04				

Table 3.12. The *p* values of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile (Continued).

SNP	Chr[a]	Position	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
IWA2731	2A	77.83			7.68E-04				
IWA8424	2A	78.10			7.68E-04				
IWA6565	2A	78.49						5.92E-04	
IWA690	2A	88.27							2.77E-03
IWA627	2A	94.70			1.33E-03				
IWA5870	2A	117.57			1.28E-03				
IWA3086	2A	117.74					1.34E-03		
IWA240	2A	127.40	**9.08E-05**	4.74E-03					
IWA241	2A	127.40	**9.08E-05**	4.74E-03					
IWA718	2A	140.95						4.49E-03	
IWA4562	2A	168.91				5.94E-04			
IWA7339	2A	168.91				2.72E-03			
IWA5872	2A	210.75		1.86E-03	3.34E-04				
IWA7633	2B	1.94					3.63E-04		
IWA1930	2B	43.99			3.75E-04	3.46E-03			
IWA2115	2B	44.30	7.64E-04					4.26E-03	
IWA2116	2B	44.30	1.26E-03					4.54E-03	
IWA5708	2B	45.94	2.42E-03						
IWA7120	2B	47.45							9.77E-04
IWA8599	2B	70.19						3.44E-03	
IWA1204	2B	92.63						4.05E-03	
IWA4135	2B	117.14		5.52E-04					
IWA4606	2B	126.28		7.67E-04					
IWA4605	2B	126.28		1.66E-03					
IWA5254	2B	139.35						2.11E-03	
IWA2189	2B	165.61							1.51E-03
IWA3935	2B	180.15						4.22E-03	
IWA840	2B	192.19			7.46E-04				
IWA4294	2B	192.19			1.75E-03				
IWA8589	2B	229.67					4.89E-04		
IWA571	2B	230.09					4.89E-04		
IWA7552	3A	42.37						4.30E-03	4.18E-03
IWA6108	3A	74.98							**2.97E-04**

Table 3.12. The *p* values of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile (Continued).

SNP	Chr[a]	Position	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
IWA6783	3A	81.44							4.07E-03
IWA523	3A	131.68	3.01E-03						
IWA4800	3B	14.49			1.08E-03				
IWA8522	3B	57.74		2.50E-04		1.19E-03			
IWA2492	3B	72.53					3.78E-03		
IWA6793	3B	80.93	2.81E-03			2.93E-03			
IWA6794	3B	80.93	2.81E-03			2.93E-03			
IWA7595	3B	80.93	2.81E-03			2.93E-03			
IWA4452	3B	80.93	2.25E-03						
IWA3997	3B	82.66	1.71E-03				8.75E-04	**8.19E-05**	
IWA1898	3B	84.19	1.03E-03					**4.35E-05**	
IWA1607	3B	84.19	1.71E-03				8.75E-04	**8.19E-05**	
IWA2800	3B	84.19	1.71E-03				8.75E-04	**8.19E-05**	
IWA7333	3B	84.19		5.53E-03			6.31E-04	3.75E-03	
IWA7247	3B	84.19		5.53E-03			6.31E-04	3.75E-03	
IWA7534	3B	84.35					1.80E-03		
IWA4613	3B	85.83	1.68E-03						
IWA4439	3B	88.51			8.82E-04				
IWA7510	3B	88.51		5.70E-03	1.36E-03				
IWA2782	3B	88.51		4.07E-03					
IWA3834	3B	101.97							3.32E-03
IWA2124	3B	101.97					2.21E-03		
IWA3046	3B	105.91							9.89E-04
IWA7679	3B	127.10					2.92E-03		
IWA7680	3B	127.10					2.92E-03		
IWA7973	3B	127.87		3.64E-03		2.98E-03			
IWA5982	3B/3A	127.10/127.51					4.10E-03	6.73E-04	
IWA3902	4A	38.43				1.25E-03			
IWA750	4A	51.63							3.39E-03
IWA912	4A	51.63							3.39E-03
IWA3763	4A	51.63							3.39E-03
IWA5127	4A	51.63							3.39E-03
IWA5490	4A	51.63							3.39E-03

Table 3.12. The *p* values of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile (Continued).

SNP	Chr[a]	Position	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
IWA8265	4A	51.63							3.39E-03
IWA8494	4A	51.63							3.39E-03
IWA3818	4A	51.63							3.83E-03
IWA6659	4A	51.92							3.39E-03
IWA3792	4A	61.12				2.02E-03			
IWA1793	4A	73.84	**3.15E-04**					3.12E-03	
IWA8341	4A	73.84	**3.15E-04**					3.12E-03	
IWA4471	4A	74.91					3.55E-03		
IWA2106	4A	101.96			8.23E-04	2.05E-03			
IWA2107	4A	101.96			8.23E-04	2.05E-03			
IWA3061	4A	146.92							8.02E-04
IWA3068	4A	148.63							**3.19E-04**
IWA6563	4A	153.72	1.37E-03		**3.57E-05**				2.57E-03
IWA4651	4A	193.19			2.99E-04				
IWA6457	4B	0.00					**4.25E-05**		
IWA453	4B	43.74				1.91E-03			
IWA3400	4B	64.50			**2.96E-05**				
IWA4041	4B	80.00				2.48E-03			
IWA3279	4B	92.78					2.23E-03		
IWA4115	4B	95.11					2.62E-03		
IWA1798	4B	124.26	2.84E-03						
IWA7299	4B	124.94	2.84E-03						
IWA6859	5A	11.22			3.35E-04				
IWA4870	5A	12.37							1.53E-03
IWA5368	5A	27.19	1.49E-03						
IWA1988	5A	45.95			**1.81E-04**				
IWA291	5A	45.95			**2.93E-04**				
IWA1253	5A	45.95			**2.93E-04**				
IWA114	5A	45.95			3.54E-04				
IWA5538	5A	59.97						1.08E-03	**3.09E-04**
IWA5539	5A	59.97						1.08E-03	**3.09E-04**
IWA3975	5A	59.97						9.10E-04	5.64E-04
IWA3646	5A	75.19		5.07E-03			1.38E-03		

Table 3.12. The *p* values of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile (Continued).

SNP	Chr[a]	Position	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
IWA7529	5A	83.34						1.49E-03	
IWA5118	5A	83.34						5.15E-03	
IWA1486	5A	113.01	2.22E-03						
IWA4648	5A	113.01	2.22E-03						
IWA4860	5A	118.23						3.76E-03	
IWA46	5A	122.84			7.00E-04				
IWA590	5A	123.21			7.00E-04				
IWA4447	5A	135.98			1.17E-03				
IWA2412	5A	145.54		1.09E-03					
IWA4237	5A	155.73					1.15E-03		
IWA3283	5A	156.02					1.70E-03		
IWA6251	5B	18.09		9.81E-04					
IWA2610	5B	39.37	2.56E-03		**3.31E-06**	7.06E-04			
IWA5784	5B	65.13	1.18E-03						
IWA2536	5B	93.99			1.18E-03				
IWA1755	5B	130.39				3.83E-03			
IWA3002	5B	137.46			9.27E-04	7.47E-04			
IWA3800	5B	161.48							2.10E-03
IWA6782	5B	170.55							4.01E-04
IWA7494	5B	172.48		5.08E-03					
IWA3972	5B	193.79		5.72E-03					
IWA6393	5B	194.03		5.72E-03					
IWA3360	5B	212.38		**1.79E-04**	3.93E-04				
IWA1061	5B	212.94		**1.79E-04**	3.93E-04				
IWA2865	5B	223.11		1.66E-03					
IWA2099	5B	223.94	1.22E-03						
IWA3645	5B/5A	93.25/75.19					1.67E-03		
IWA4147	6A	79.08							3.86E-03
IWA2812	6A	106.04			8.23E-04	2.05E-03			
IWA6962	6A	106.04			8.23E-04	2.05E-03			
IWA3024	6A	135.94						3.99E-03	**7.15E-06**
IWA4951	6A	141.64	2.42E-03						
IWA6775	6A	141.64	2.42E-03						

Table 3.12. The *p* values of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile (Continued).

SNP	Chr[a]	Position	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
IWA2705	6A	180.19	1.82E-03						
IWA3487	6A	180.19	1.82E-03						
IWA3488	6A	180.19	1.82E-03						
IWA504	6A	191.41							4.00E-04
IWA8595	6A	204.49		5.19E-03					
IWA860	6B	0.31		5.34E-03					
IWA4610	6B	14.54	2.05E-03						
IWA1721	6B	37.85							1.93E-03
IWA7897	6B	40.68					3.82E-03		
IWA3424	6B	51.39					**1.90E-04**		
IWA2307	6B	51.39					5.54E-04		
IWA5345	6B	82.68					**5.49E-05**		1.61E-03
IWA8165	6B	82.68						2.18E-03	
IWA5346	6B	82.68		2.91E-03			**3.34E-04**		
IWA7901	6B	82.68					2.40E-03		
IWA3030	6B	82.68					3.55E-03		
IWA4590	6B	83.04					2.40E-03		
IWA3354	6B	88.50		4.61E-03					
IWA800	6B	88.82		4.61E-03					
IWA3679	6B	90.23		4.61E-03					
IWA4382	6B	90.36						**4.08E-05**	1.03E-03
IWA8462	6B	90.36						**4.08E-05**	1.03E-03
IWA7384	6B	94.68				2.40E-03			
IWA7954	6B	94.96				2.40E-03			
IWA8064	6B	119.84	8.85E-04						
IWA1022	7A	14.01						7.69E-04	
IWA472	7A	41.04		4.17E-03			7.28E-04		
IWA473	7A	41.04					9.41E-04		
IWA1759	7A	46.57						**1.70E-05**	3.58E-04
IWA3831	7A	48.00						1.97E-04	
IWA3832	7A	48.00						1.97E-04	
IWA2535	7A	48.90						1.97E-04	
IWA2042	7A	63.61							2.81E-03

Table 3.12. The *p* values of significant SNPs associated with resistance to seven *P. graminis* f. sp. *tritici* (*Pgt*) races at the significant level of 1 percentile (Continued).

SNP	Chr[a]	Position	TTKSK	TRTTF	TTTTF	TPMKC	RKQQC	QTHJC	MCCFC
IWA3843	7A	82.34				1.12E-03			
IWA2082	7A	82.34	2.06E-03						
IWA1491	7A	87.16				3.74E-03			
IWA7600	7A	87.49				1.39E-03			
IWA1946	7A	100.51				9.29E-04			
IWA923	7A	100.84	7.57E-04		1.56E-03	1.32E-03			
IWA3639	7A	101.83	**5.69E-04**			9.36E-04			
IWA7749	7A	102.48	2.23E-03			**2.03E-04**			
IWA3925	7A	102.48	3.12E-03			6.13E-04			
IWA4411	7A	102.48	7.57E-04		1.56E-03	1.32E-03			
IWA6940	7A	103.71		3.16E-03		2.49E-03			
IWA4910	7A	127.29						1.89E-03	1.93E-03
IWA4438	7A	158.90							6.36E-04
IWA4437	7A	159.52				2.89E-03			
IWA3393	7B	54.08			1.31E-03				
IWA4250	7B	65.56			5.56E-04				
IWA446	7B	98.78	1.80E-03						
IWA18	NA	NA	2.41E-03	**4.04E-05**	1.31E-03	**1.69E-04**			2.53E-03
IWA6340	NA	NA						4.31E-03	
IWA3943	NA	NA				2.02E-03	2.89E-03		
IWA2468	NA	NA					4.06E-03		
IWA2471	NA	NA					4.06E-03		
IWA100	NA	NA			7.59E-04				
IWA517	NA	NA		5.96E-03					

[a]Chr, Chromosome. NA, Not available. [b]The bold *p*-values are smaller than the cutoff *p* values at the significant level of 0.1 percentile.

Supplementary Figures

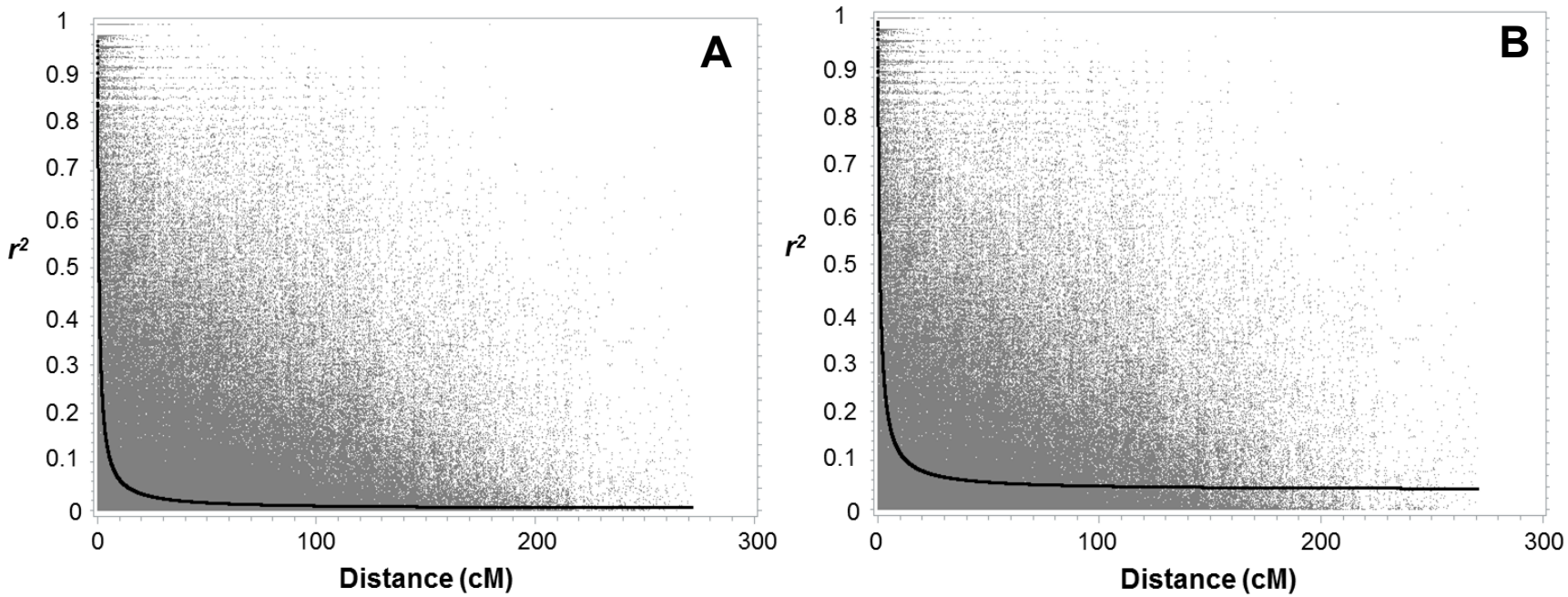

Figure 3.5. Genome-wide linkage disequilibrium (LD) decay plots for 178 emmer wheat accessions. Linkage disequilibrium, measured as r^2 between pairs of polymorphic marker loci, was plotted against the genetic distance (cM). Figure A and B were obtained based on polymorphic SNPs with MAF cutoff value of 0.05 and 0.1, respectively.

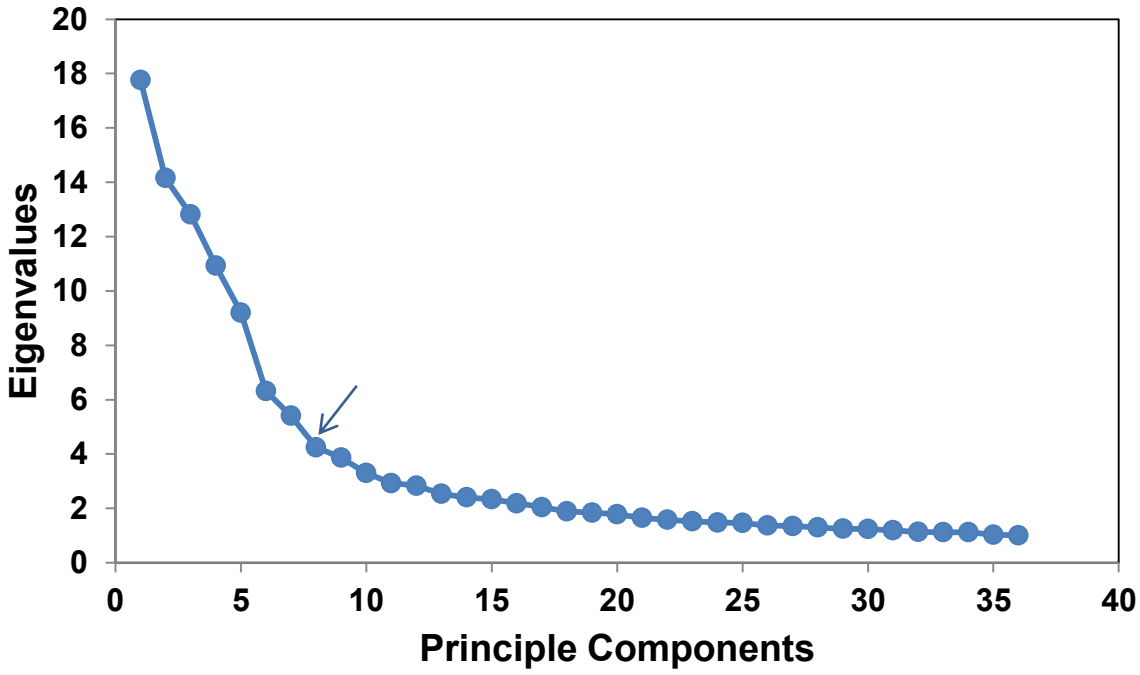

Figure 3.6. Scree plot produced by principle component analysis. The arrow means the 'elbow' of the scree plot, after which the changes of eigenvalues are much smaller than those before the 8th PCs.

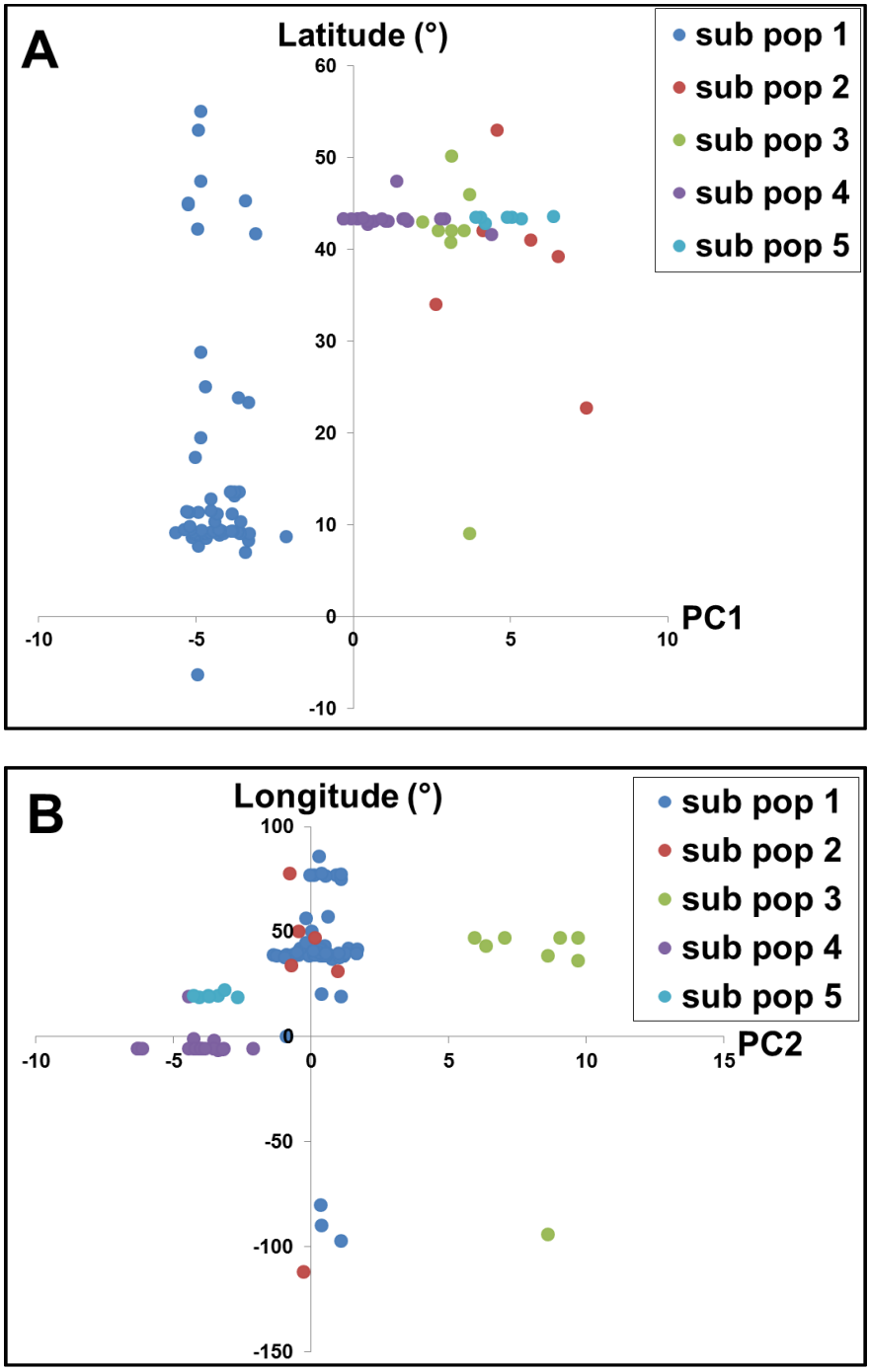

Figure 3.7. Scatter plots of the first two principle components against latitude and longitude. Figure A and B were produced by PC1 versus latitude degree and PC2 versus longitude degree, respectively. Relationship between the sub-populations and their geographic distributions were shown. One dot represented one accession. Only accessions with known latitude and longitude degrees were included in the plots. Different colors indicated different sub-populations.

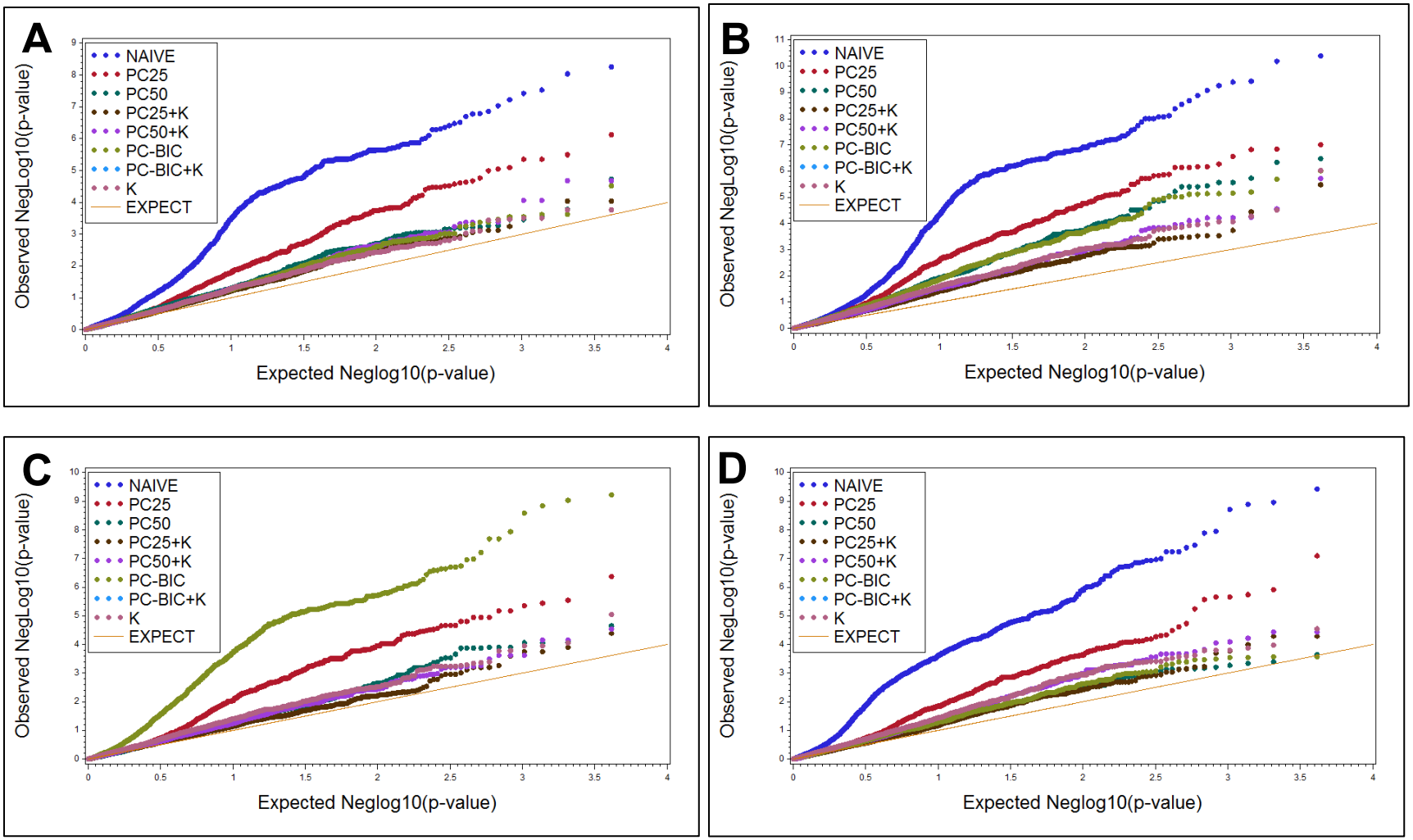

Figure 3.8. Comparison of Quantile-Quantile (Q-Q) plots for the different eight GWAS models tested for each phenotype for stem rust. Figure A, B, C, D, E, F, and G were produced for race TTKSK, TTTTF, TRTTF, TPMKC, RKQQC, QTHJC, and MCCFC, respectively. In each figure, the X axis is the expected $-\text{Log}_{10}(p\text{-value})$, and the Y axis is the observed $-\text{Log}_{10}(p\text{-value})$. One naïve model and seven models with different methods of adjusting population structures were compared.

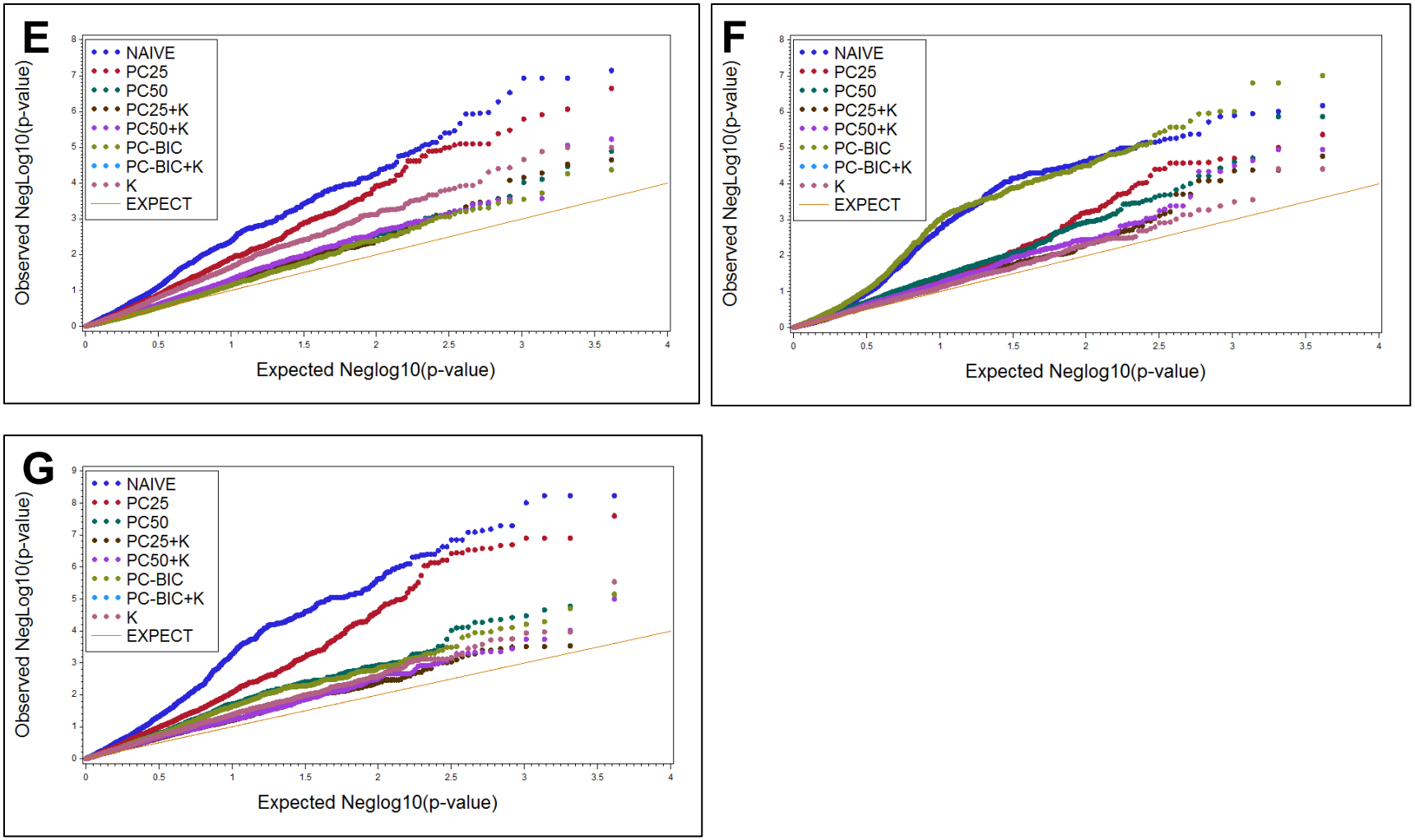

Figure 3.8. Comparison of Quantile-Quantile (Q-Q) plots for the different eight GWAS models tested for each phenotype for stem rust (Continued). Figure A, B, C, D, E, F, and G were produced for race TTKSK, TTTTF, TRTTF, TPMKC, RKQQC, QTHJC, and MCCFC, respectively. In each figure, the X axis is the expected $-\text{Log}_{10}(p\text{-value})$, and the Y axis is the observed $-\text{Log}_{10}(p\text{-value})$. One naïve model and seven models with different methods of adjusting population structures were compared.

Literature Cited

Beteselassie, N., C. Fininsa and A. Badebo. 2007. Sources of resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) in Ethiopian tetraploid wheat accessions. Genetic Resources and Crop Evolution 54: 337-343.

Bradbury, P.J., Z. Zhang, D.E. Kroon, T.M. Casstevens, Y. Ramdoss and E.S. Buckler. 2007. TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23: 2633-2635.

Breseghello, F. and M.E. Sorrells. 2006. Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172: 1165-1177.

Cavanagh, C.R., S. Chao, S. Wang, B.E. Huang, S. Stephen, S. Kiani, et al. 2013. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proceedings of the National Academy of Sciences 110: 8057-8062.

Chao, S., J. Dubcovsky, J. Dvorak, M.C. Luo, S.P. Baenziger, R. Matnyazov, et al. 2010. Population- and genome-specific patterns of linkage disequilibrium and SNP variation in spring and winter wheat (*Triticum aestivum* L.). BMC Genomics 11: 727.

Chu, C., Z. Niu, S. Zhong, S. Chao, T.L. Friesen, S. Halley, et al. 2011. Identification and molecular mapping of two QTLs with major effects for resistance to Fusarium head blight in wheat. Theoretical and Applied Genetics 123: 1107-1119.

Chu, C., S. Chao, T.L. Friesen, J.D. Faris, S. Zhong and S.S. Xu. 2010. Identification of novel tan spot resistance QTLs using an SSR-based linkage map of tetraploid wheat. Molecular Breeding 25: 327-338.

Chu, C., T.L. Friesen, S.S. Xu and J.D. Faris. 2008. Identification of novel tan spot resistance loci beyond the known host-selective toxin insensitivity genes in wheat. Theoretical and Applied Genetics 117: 873-881.

Crossa, J., J. Burgueno, S. Dreisigacker, M. Vargas, S.A. Herrera-Foessel, M. Lillemo, et al. 2007. Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177: 1889-1913.

de la Fuente van Bentem, S. and H. Hirt. 2009. Protein tyrosine phosphorylation in plants: more abundant than expected? Trends in Plant Science 14: 71-76.

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

Gao, X., J. Starmer and E.R. Martin. 2008. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. Genetic Epidemiology 32: 361-369.

Gupta, P.K., S. Rustgi and P.L. Kulwal. 2005. Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Molecular Biology 57: 461-485.

Gurung, S., S. Mamidi, J.M. Bonman, M. Xiong, G. Brown-Guedira and T.B. Adhikari. 2014. Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. PloS One 9: e108179.

Hamrick, J.L. and M.J.W. Godt. 1996. Effects of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 351: 1291-1298.

Hardy, O.J. and X. Vekemans. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2: 618-620.

Hiebert, C., T. Fetch, Jr. and T. Zegeye. 2010. Genetics and mapping of stem rust resistance to Ug99 in the wheat cultivar Webster. Theoretical and Applied Genetics 121: 65-69.

Hodson, D.P., J. Grønbech-Hansen, P. Lassen, Y. Alemayehu, J. Arista, K. Sonder, et al. 2012. Tracking the wheat rust pathogens. In: R. McIntosh, editor Borlaug Global Rust Initiative 2012 Technical Workshop. Beijing, China.

Jin, Y., M. Rouse, P.D. Olivera and B.J. Steffenson. 2009. Progress and prospects in discovery and use of novel sources of stem rust resistance. In: R. McIntosh, editor, Borlaug Global Rust Initiative Technical Workshop. Cd. Obregón, Sonora, Mexico. p. 89-91.

Jin, Y. and R.P. Singh. 2006. Resistance in US wheat to recent eastern African isolates of *Puccinia graminis* f. sp. *tritici* with virulence to resistance gene *Sr31*. Plant Disease 90: 476-480.

Jin, Y., R.P. Singh, R.W. Ward, R. Wanyera, M. Kinyua, P. Njau, et al. 2007. Characterization of seedling infection types and adult plant infection responses of monogenic *Sr* gene lines to race TTKS of *Puccinia graminis* f. sp. *tritici*. Plant Disease 91: 1096-1099.

Jin, Y., L.J. Szabo and Z.A. Pretorius. 2008. Virulence variation within the Ug99 lineage. In: R. Appels, R. Eastwood, E. Lagudah, P. Langridge, M. Mackay, L. McIntye and P. Sharp, editors, 11th International Wheat Genet Symposium, Sydney, Australia. Sydney University Press. p. 4-6.

Kang, H.M., N.A. Zaitlen, C.M. Wade, A. Kirby, D. Heckerman, M.J. Daly, et al. 2008. Efficient control of population structure in model organism association mapping. Genetics 178: 1709-1723.

Kaur, J., U.K. Bansal, R. Khanna, R.G. Saini and H.S. Bariana. 2009. Molecular mapping of stem rust resistance in HD2009/WL711 recombinant inbred line population. International Journal of Plant Breeding 3: 28-33.

Kawahara, Y., M. de la Bastide, J. Hamilton, H. Kanamori, W. McCombie, S. Ouyang, et al. 2013. Improvement of the *Oryza sativa* Nipponbare reference genome using next generation sequence and optical map data. Rice 6: 1-10.

Khan, R.R., H.S. Bariana, B.B. Dholakia, S.V. Naik, M.D. Lagu, A.J. Rathjen, et al. 2005. Molecular mapping of stem and leaf rust resistance in wheat. Theoretical and Applied Genetics 111: 846-850.

Klindworth, D.L., J.D. Miller and S.S. Xu. 2006. Registration of rusty durum wheat. Crop Science 46: 1012-1013.

Knott, D.R. 1962. The inheritance of rust resistance: IX. The inheritance of resistance to races 15B and 56 of stem rust in the wheat variety Khapstein. Canadian Journal of Plant Science 42: 415-419.

Leonard, K.J. and L.J. Szabo. 2005. Stem rust of small grains and grasses caused by *Puccinia graminis*. Molecular Plant Pathology 6: 99-111.

Letta, T., M. Maccaferri, A. Badebo, K. Ammar, A. Ricci, J. Crossa, et al. 2013. Searching for novel sources of field resistance to Ug99 and Ethiopian stem rust races in durum wheat via association mapping. Theoretical and Applied Genetics 126: 1237-1256.

Li, Z. and R.L. Nelson. 2001. Genetic diversity among soybean accessions from three countries measured by RAPDs. Crop Science 41: 1337-1347.

Lipka, A.E., F. Tian, Q. Wang, J. Peiffer, M. Li, P.J. Bradbury, et al. 2012. GAPIT: genome association and prediction integrated tool. Bioinformatics 28: 2397-2399.

Luo, M.C., Z.L. Yang, F.M. You, T. Kawahara, J.G. Waines and J. Dvorak. 2007. The structure of wild and domesticated emmer wheat populations, gene flow between them, and the site of emmer domestication. Theoretical and Applied Genetics 114: 947-959.

Maccaferri, M., A. Ricci, S. Salvi, S.G. Milner, E. Noli, et al. 2014. A high-density, SNP-based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. Plant Biotechnology Journal: n/a-n/a. doi: 10.1111/pbi.12288.

Maccaferri, M., M.C. Sanguineti, V. Natoli, J.L.A. Ortega, M.B. Salem, J. Bort, et al. 2006. A panel of elite accessions of durum wheat (*Triticum durum* Desf.) suitable for association mapping studies. Plant Genetic Resources 4: 79-85.

Mamidi, S., S. Chikara, R.J. Goos, D.L. Hyten, D. Annam, S.M. Moghaddam, et al. 2011. Genome-wide association analysis identifies candidate genes associated with iron deficiency chlorosis in soybean. Plant Genome 4: 154-164.

Mamidi, S., R.K. Lee, J.R. Goos and P.E. McClean. 2014. Genome-wide association studies identifies seven major regions responsible for iron deficiency chlorosis in soybean (*Glycine max*). PloS One 9(9): e107469.

McFadden, E.S. 1930. A successful transfer of emmer characters to *vulgare* wheat. Journal of the American Society of Agronomy 22: 1020-1034.

McIntosh, R. 1980. Chromosome location and linkage studies involving the wheat stem rust resistance gene *Sr14*. Cereal Research Communications 8: 315-320.

McIntosh, R.A. 1972. Cytogenetical studies in wheat VI. Chromosome location and linkage studies involving *Sr13* and *Sr8* for reaction to *Puccinia graminis* f. sp. *tritici*. Australian Journal of Biological Sciences 25: 765-774.

McIntosh, R.A., N.H. Luig and E.P. Baker. 1967. Genetic and cytogenetic studies of stem rust, leaf rust, and powdery mildew resistances in Hope and related wheat cultivars. Australian Journal of Biological Sciences 20: 1181-1192.

McIntosh, R.A., Y. Yamazaki, J. Dubcovsky, J. Rogers, C. Morris, R. Appels, et al. 2013. Catalogue of gene symbols for wheat. 12th International Wheat Genetics Symposium, Yokohama, Japan

Nazari, K., M. Mafi, A. Yahyaoui, R.P. Singh and R.F. Park. 2009. Detection of wheat stem rust (*Puccinia graminis* f. sp. *tritici*) race TTKSK (Ug99) in Iran. Plant Disease 93: 317-317.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individials. Genetics 89: 583-590.

Nesbitt, M., and D. Samuel. 1996. From staple crop to extinction? The archaeology and history of hulled wheats, pp. 41-100 in 1st Internatl Workshop on hulled wheats, edited by S. Padulosi, K. Hammer and J. Heller. International Plant Genetic Resources Institute, Rome, Italy, Castelvecchio Pacoli, Tuscany, Italy.

Newcomb, M., M. Acevedo, H.E. Bockelman, G. Brown-Guedira, B.J. Goates, E.W. Jackson, et al. 2013. Field resistance to the Ug99 race group of the stem rust pathogen in spring wheat landraces. Plant Disease 97: 882-890.

Olivera, P.D., A. Badebo, S.S. Xu, D.L. Klindworth and Y. Jin. 2012a. Resistance to race TTKSK of *Puccinia graminis* f. sp. *tritici* in emmer wheat. Crop Science 52: 2234-2242.

Olivera, P.D., Y. Jin, M. Rouse, A. Badebo, T. Fetch, R.P. Singh, et al. 2012b. Races of *Puccinia graminis* f. sp. *tritici* with combined virulence to *Sr13* and *Sr9e* in a field stem rust screening nursery in Ethiopia. Plant Disease 96: 623-628.

Olivera, P.D., Z.A. Pretorius, A. Badebo and Y. Jin. 2013. Identification of resistance to races of *Puccinia graminis* f. sp. *tritici* with broad virulence in *Triticale (×Triticosecale)*. Plant Disease 97: 479-484.

Paterson, A.H., J.E. Bowers, R. Bruggmann, I. Dubchak, J. Grimwood, H. Gundlach, et al. 2009. The *Sorghum bicolor* genome and the diversification of grasses. Nature 457: 551-556.

Periyannan, S.K., Z.U. Qamar, U.K. Bansal and H.S. Bariana. 2014. Development and validation of molecular markers linked with stem rust resistance gene *Sr13* in durum wheat. Crop and Pasture Science 65: 74-79.

Peterson, R.F., A.B. Campbell and A.E. Hannah. 1948. A diagrammatic scale for estimating rust severity on leaves and stems of cereals. Canadian Journal of Genetics and Cytology C26:496–500.

Pretorius, Z.A., R.P. Singh, W.W. Wagoire and T.S. Payne. 2000. Detection of virulence to wheat stem rust resistance gene *Sr31* in *Puccinia graminis*. f. sp. *tritici* in Uganda. Plant Disease 84: 203-203.

Price, A.L., N.J. Patterson, R.M. Plenge, M.E. Weinblatt, N.A. Shadick and D. Reich. 2006. Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics 38: 904-909.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M.A.R. Ferreira, D. Bender, et al. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics 81: 559-575.

Pyhajarvi, T., M.R. García-Gil, T. Knürr, M. Mikkonen, W. Wachowiak and O. Savolainen. 2007. Demographic history has influenced nucleotide diversity in european *Pinus sylvestris* Populations. Genetics 177: 1713-1724.

Remington, D.L., J.M. Thornsberry, Y. Matsuoka, L.M. Wilson, S.R. Whitt, J. Doebley, et al. 2001. Structure of linkage disequilibrium and phenotypic associations in the maize genome. Proceedings of the National Academy of Sciences 98: 11479-11484.

Ren, J., D. Sun, L. Chen, F.M. You, J. Wang, Y. Peng, et al. 2013. Genetic diversity revealed by single nucleotide polymorphism markers in a worldwide germplasm collection of durum wheat. International Journal of Molecular Sciences 14: 7061-7088.

Roelfs, A.P. 1985. Wheat and rye stem rust. In: W. R. Bushnell and A. P. Roelfs, editors, The cereal rusts. Academic Press, Orlando, Florida, USA. p. 3-37.

Roelfs, A.P. and J.W. Martens. 1988. An international system of nomenclature for *Puccinia graminis* f. sp. *tritici*. Phytopathology 78: 526-533.

Rosewarne, G.M., R.P. Singh, J. Huerta-Espino, H.M. William, S. Bouchet, S. Cloutier, et al. 2006. Leaf tip necrosis, molecular markers and β1-proteasome subunits associated with the slow rusting resistance genes *Lr46/Yr29*. Theoretical and Applied Genetics 112: 500-508.

Rouse, M.N., J. Nirmala, Y. Jin, S. Chao, T.G. Fetch Jr., Z.A. Pretorius, et al. 2014. Characterization of *Sr9h*, a wheat stem rust resistance allele effective to Ug99. Theoretical and Applied Genetics 127:1681-1688.

Saintenac, C., D. Jiang, S. Wang and E. Akhunov. 2013. Sequence-based mapping of the polyploid wheat genome. G3: Genes|Genomes|Genetics 3: 1105-1114.

Salvi, S. and R. Tuberosa. 2005. To clone or not to clone plant QTLs: present and future challenges. Trends in Plant Science 10: 297-304.

SAS Institute. 2011. The SAS system for Windows. SAS Inst., Cary, NC.

Scheet, P. and M. Stephens. 2006. A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase. The American Journal of Human Genetics 78: 629-644.

Schwarz, G. 1978. Estimating the dimension of a model. The Annals of Statistics 6: 461-464.

Singh, R.P., S.A. Herrera-Foessel, J. Huerta-Espino, C.X. Lan, B.R. Basnet et al., 2013 Pleiotropic gene *Lr46/Yr29/Pm39/Ltn2* confers slow rusting, adult plant resistance to wheat stem rust fungus, pp. 19-22 in Proceedings Borlaug Global Rust Initiative, 2013 Technical Workshop.

Singh, R.P., D.P. Hodson, J. Huerta-Espino, Y. Jin, S. Bhavani, P. Njau, et al. 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annual Review of Phytopathology 49: 465-481.

Singh, R.P., D.P. Hodson, Y. Jin, J. Huerta-Espino, M.G. Kinyua, R. Wanyera, et al. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 54: 1-13.

Smith, G.S. 1957. Inheritance of stem rust reaction in tetraploid wheat hybrids: I. allelic genes in Mindum durum × Vernal emmer. Agronomy Journal 49:134–137.

Stakman, E.C., D.M. Stewart and W.Q. Loegering. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici* USDA ARS E-617. U.S. Gov. Print. Off., Washington, DC.

Stich, B., J. Möhring, H.P. Piepho, M. Heckenberger, E.S. Buckler and A.E. Melchinger. 2008. Comparison of mixed-model approaches for association mapping. Genetics 178: 1745-1754.

The International Brachypodium Initiative. 2010. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463: 763-768.

Thornsberry, J.M., M.M. Goodman, J. Doebley, S. Kresovich, D. Nielsen and E.S. Buckler. 2001. *Dwarf8* polymorphisms associate with variation in flowering time. Nature Genetics 28: 286-289.

Tsilo, T.J., Y. Jin and J.A. Anderson. 2008. Diagnostic microsatellite markers for the detection of stem rust resistance gene *Sr36* in diverse genetic backgrounds of wheat. Crop Science 48: 253-261.

Wu, S., M. Pumphrey and G. Bai. 2009. Molecular mapping of stem rust resistance gene *Sr40* in wheat. Crop Science 49: 1681-1686.

Xu, S.S., Y. Jin, D.L. Klindworth, R.R.C. Wang and X. Cai. 2009. Evaluation and characterization of seedling resistances to stem rust Ug99 races in wheat-alien species derivatives. Crop Science 49: 2167-2175.

Yu, L.X., H. Barbier, M. Rouse, S. Singh, R. Singh, S. Bhavani, et al. 2014. A consensus map for Ug99 stem rust resistance loci in wheat. Theoretical and Applied Genetics 127: 1561-1581.

Yu, L.X., S. Liu, J.A. Anderson, R.P. Singh, Y. Jin, J. Dubcovsky, et al. 2010. Haplotype diversity of stem rust resistance loci in uncharacterized wheat lines. Molecular Breeding 26: 667-680.

Yu, L.X., A. Lorenz, J. Rutkoski, R.P. Singh, S. Bhavani, J. Huerta-Espino, et al. 2011. Association mapping and gene-gene interaction for stem rust resistance in CIMMYT spring wheat germplasm. Theoretical and Applied Genetics 123: 1257-1268.

Yu, L.X., A. Morgounov, R. Wanyera, M. Keser, S.K. Singh and M. Sorrells. 2012. Identification of Ug99 stem rust resistance loci in winter wheat germplasm using genome-wide association analysis. Theoretical and Applied Genetics 125: 749-758.

Zaharieva, M., N.G. Ayana, A. Al Hakimi, S.C. Misra and P. Monneveux. 2010. Cultivated emmer wheat (*Triticum dicoccon* Schrank), an old crop with promising future: a review. Genetic Resources and Crop Evolution 57: 937-962.

Zhang, D., G. Bai, C. Zhu, J. Yu and B.F. Carver. 2010. Genetic diversity, population structure, and linkage disequilibrium in U.S. elite winter wheat. Plant Genome 3: 117-127.

Zhang, D., R.L. Bowden, J. Yu, B.F. Carver and G. Bai. 2014. Association analysis of stem rust resistance in U.S. winter wheat. PloS One 9: e103747.

Zhang, Y. and X. Li. 2005. A putative nucleoporin 96 is required for both basal defense and constitutive resistance responses mediated by *suppressor of npr1-1, constitutive 1*. The Plant Cell Online 17: 1306-1316.

Zhu, C., M. Gore, E.S. Buckler and J. Yu. 2008. Status and prospects of association mapping in plants. The Plant Genome 1: 5-20.

Zondervan, K.T. and L.R. Cardon. 2004. The complex interplay among factors that influence allelic association. Nature Reviews Genetics 5: 89-100.

CHAPTER 4. ASSOCIATION ANALYSIS OF LEAF RUST RESISTANCE IN CULTIVATED EMMER WHEAT

Abstract

Cultivated emmer wheat (*Triticum turgidum* ssp. *dicoccum*) is known to be a good source of genes for resistance to wheat leaf rust (caused by *Puccinia triticina* Eriks.). Two major *Lr* genes/alleles, *Lr14a* and *Lr27*, derived from cultivated emmer are currently deployed in modern wheat cultivars and germplasm. A panel of 180 emmer wheat accessions previously assembled for association mapping of stem rust resistance genes were genotyped with 9K SNP Infinium array. The objective of this study was to use this emmer panel for association mapping in order to detect genomic regions with leaf rust resistance. A total of 4,134 polymorphic SNPs and 178 emmer wheat accessions were selected for association analysis after filtering for missing data points and minor allele frequency. Genome-wide association analysis revealed 42 SNP markers that were significantly associated with the leaf rust resistance at the seedling stage. Among the five significant markers at the significant level of 0.1 percentile, one co-located with leaf rust resistance gene *Lr16*. The remaining four markers, located on chromosomes 5B and 7A, were found in the genomic regions where no known *Lr* genes were previously identified, suggesting that some of the emmer wheat accessions carry novel *Lr* genes. The significant markers with major effects were selected based on stepwise regression. Their allelic combinations are potentially useful for marker-assisted selection of leaf rust resistance genes in wheat breeding programs. This study also provides preliminary evidence for discovering novel leaf rust resistance genes in cultivated emmer wheat germplasm.

Introduction

Wheat leaf rust or brown rust, caused by the fungus *Puccinia triticina* Eriks., is one of the three most important rust diseases that challenge wheat production (*Triticum aestivum* L.) (Anikster et al., 1997). Although leaf rust is less damaging than stem rust and stripe rust, it causes greater annual losses globally because of the frequency and widespread occurrence of epidemics (Huerta-Espino et al., 2011). In North America, leaf rust has been of great importance. In the U.S., losses due to leaf rust from 2000 to 2004 were estimated at three million tons, this being a loss of over $350 million for farmers. In Canada, losses caused by leaf rust reached up to 10% of total yield per year between 2000 and 2009 (Huerta-Espino et al., 2011). In northwestern Mexico, a severe leaf rust epidemic on a bread wheat cultivar during the 1976-1977 triggered emergency implementation of a fungicide control program (Dubin and Torres, 1981; Huerta-Espino et al., 2011).

Control of leaf rust in wheat relies on host plant resistance. The wheat community has made on-going efforts to identify *Resistance* genes in wheat and related wild or cultivated species that provide protection against leaf rust, with these genes referred to as *Lr* genes. To date, over 100 genes and numerous QTL associated with resistance to leaf rust have been identified, with 71 genes designated *Lr1- Lr71* (McIntosh et al., 2013). Many of the *Lr* genes, such as *Lr21*, *Lr27*, *Lr31*, and *Lr34*, have been deployed in wheat cultivars and have played major roles in protecting wheat crops from leaf rust.

Unfortunately, *P. triticina* has exhibited a remarkable ability to evolve virulence to wheat resistance conferred by *Lr* genes. *P. triticina* populations have a high degree of genetic diversity (Huerta-Espino, 1992). As many as 70 *P. triticina* races of leaf rust have been identified annually in the U.S. (Kolmer et al., 2007). New virulence races are constantly emerging. For example, two

new races, TFBJQ and TFBGQ, with virulence to *Lr21* were first detected in 2010 in the hard red spring wheat region of the U.S. (Kolmer and Anderson, 2011). In this region, the two races posed a significant threat because more than 50% of the hard red spring wheat acreage in Minnesota and North Dakota relied on *Lr21* for controlling leaf rust (Kolmer and Anderson, 2011). In 2001, a new race, BBG/BN, detected in northwestern Mexico overcame the resistance of widely adapted durum cultivars in the region (Singh et al., 2004, 2013a). Singh et al. (2004) reported that 93.1% of 1,160 elite lines from the CIMMYT Durum Wheat Breeding Program, and most of durum cultivars from 31 countries including U.S. and Canada, were extremely susceptible to the BBG/BN race in greenhouse testing. The leaf rust epidemics caused by this race in northwestern Mexico caused heavy yield losses of durum wheat in 2000-2003, worth an estimated $32 million (Singh et al., 2004).

The BBG/BN race of leaf rust continues to be a serious threat to durum production in Mexico. This race is avirulent *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3*, *Lr3bg*, *Lr3ka*, *Lr9*, *Lr12*, *Lr13*, *Lr14a*, *Lr15*, *Lr16*, *Lr17*, *Lr18*, *Lr19*, *Lr21*, *Lr22a*, *Lr24*, *Lr25*, *Lr26*, *Lr27+31*, *Lr28*, *Lr29*, *Lr30*, *Lr32*, *Lr35*, *Lr36*, and *Lr37*, and virulent to *LrB*, *Lr10*, *Lr11*, *Lr14b*, *Lr20*, *Lr23*, *Lr33*, *Lr39/41*, *Lr44*, and *Lr64* (Singh et al., 2004; Kolmer, 2015). BBG/BN evolves fast and its new variant BBG/BP with virulence to *Lr12*, *Lr27*, and *Lr31* present in durum cultivars in Mexico was detected during the 2007–2008 crop season (Huerta-Espino et al., 2009). BBG/BN race also threatens durum wheat in the U.S. and Canada. In the U.S., a *P. triticina* isolate with a similar virulence pattern and genotype to BBG/BN was first identified on a hard red winter wheat cultivar Overley grown in Kansas in 2013 (Kolmer, 2015). The isolate was found to have high virulence to the U.S. and Canadian durum cultivars tested and it most likely migrated to the southern Great Plains region from the durum-growing regions in Mexico (Kolmer, 2015). This isolate was designated as

BBBQD based on the North American nomenclature system introduced by Long and Kolmer (1989). It is predicted that BBBQD will move from the southern Great Plains region to the major durum producing areas of North Dakota and Saskatchewan (Kolmer, 2015).

To prevent a potential leaf rust epidemic caused by BBBQD in the major durum-growing regions in the U.S. and Canada, it is necessary to search for resistant genes against BBBQD among existing wheat gene pools. Cultivated emmer wheat (*T. turgidum* ssp. *dicoccom,* $2n = 4x = 28$, AABB) is an important genetic resource for the improvement of common and durum wheat. Several breeding programs have developed many cultivars with good disease resistance derived from cultivated emmer (Zaharieva et al., 2010). For resistance to leaf rust, *Lr14a* and *Lr27* were derived from cultivated emmer (McIntosh et al., 2013). Both *Lr* genes were initially transferred from Yaroslav emmer to the common wheat variety Marquis (McFadden, 1930; McIntosh et al., 1967; Singh and McIntosh, 1984). *Lr14a* is one major allele of locus *Lr14* on 7BL. *Lr27* on 4BS is one of two complementary genes together with *Lr31* (McIntosh et al., 2013). Both *Lr14a* and *Lr27* have been deployed for leaf rust resistance in some common wheat cultivars and a small number of durum cultivars (McIntosh et al., 2013). Because *Lr14a* and *Lr27* were derived from a single cultivar of cultivated emmer, it is clear that the cultivated emmer accessions that are maintained in various genetic resource centers provide a valuable unexplored resource worldwide for leaf rust resistance.

In recent years, association mapping has become an important strategy for identifying major genes and quantitative trait loci (QTL) in various crop species such as rice, maize, soybean, and wheat (Salvi and Tuberosa, 2005; Thornsberry et al., 2001; Gupta et al., 2005; Zondervan and Cardon, 2004). In durum wheat, genome-wide association studies have been used to identify the molecular markers significantly associated with leaf rust resistance genes.

Maccaferri et al. (2010) performed association mapping using 164 elite durum accessions genotyped with 225 mapped SSR loci that were evaluated for reactions to 25 leaf rust isolates in greenhouse and field studies in Italy and Mexico. They refined the map region of *Lr14/QLr.ubo-7B.2*, and found significant associations between markers and leaf rust resistance on chromosomes 2A and 2B. They also revealed several regions on chromosomes 2BL, 3BS, and 7BS that harbor minor QTL associated with slow rusting resistance.

The cultivated emmer panel consisting of 180 accessions and the SNP genotypic data used for association mapping of the stem rust resistance in Chapter 3 are useful genetic and genomic resource for identification of leaf rust resistance genes present in the cultivated emmer germplasm. The objectives of present study are to detect genomic regions associated with leaf rust resistance in the cultivated emmer population through GWAS.

Materials and Methods

Plant Material

A panel of 180 cultivated emmer wheat accessions (Table A1) for association mapping of the stem rust resistance in Chapter 3 was used for association mapping of the leaf rust resistance in this study. These cultivated emmer wheat accessions are currently maintained at USDA-ARS National Small Grains Collection (Aberdeen, ID). Their information was retrieved from USDA-ARS Germplasm Resources Information Network (http://www.ars-grin.gov/npgs/searchgrin.html). This emmer wheat panel had proved to have both geographic and genetic diversity in a previous analysis in Chapter 3. The polymorphic SNPs in the panel were dense enough and distributed evenly on 14 chromosomes based on the 9K consensus map developed by Cavanagh et al. (2013). Thus, this emmer population is an appropriate natural population for genome-wide association analysis.

Leaf Rust Resistance Evaluation

The 180 emmer accessions were evaluated for their reactions to race BBBQD at seedling stage in greenhouse. All the seedlings were screened with three replicates at North Dakota Agricultural Experiment Station (AES) Research Greenhouse Complex in Fargo, ND. Five seeds per accession were planted in a single cell in 50-cell trays containing sunshine mix #1 (Sungro Horticulture Distribution Inc., Quincy, MI, USA) and slow-release commercial fertilizer Osmocote 15-9-12, N-P-K (Everris NA Inc., OH, USA) in a rust-free greenhouse set to 22 °C/18 °C (day/night) with 16-h photoperiod. In each tray, common wheat cultivar 'Little Club' and durum wheat line RL 6089 were used as susceptible checks. After seedling emergence, a foliar fertilizer, Peat Lite 20-20-20, was applied once a week. The seedlings at two-leaf stage were inoculated with fresh rust spores suspended in Soltrol-170 oil (Phillips Petroleum, Bartlesville, OK, U.S.A) at a rate of 0.01 g/mL and then left to air dry.

After inoculation, plants were placed in a dark dew chamber for 16-18 h at 20 °C and were then relocated to the greenhouse. About 12 to 14 days after inoculation, the infection types (ITs) were scored using 0-4 scale (McIntosh et al., 1995), where IT with 0 represents no visible sign or symptom; 1 represents small uredinia with necrosis; 2 represents small to medium sized uredinia with green islands and surrounded by necrosis or chlorosis; 3 represents medium sized uredinia with or without chlorosis; 4 represents large uredinia without chlorosis. Two additional signs "-" and "+" indicate smaller and larger uredinia, respectively, for each basic level. ITs of 0 to 2 were considered as resistant reaction while 3 to 4 were considered as susceptible reaction. A combination of ITs was used for representing the disease reactions of a single genotype, and the predominant type was ordered first. When there was a mixture of resistant and susceptible ITs on the same leaf of single plant, the predominant IT would be used for deciding whether the plant

was resistant or susceptible. For association analysis, ITs of each accession were converted to a single value using the method described by Zhang et al. (2014). The converting method was detailed described in GWAS for stem rust resistance in Chapter 3.

All the statistical analyses for phenotypic data were performed using Statistical Analysis System (SAS) version 9.3 (SAS Institute Inc., Gary, NC, U.S.A). Brown and Forsythe's test was performed to test homogeneity of error variances among the replicates using SAS PROC GLM for linearized ITs from the three replicates in greenhouse. Then the Spearman correlation coefficient was estimated for different replicates using SAS PROC CORR. The data of homogeneous or significantly correlated replications was then pooled together and used for further analysis (Chu et al., 2008, 2010).

The analysis of variance (ANOVA) of the reactions in the three replicates in greenhouse was performed in SAS PROC ANOVA. Based on this ANOVA and Equation 4.1, the broad-sense heritability (H^2) of leaf rust resistance to BBBQD in greenhouse was estimated (Letta et al., 2014). In Equation 4.1, σ_G^2, σ_E^2, and r represent the mean square value among accessions, the mean square value of error, and the number of replications, respectively.

$$H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_E^2/r) \quad \text{(Eq. 4.1)}$$

For association analysis, the method of filtering raw genotypic data, model selection, marker-trait association, LD block identification, and stepwise regression analysis followed the procedure described in Chapter 3.

Results and Discussion

The panel of 180 emmer accessions that were used for GWAS of stem rust resistance in Chapter 3 was further evaluated for resistance to leaf rust at seedling stage in greenhouse. The

leaf rust evaluation data and the SNP marker data were used for GWAS to identify loci for resistance to leaf rust in this study.

Reaction of Emmer Wheat Accessions to Leaf Rust at Seedling Stage

For seedling reactions, 46 (26%), 12 (6%), and 122 (68%) accessions showed resistant, heterogeneous (resistant/susceptible), and susceptible reactions to race BBBQD, respectively, based on the predominant ITs in three replicates of the 180 emmer accessions (Table 4.1). Leaf samples with different ITs are shown in Figure 4.1. The analysis of variances (ANOVA) for the linearized ITs in three replicates revealed that the disease reactions of different accessions were significantly different ($p < 0.0001$), while the disease reactions of the three replicates were not significantly different ($p = 0.0597$) (Table 4.4). Based on this ANOVA, the broad-sense heritability (H^2) of the resistance to race BBBQD across the three replications in greenhouse was estimated at 0.9728. The Brown and Forsythe's test of homogeneity showed that the three replicates were homogeneous ($p = 0.2951$). The Spearman correlation coefficients among the reactions in three replications had a range from 0.59 to 0.71 with $P < 0.0001$, indicating that they were highly correlated with each other. Therefore, the three replicates could be pooled together for further analysis.

Table 4.1. Reactions of 180 emmer wheat accessions to *P. triticina* race BBBQD in greenhouse.

Accession	Seedling reaction			Accession	Seedling reaction		
	Rep1[a]	Rep2	Rep3		Rep1[a]	Rep2	Rep3
CItr 7687-1	3	3	3	PI 60704	3	3	3
CItr 12213-1	3	3	3	PI 74106	NA	3	23
CItr 14133-1	;	;	;	PI 94617	3	3	3
CItr 14621-1	3	3-	3	PI 94630	3-	3	3
CItr 14637-1	3	3-	3	PI 94654	3-	3	3
CItr 14916-1	;1	;1	1	PI 94663	21;	3-	3
CItr 14917-1	;1	;1	;1	PI 94665	1	3-	3
CItr 14919-1	2	1	2	PI 94668	3	3+	3
PI 41024-1	3	3	3	PI 94680	3	3-	3+
PI 74108-1	3-	3-	3-	PI 113961	3	3	3
PI 94616-1	3	3	3	PI 168675	3	3	3
PI 94621-1	;1	1	1	PI 190920	3	3	3
PI 94625-1	3	3	3	PI 190926	3	3	3
PI 94626-1	;	NA[b]	3	PI 191091	3	3	;1
PI 94627-1	3+	3	3	PI 193643	3	3	3
PI 94631-1	3	3	3	PI 193878	1	1;	1
PI 94634-1	4	3	3	PI 195721	1	3	3
PI 94635-1	3	3-	3+	PI 196100	3	3	3
PI 94638-1	4	3	3	PI 196905	NA	NA	3-
PI 94648-1	3	3	3	PI 197482	1+	3	NA
PI 94656-1	3	3	3	PI 197490	3	3	3
PI 94664-1	3	3-	3	PI 221401	3	3	3
PI 94666-1	1	;1	;	PI 226951	3	3	3-
PI 94673-1	3	3	3	PI 248991	4	3	3-
PI 94674-1	1;	;	;1	PI 254146	3	3	3
PI 94675-1	2	3-	NA	PI 254163	3	3	3-
PI 94676-1	1	2+	1	PI 254188	3	3	3
PI 94738-1	2	2-	;	PI 254193	3	3	3
PI 94747-1	1	1+	;	PI 272527	;1	1	;1
PI 101971-1	3	3	3	PI 273982	3	3	33
PI 133134-1	1	2+	3	PI 275997	3	3-	NA
PI 154582-1	3	3	3-	PI 275998	;1	3	3-
PI 164578-1	3	3-	3	PI 275999	;1	1;	;1
PI 168673-1	3	3	3	PI 276000	;13-	13-	23
PI 193641-1	3	3	3	PI 276005	NA	NA	;1
PI 193873-1	3	3	3	PI 276006	3	3	3
PI 193879-1	2	2	3	PI 276007	3	3	NA
PI 193880-1	3	3	3	PI 276012	3+	3	3
PI 193882-1	3+	3	3	PI 276014	3	13	3
PI 193883-1	3	3	3	PI 277670	3	3	3+
PI 194042-1	1	1	1+	PI 277671	3	3	3-
PI 194375-1	1	;	;	PI 277677	3-	3	3
PI 197483-1	1	;1	;1	PI 286061	3	3-	3

Table 4.1. Reactions of 180 emmer wheat accessions to *P. triticina* race BBBQD in greenhouse (Continued).

Accession	Seedling reaction			Accession	Seedling reaction		
	Rep1[a]	Rep2	Rep3		Rep1[a]	Rep2	Rep3
PI 197485-1	;	;	NA	PI 289603	3+	3	3
PI 217637-1	3	3	3	PI 295065	3	3	3+
PI 217639-1	3	3	3	PI 297830	3	3	3
PI 217640-1	3	3	3	PI 298543	NA	3	4
PI 221400-1	3	3	3	PI 298548	3	3	3-
PI 225332-1	3+	3	3	PI 306536	3	3+	3+
PI 244341-1	;1	1	NA	PI330544	3-	3-	NA
PI 254165-1	3	3	3	PI 349045	3-	3	NA
PI 254167-1	1;	;13-	;1	PI 350001	3	3	NA
PI 254189-1	;1	1	1	PI 352335	3+	3	NA
PI 254190-1	;	1;	1	PI 352337	;13-	3-	NA
PI 272533-1	3-	3	3	PI 352338	;3	1	3
PI 273981-1	1	1	1	PI 352341	3-	3	3
PI 275996-1	1	1	1	PI 352342	3-	3-	3
PI 298582-1	3-	3	3-	PI 352358	3	3	3
PI 310471-1	3	3	3	PI 352365	3+	3-	3-
PI 319869-1	3	3	3	PI 355460	4	3	;1
PI 322232-1	2	3-	1	PI 355461	3	3	3-
PI 324076-1	3	3	3	PI 355470	4	3	3
PI 349043-1	3-	3	3	PI 355475	4	3	3+
PI 349046-1	;1	;	NA	PI 355483	3-	;13	;3
PI 352548-1	3	3+	3	PI 355485	3-	NA	NA
PI 355477-1	3	3-	3	PI 355486	3	4	3
PI 355507-1	3	3-	3	PI 355489	3	3	4
PI 377655-1	3	3	3	PI 355497	4	3	4
PI 377657-1	1	1	1	PI 355505	4	3	4
PI 384332-1	3	3	3	PI 361833	;1	1	;
PI 434992-1	;1	3	3	PI 362438	3-	3	3-
PI 480460-1	3	3	4	PI 362500	3	3	3-
PI 532305-1	3	2-	3	PI 362697	NA	3	3
CItr 3686	3	3	3	PI 374685	3	3	3
CItr 4013	;	;1	;1	PI 377650	3	3	3-
CItr 7685	3	3-	3;	PI 377672	3	3	3
CItr 7686	;13-	;13-	3	PI 384297	3	3	3
CItr 7779	3-	3	3	PI 384302	NA	3+	3
CItr 7962	3	3	3	PI 384318	1	1	;1
CItr 14085	3+	3	3+	PI 384331	3	3	1
CItr 14086	3+	3	3	PI 434996	3	3	3
CItr 14098	3+	3	NA	PI 470737	2-	NA	NA
CItr 14639	;1+	1	1+	PI 479957	1	1;	;
CItr 14751	;	1	NA	PI 479965	1	1;	NA
CItr 14822	3	3	3-	PI 480307	1	1	NA

Table 4.1. Reactions of 180 emmer wheat accessions to *P. triticina* race BBBQD in greenhouse (Continued).

Accession	Seedling reaction			Accession	Seedling reaction		
	Rep1[a]	Rep2	Rep3		Rep1[a]	Rep2	Rep3
CItr 14834	3	3	3	PI 480312	1	3-	NA
CItr 14866	3	3	3	PI 480313	NA	;	;1
CItr 14971	;13-	3	2	PI 480461	3	3	3-
PI 41025	2	3	3-	PI 480462	3	3	3+
PI 58788	3+	3	3	PI 532304	3	3	3

[a]Rep1, 2, and 3 represent the three replicates in greenhouse. [b]NA, Not available.

Figure 4.1. Representative infection types of cultivated emmer to *P. triticina* race BBBQD. The infection type for each leaf sample was shown on the top of figure. Infection types ';' to '2' are considered resistant. Infection types '3' to '4' are considered high-susceptible.

Association Analysis

A best linear model was first chosen based on MSD values (Table 4.5) and Q-Q plot (Figure 4.2) of eight different models. The mixed linear model "PC25+K" is the best model

among the eight tested models. At the significant level of 0.1 percentile, the cutoff *p* values were estimated at 1.56×10^{-3}. At the significant level of 1 percentile, the cutoff *p* values were estimated at 8.20×10^{-3}. The SNP with a *p* value smaller than the cutoff *p* value identified by the best model was considered to be significantly associated with resistance to race BBBQD. A total of 42 significant markers (1 percentile) were detected (Tables 4.2). These markers were located on 11 A- and B-genome chromsomes. Their genomic positions and significant levels were shown in the Manhattan plots (Figure 4.3).

The phenotypic variation (R^2) explained by each marker ranged from 0.05% to 11.82% (Table 4.2). Stepwise regression analysis showed that 11 significant markers had a major effect on the seedling resistance to race BBBQD, and they together explained phenotypic variation of 56.34%. Among the five significant markers at the siginificant level of 0.1 percentile, *IWA2482* on chromosome 2B was near the genomic region containing *Lr16*. The remaining four significant markers on chromosomes 5B and 7A did not cover the genomic regions with known leaf rust resistance genes or QTL, based on the three genetic maps that were used. The genomic regions with those markers likely harbor novel leaf rust resistance genes. At the significant level of 0.1 percentile, three LD blocks with significant SNPs were identified and located on two chromosomes (Table 4.3). The lengthes of the three blocks have a range from 1.05 cM to 3.14 cM. Based on the mapped positions of the significant markers in LD blocks, all the three LD blocks were at genomic regions without previously identified genes or QTL for resistance to leaf rust. Based on the result of stepwise regression analysis, the LD block on chromosome 7A harboring a significant marker with major effect was a putative major QTL, and the remaining two were putative minor QTL.

Table 4.2. Significant markers associated with resistance to *P. triticina* race BBBQD at the significant level of 1 percentile.

SNP[a]	Chr[b]	Pos[c]	MAF[d]	NegLog$_{10}$(p)	R^2 (%)	SRI[e]
IWA2482	2B	4.98	0.35	3.82	4.22	
IWA8451	NA[f]	NA	0.07	3.53	10.78	Yes
IWA6642	7A	9.35	0.22	3.17	6.37	Yes
IWA2454	5B	100.56	0.06	3.11	11.32	
IWA2455	5B	100.56	0.49	2.81	0.11	
IWA7306	7A	6.21	0.19	2.75	4.26	
IWA2335	5B	116.65	0.21	2.73	2.76	
IWA2336	5B	116.65	0.21	2.73	2.76	
IWA6798	2A	219.15	0.29	2.68	1.72	
IWA2624	2B	72.76	0.35	2.55	1.87	Yes
IWA6797	2B/2A	247.02/219.15	0.28	2.54	1.88	Yes
IWA2453	5B	100.56	0.05	2.52	9.73	
IWA6838	2B	72.76	0.36	2.51	1.88	
IWA435	1B	30.47	0.16	2.50	0.52	Yes
IWA1374	5B	99.94	0.06	2.47	11.82	Yes
IWA2135	6B	59.49	0.49	2.44	7.21	Yes
IWA8283	3A	75.24	0.13	2.35	5.82	
IWA4724	2B	4.98	0.39	2.33	3.26	Yes
IWA6054	2B	192.19	0.20	2.29	2.72	
IWA728	2B	183.10	0.17	2.26	7.22	
IWA4688	4A	206.12	0.49	2.26	8.38	Yes
IWA4690	4A	205.62	0.49	2.26	8.38	
IWA8585	6A	117.04	0.48	2.26	0.20	
IWA553	2B	158.40	0.17	2.20	2.64	
IWA4717	6B	127.53	0.18	2.17	4.98	Yes
IWA4784	4A	77.08	0.08	2.16	4.74	
IWA4785	4A	77.08	0.08	2.16	4.74	
IWA4786	4A	77.08	0.08	2.16	4.74	
IWA4787	4A	77.08	0.08	2.16	4.74	
IWA7615	2B	192.19	0.19	2.16	2.36	
IWA2045	4A	74.91	0.13	2.16	5.60	
IWA5269	4A	74.91	0.13	2.16	5.60	
IWA8550	7B	100.37	0.40	2.15	0.28	
IWA6401	7B	63.40	0.06	2.14	1.24	
IWA1361	7B	64.03	0.06	2.14	1.24	
IWA4290	6B	21.76	0.43	2.12	0.05	
IWA7725	6B	21.76	0.43	2.12	0.05	
IWA5202	3B	3.87	0.38	2.11	0.14	
IWA3211	5B/5A	32.02/11.22	0.24	2.09	2.63	
IWA6947	5B	32.02	0.24	2.09	2.63	
IWA6946	5B	32.02	0.24	2.09	2.63	Yes
IWA8064	6B	119.84	0.28	2.09	0.72	

[a]The bold SNPs are significant at the significant level of 0.1 percentile; [b]Chr, Chromosome; [c]Pos, Positions; [d]MAF, Minor allele frequency; [e]SRI, Stepwise regression included. [f]NA, Not available.

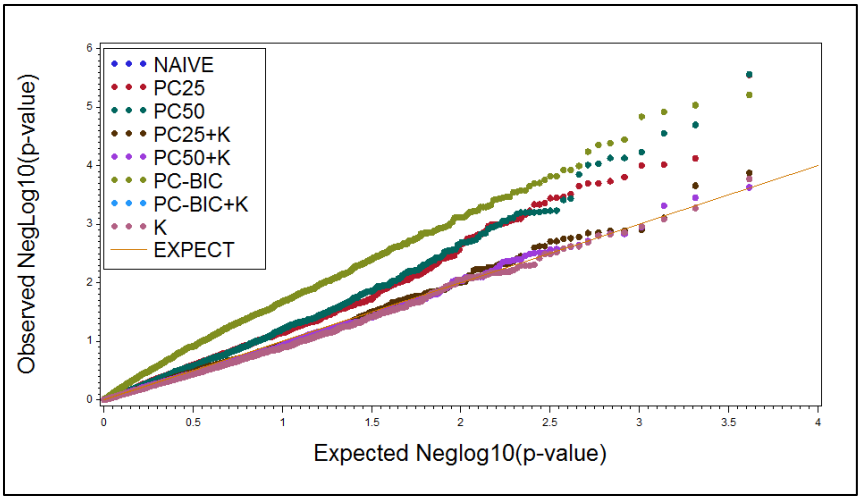

Figure 4.2. Comparison of Quantile-Quantile (Q-Q) plots for the different eight GWAS models tested for leaf rust reactions. The X axis is the expected $-Log_{10}(p\text{-value})$, and the Y axis is the observed $-Log_{10}(p\text{-value})$.

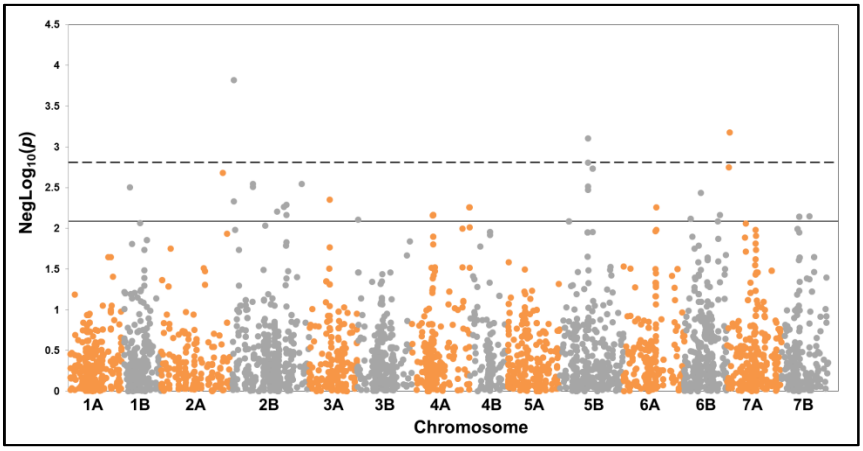

Figure 4.3. Manhattan plots of $-\log_{10}(p\text{-value})$ of all SNP markers used in association analysis with 178 cultivated emmer accessions for leaf rust reactions. The black solid and dashed horizontal lines indicate the significant levels of 1 percentile and 0.1 percentile, respectively. The dots above different horizontal lines represent the identified significant markers associated with disease resistance at different significant levels. Y axis indicates $-\log_{10}(p\text{-value})$ and X axis indicates the chromosomes. The position for each marker was based on the wheat consensus SNP map (Cavanagh et al., 2013).

Based on the number of markers fitted into the stepwise regression, 57 allelic combinations of the significant markers (1 percentile) with major effects on phenotypic variation to leaf rust were identified (Table 4.6). The average reaction of the accessions with specific allelic combination could be used for inferring the resistance or susceptibility of an accession (Table 4.6). Among the 57 allelic combinations, 30 and 27 associated with resistant and susceptible reactions, respectively, in the population (Table 4.6). The combinations of significant markers associated with resistance could be used for marker-assisted selection in breeding programs.

Table 4.3. Linkage disequilibrium (LD) blocks including one or several significant markers associated with resistance to leaf rust at the significant level of 0.1 percentile.

LD block	Chr[a]	Pos[b](cM)	SNP	Extented length(cM)
1	7A	6.21	*IWA7306*	3.14
		9.35	***IWA6642****	
2	5B	98.62	*IWA5048*	1.94
		99.47	*IWA4074, IWA5283*	
		99.94	*IWA1374*	
		100.56	*IWA2453, IWA2454**	
3	5B	100.56	*IWA2455**	1.05
		101.61	*IWA265*	

*Significant markers. The bold significant markers are stepwise regression model included markers. [a]Chr, Chromosome. [b]Pos, Positions.

In the primary gene pool of cultivated wheat, cultivated emmer wheat is a good source for leaf rust resistance. In the emmer panel used in this study, 46 (26%) accessions showed seedling resistance to race BBBQD. Through GWAS, 42 SNPs were identified to significantly associate with leaf rust resistance. Based on the genomic position of the five significant markers at the significant level of 0.1 percentile, it was found that significant SNP *IWA2482* on chromosome 2B was near the genomic region containing *Lr16*. Because *Lr16* was not originally derived from cultivated emmer, the genomic regions containing *IWA2482* unlikely harbor the locus *Lr16* in most cases, and may contain unknown *Lr* genes near to *Lr16*.

Except for *IWA2482* associated with *Lr16*, the remaining four significant SNPs (0.1 percentile) located to the genomic regions where there are no known *Lr* genes were identified on chromosomes 5B and 7A. The genomic regions on chromosome arms 5BL, 7AS, and 7AL were also identified to associate with leaf rust resistance in association mapping of leaf rust resistance in durum by Maccaferri et al. (2010). These genomic regions most likely harbor the novel leaf rust resistance genes. The cultivated emmer accessions with high levels of broad-spectrum resistance and favorable alleles in the marker loci at these regions will be useful materials for new *Lr* gene identification through linkage mapping analysis using bi-parental mapping population.

The results from GWAS in this study provide preliminary evidence of the genomic regions associated with leaf rust resistance in cultivated emmer wheat. The precise identification and verification of the *Lr* genes will require linkage analysis using bi-parental mapping populations. However, the results from GWAS provide guidance in searching for genomic regions harboring resistance genes. The identified LD blocks especially for the LD block with major effect will be good candidate regions. The allelic combinations of significant markers selected by stepwise regression could be used for identifying wheat genotypes with *Lr* genes located in targeted genomic regions. Therefore, our genome-wide association study provides the first step towards identifying novel *Lr* genes in emmer wheat and pyramiding resistance loci from emmer wheat for MAS breeding. It will eventually increase the genetic diversity for resistance to leaf rust in modern durum and bread wheat germplasm.

Supplementary Tables

Table 4.4. Analysis of variances (ANOVA) of reactions in three replicates to *P. triticina* race BBBQD at seedling stage in greenhouse for 180 cultivated emmer accessions.

Source of variation	DF[a]	SS[b]	MS[c]	F value	*p* value
Accession	179	3461.270	19.3367	11.91	<.0001
Replication	2	9.235	4.6175	2.84	0.0597
Error	327	531.061	1.6240		
Total	508	4001.570			

[a]DF, Degree of freedom.
[b]SS, Sum of squares.
[c]MS, Mean squares.

Table 4.5. Mean square difference (MSD) values of eight different GWAS models used for identifying significant associations between SNPs and leaf rust resistance at seedling stage.

Model	Greenhouse
Naïve	3.17E-02
PC25	2.07E-03
PC50	1.70E-03
PC25+K	1.98E-04
PC50+K	4.89E-04
PC-BIC	3.17E-02
PC-BIC+K	1.11E-03
K	1.11E-03

Table 4.6. Allelic combinations of stepwise included significant markers associated with leaf rust resistance and the sample statistics of each allelic combination's phenotypic data.

Allelic combination[a]	No. of obs[b]	Mean (IT)	Std dev[c]	Min[d]	Max[e]	Reaction[f]
BBAAABBAABA	1	1.39	.	1.39	1.39	R
BBBAABBAABA	3	1.64	0.23	1.44	1.89	R
ABBBBAAABAA	1	2.27	.	2.27	2.27	R
BBABAABAABA	5	2.61	1.28	1.67	4.73	R
BBAAAABAABA	2	2.63	1.46	1.60	3.67	R
ABBABAAABAA	4	2.67	1.05	1.42	3.61	R
ABBBBBBAABA	1	2.73	.	2.73	2.73	R
ABBABAAABBA	3	3.07	1.31	1.87	4.47	R
BBBBAABBAAA	4	3.24	1.88	0.67	5.17	R
BBBBABBAABA	2	3.45	1.48	2.40	4.50	R
BBBBAAABAAA	2	3.48	1.91	2.13	4.83	R
BBBABAAABAA	1	3.61	.	3.61	3.61	R
BBBBABBBABA	2	4.25	3.18	2.00	6.50	R
BBBBAABAABA	6	4.30	2.68	1.67	7.92	R
BBABAABAABB	2	4.83	2.83	2.83	6.83	R
BBBBAABBAAB	5	5.03	1.41	3.27	6.94	R
BABAAABAABA	1	5.27	.	5.27	5.27	R
BBBAAABABBA	1	5.78	.	5.78	5.78	R
BBBAAAAABAA	3	5.81	2.72	2.67	7.50	R
BBBBAABBABB	1	5.83	.	5.83	5.83	R
BBBBBAAABAA	2	5.83	1.34	4.89	6.78	R
BAABAABAABA	3	6.13	1.79	4.17	7.67	R
BBBBAABBABA	11	6.27	2.39	2.27	8.17	R
BBBBAABABBA	1	6.28	.	6.28	6.28	R
ABBBBAAABBA	1	6.44	.	6.44	6.44	R
BBABAAAAABB	1	6.44	.	6.44	6.44	R
BBBBAAABABA	1	6.50	.	6.50	6.50	R
BAABAABAABB	10	6.72	1.20	4.44	8.00	R
BAABAABBABA	1	6.87	.	6.87	6.87	R
ABBAAAAABBA	2	6.96	1.19	6.11	7.80	R
BAABAAAABBB	1	7.00	.	7.00	7.00	S
BBBAAAAABBA	24	7.11	1.51	2.20	8.33	S
BBBBAABBBAB	1	7.20	.	7.20	7.20	S
BBABAAABBBB	1	7.22	.	7.22	7.22	S
BBABAABBABA	4	7.31	0.74	6.67	8.25	S
BBBBAAAAABA	1	7.33	.	7.33	7.33	S
BBBBBABBBBA	3	7.37	0.42	6.94	7.78	S
BAABAAAAABB	9	7.58	0.52	6.28	8.00	S
BABBABBBABA	1	7.60	.	7.60	7.60	S
BAABAABBBBA	2	7.61	0.00	7.61	7.61	S
BBABBAABBBA	5	7.67	0.35	7.17	8.00	S
BBBAAABABBB	1	7.67	.	7.67	7.67	S
BBABAABBBBA	3	7.68	0.42	7.20	8.00	S

Table 4.6. Allelic combinations of stepwise included significant markers associated with leaf rust resistance and the sample statistics of each allelic combination's phenotypic data (Continued).

Allelic combination[a]	No. of obs[b]	Mean (IT)	Std dev[c]	Min[d]	Max[e]	Reaction[f]
BBBBAAABAAB	9	7.71	0.55	6.53	8.25	S
BBBBAABBBBA	2	7.75	0.12	7.67	7.83	S
BAABAABABBA	6	7.76	0.29	7.50	8.22	S
BBBBBAABBBA	4	7.80	0.37	7.47	8.17	S
BBBBAAAABBA	7	7.86	0.26	7.44	8.17	S
BBABAABABBA	2	7.89	0.51	7.53	8.25	S
BBABAAABBBA	1	7.93	.	7.93	7.93	S
BBABBAAABBA	1	8.00	.	8.00	8.00	S
BBABAAAABBA	1	8.17	.	8.17	8.17	S
BBBAAAABBBA	1	8.17	.	8.17	8.17	S
BAABBAABBBA	1	8.28	.	8.28	8.28	S
BABABAABBBA	2	8.44	0.08	8.39	8.50	S
BABAAABBABA	1	8.50	.	8.50	8.50	S
BAABAAABABB	1	8.67	.	8.67	8.67	S

[a]A and B refer to the alleles in the 9K SNP wheat chip.
Orders of SNPs: *IWA8451, IWA6642, IWA2624, IWA6797, IWA435, IWA1374, IWA2135, IWA4724, IWA4688, IWA4717, IWA6946*.
[b]Number of observations; [c]Standard deviation; [d]Min, minimum; [e]Max, Maximum; [f]R, Resistant; S, Susceptible.

Literature Cited

Anikster, Y., W.R. Bushnell, A.P. Roelfs, T. Eilam and J. Manisterski. 1997. *Puccinia recondita* causing leaf rust on cultivated wheats, wild wheats, and rye. Canadian Journal of Botany 75: 2082-2096.

Bansal, U.K., M.J. Hayden, B.P. Venkata, R. Khanna, R.G. Saini and H.S. Bariana. 2008. Genetic mapping of adult plant leaf rust resistance genes *Lr48* and *Lr49* in common wheat. Theoretical and Applied Genetics 117: 307-312.

Cavanagh, C.R., S. Chao, S. Wang, B.E. Huang, S. Stephen, S. Kiani, et al. 2013. Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. Proceedings of the National Academy of Sciences 110: 8057.

Chu, C.G., S. Chao, T.L. Friesen, J.D. Faris, S. Zhong and S.S. Xu. 2010. Identification of novel tan spot resistance QTLs using an SSR-based linkage map of tetraploid wheat. Molecular Breeding 25: 327-338.

Chu, C.G., T.L. Friesen, S.S. Xu and J.D. Faris. 2008. Identification of novel tan spot resistance loci beyond the known host-selective toxin insensitivity genes in wheat. Theoretical and Applied Genetics 117: 873-881.

Chu, C.G., T.L. Friesen, S.S. Xu, J.D. Faris and J.A. Kolmer. 2009. Identification of novel QTLs for seedling and adult plant leaf rust resistance in a wheat doubled haploid population. Theoretical and Applied Genetics 119: 263-269.

Dubin, H.J. and E. Torres. 1981. Causes and consequences of the 1976-1977 wheat leaf rust epidemic in northwest Mexico. Annual Review of Phytopathology 19: 41-49.

Gupta, P.K., S. Rustgi and P.L. Kulwal. 2005. Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Molecular Biology 57: 461-485.

Herrera-Foessel, S.A., R.P. Singh, J. Huerta-Espino, H.M. William, V. Garcia, A. Djurle, et al. 2008. Identification and molecular characterization of leaf rust resistance gene *Lr14a* in durum wheat. Plant Disease 92: 469-473.

Huerta-Espino J. 1992. Analysis of wheat leaf and stem rust virulence on a worldwide basis. PhD thesis, University of Minnesota, St. Paul, MN

Huerta-Espino, J., R.P. Singh, S. Germán, B.D. McCallum, R.F. Park, W.Q. Chen, et al. 2011. Global status of wheat leaf rust caused by *Puccinia triticina*. Euphytica 179: 143-160.

Kolmer, J. 2015. First report of a wheat leaf rust (*Puccinia triticina*) phenotype with high virulence to durum wheat in the great plains region of the United States. Plant Disease 99: 156-156.

Kolmer, J.A. 2013. Leaf rust of wheat: pathogen biology, variation and host resistance. Forests 4: 70-84.

Kolmer, J. A. and J.A. Anderson. 2011. First detection in North America of virulence in wheat leaf rust (*Puccinia triticina*) to seedling plants of wheat with *Lr21*. Plant Disease 95: 1032-1032.

Kolmer J.A., Y. Jin and D.L. Long. 2007. Leaf and stem rust of wheat in the United States. Australian Journal of Agricultural Research 58:631–638.

Lehti-Shiu, M.D., C. Zou, K. Hanada and S.-H. Shiu. 2009. Evolutionary history and stress regulation of plant receptor-like kinase/pelle genes. Plant Physiology 150: 12-26.

Letta, T., P. Olivera, M. Maccaferri, Y. Jin, K. Ammar, A. Badebo, et al. 2014. Association mapping reveals novel stem rust resistance loci in durum wheat at the seedling stage. Plant Genome 7: -. doi:10.3835/plantgenome2013.08.0026.

Li, T. and G. Bai. 2009. Lesion mimic associates with adult plant resistance to leaf rust infection in wheat. Theoretical and Applied Genetics 119: 13-21.

Long, D.L. and J.A. Kolmer. 1989. A North American system of nomenclature for *Puccinia recondita* f. sp. *tritici*. Phytopathology 79: 525-529.

Maccaferri, M., M.C. Sanguineti, P. Mantovani, A. Demontis, A. Massi, K. Ammar, et al. 2010. Association mapping of leaf rust response in durum wheat. Molecular Breeding 26: 189-228.

Mantovani, P., M. Maccaferri, R. Tuberosa and J. Kolmer. 2010. Virulence phenotypes and molecular genotypes in collections of *Puccinia triticina* from Italy. Plant Disease 94: 420-424.

McCartney, C.A., D.J. Somers, B.D. McCallum, J. Thomas, D.G. Humphreys, J.G. Menzies, et al. 2005. Microsatellite tagging of the leaf rust resistance gene *Lr16* on wheat chromosome 2BSc. Molecular Breeding 15: 329-337.

McFadden, E.S. 1930. A successful transfer of emmer characters to *vulgare* wheat. Journal of the American Society of Agronomy 22: 1020-1034.

McIntosh, R.A., N.H. Luig and E.P. Baker. 1967. Genetic and cytogenetic studies of stem rust, leaf rust and powdery mildew resistances in Hope and related wheat cultivars. Australian Journal of Biological Sciences 20: 1181-1192.

McIntosh, R.A., C.R. Wellings and R.F. Park. 1995. Wheat rusts: an atlas of resistance genesCSIRO Publications, East Melbourne, Australia.

McIntosh, R.A., Y. Yamazaki, J. Dubcovsky, J. Rogers, C. Morris, R. Appels, et al. 2013. Catalogue of gene symbols for wheat. 12th International Wheat Genetics Symposium, Yokohama, Japan

Messmer, M.M., R. Seyfarth, M. Keller, G. Schachermayr, M. Winzeler, S. Zanetti, et al. 2000. Genetic analysis of durable leaf rust resistance in winter wheat. Theoretical and Applied Genetics 100: 419-431.

Newcomb, M., M. Acevedo, H.E. Bockelman, G. Brown-Guedira, B.J. Goates, E.W. Jackson, et al. 2013. Field resistance to the Ug99 race group of the stem rust pathogen in spring wheat landraces. Plant Disease 97: 882-890.

Peterson, R.F., A.B. Campbell and A.E. Hannah. 1948. A diagrammatic scale for estimating rust severity on leaves and stems of cereals. Canadian Journal of Genetics and Cytology C26:496–500.

Rosewarne, G.M., R.P. Singh, J. Huerta-Espino, H.M. William, S. Bouchet, S. Cloutier, et al. 2006. Leaf tip necrosis, molecular markers and β1-proteasome subunits associated with the slow rusting resistance genes *Lr46/Yr29*. Theoretical and Applied Genetics 112: 500-508.

Salvi, S. and R. Tuberosa. 2005. To clone or not to clone plant QTLs: present and future challenges. Trends in Plant Science 10: 297-304.

SAS Institute. 2011. The SAS system for Windows. SAS Inst., Cary, NC.

Singh, A., J.K. Pallavi, P. Gupta and K.V. Prabhu. 2011. Identification of microsatellite markers linked to leaf rust adult plant resistance (APR) gene *Lr48* in wheat. Plant Breeding 130: 31-34.

Singh, A., M.P. Pandey, A.K. Singh, R.E. Knox, K. Ammar, J.M. Clarke, et al. 2013a. Identification and mapping of leaf, stem and stripe rust resistance quantitative trait loci and their interactions in durum wheat. Molecular Breeding 31: 405-418.

Singh, D., V. Mohler and R.F. Park. 2013. Discovery, characterisation and mapping of wheat leaf rust resistance gene *Lr71*. Euphytica 190: 131-136.

Singh, R.P., J. Huerta-Espino, W. Pfeiffer and P. Figueroa-Lopez. 2004. Occurrence and impact of a new leaf rust race on durum wheat in northwestern Mexico from 2001 to 2003. Plant Disease 88: 703-708.

Singh, R.P. and R.A. McIntosh. 1984. Complementary genes for resistance to *Puccinia recondita tritici* in *Triticum aestivum* II. Cytogenetic studies. Canadian Journal of Genetics and Cytology 26: 736-742.

Takahashi, A., C. Casais, K. Ichimura and K. Shirasu. 2003. HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in Arabidopsis. Proceedings of the National Academy of Sciences 100: 11777-11782.

Takai, R., N. Matsuda, A. Nakano, K. Hasegawa, C. Akimoto, N. Shibuya, et al. 2002. EL5, a rice *N*-acetylchitooligosaccharide elicitor-responsive RING-H2 finger protein, is a ubiquitin ligase which functions *in vitro* in co-operation with an elicitor-responsive ubiquitin-conjugating enzyme, *Os*UBC5b. The Plant Journal 30: 447-455.

Thornsberry, J.M., M.M. Goodman, J. Doebley, S. Kresovich, D. Nielsen and E.S. Buckler. 2001. *Dwarf8* polymorphisms associate with variation in flowering time. Nature Genetics 28: 286-289.

Wu, Y. and J.-M. Zhou. 2013. Receptor-like kinases in plant innate immunity. Journal of Integrative Plant Biology 55: 1271-1286.

Xu, X., G. Bai, B.F. Carver, G.E. Shaner and R.M. Hunger. 2005. Molecular characterization of slow leaf-rusting resistance in wheat. Crop Science 45: 758-765.

Yu, L.X., A. Lorenz, J. Rutkoski, R.P. Singh, S. Bhavani, J. Huerta-Espino, et al. 2011. Association mapping and gene-gene interaction for stem rust resistance in CIMMYT spring wheat germplasm. Theoretical and Applied Genetics 123: 1257-1268.

Zaharieva, M., N.G. Ayana, A. Al Hakimi, S.C. Misra and P. Monneveux. 2010. Cultivated emmer wheat (*Triticum dicoccon* Schrank), an old crop with promising future: a review. Genetic Resources and Crop Evolution 57: 937-962.

Zhang, D., R.L. Bowden, J. Yu, B.F. Carver and G. Bai. 2014. Association analysis of stem rust resistance in U.S. winter wheat. PloS One 9: e103747.

Zhao, X.L., T.C. Zheng, X.C. Xia, Z.H. He, D.Q. Liu, W.X. Yang, et al. 2008. Molecular mapping of leaf rust resistance gene *LrZH84* in Chinese wheat line Zhou 8425B. Theoretical and Applied Genetics 117: 1069-1075.

Zondervan, K.T. and L.R. Cardon. 2004. The complex interplay among factors that influence allelic association. Nature Reviews Genetics 5: 89-100.

CHAPTER 5. ASSOCIATION ANALYSIS OF TAN SPOT RESISTANCE IN CULTIVATED EMMER WHEAT

Abstract

Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr), is a major disease of both durum (*Triticum turgidum* ssp. *durum*) and common wheat (*T. aestivum*) around the world. Sources of resistance to tan spot are rarely found in adapted wheat germplasm. Cultivated emmer wheat (*T. turgidum* ssp. *dicoccum*) contains many useful genes for resistance to various forms of biotic and abiotic stresse. In order to identify new sources of resistance to tan spot, a set of 180 cultivated emmer wheat accessions that were previously genotyped with 9K SNP array was evaluated for reaction to four Ptr races, race 1 (isolate Pti2), race 2 (86-124), race 3 (331-9), and race 5 (DW5). Reaction data from this study and 4,134 polymorphic previously identified SNPs were further used for genome-wide association analysis to identify SNP markers significantly associated with resistance to tan spot. The disease evaluations showed that 22, 65, 15, and 29 accessions were resistant to races 1, 2, 3, and 5, respectively. Six accessions were highly resistant to all four races. Genome-wide association mapping revealed that 43, 42, 42, and 41 SNPs were significantly associated with resistance to races 1, 2, 3, and 5, respectively, at the significant level of 1 percentile. Among the 20 significant markers at the significant level of 0.1 percentile, nine co-located with several previously known genes or QTL conferring resistance to tan spot. The remaining 11 markers located on chromosomes 1B, 2B, 4A, 4B, 5B, and 7A were found in the genomic regions where no known tan spot resistance genes were previously identified. This evidence points to some of the emmer wheat accessions carrying novel tan spot resistance genes. This study provided preliminary evidence for discovering novel tan spot resistance genes in cultivated emmer wheat germplasm, and the accessions with allelic combinations of the

significant SNP loci with major effects are potentially useful resources for breeding and genetic studies of tan spot resistance.

Introduction

In wheat (*Triticum aestivum* L.), tan spot (also named yellow spot or yellow leaf blotch) is a disease caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs. (Abbreviated as Ptr) [anamorph *Drechslera tritici-repentis* (Died.) Shoem.], which can infect almost all cultivated wheat species (Krupinsky, 1982; De Wolf et al., 1998; Faris et al., 2013; Singh et al., 2010a). Tan spot can cause an average of 5-10% yield losses per year, but can reach up to 50% loss under favorable environmental conditions (Shabeer and Bockus, 1988). Different Ptr isolates can induce two independent symptoms, i.e., necrosis and chlorosis (Lamari and Bernier, 1989a, b). Based on virulence patterns of Ptr isolates on a differential set consisting of four common wheat cultivars Salamouni, Glenlea, 6B662, and 6B365, eight Ptr races (races 1 through 8) have been identified (Lamari et al., 1995, 2003).

The virulence of *P. tritici-repentis* is related to different host-specific/selective toxins (HSTs) and nonspecific toxins (Oliver and Solomon, 2010). HSTs are also known as necrotrophic effectors (NEs). A compatible interaction between necrotrophic pathogens and wheat involves the recognition of HSTs by the product of a host susceptibility gene, which eventually results in a susceptible reaction of the host plant (Friesen and Faris, 2010). So far, three HSTs produced by *P. tritici-repentis* have been identified, designated as Ptr ToxA, Ptr ToxB and Ptr ToxC (Tomás et al., 1990; Orolaza et al., 1995; Strelkov et al., 1999; Effertz et al., 2002). The three susceptibility genes of host plants that correspond to the three HSTs have been identified; these genes include *Tsn1* on chromosome arm 5BL for Ptr ToxA (Faris et al., 2010), *Tsc2* on 2BS for Ptr ToxB (Friesen and Faris, 2004), and *Tsc1* on 1AS for Ptr ToxC (Effertz et

al., 2002). Because the HSTs are major virulence factors, elimination of the HST-sensitive genes in wheat would improve resistance to tan spot. In addition to the three HST-insensitive genes, several other genes (*Tsr2-Tsr5*) could also confer resistance to tan spot in wheat (Faris et al., 2013). The tan spot resistance in wheat can also be inherited quantitatively (Elias et al., 1989; Nagle et al., 1982). Several QTLs with major effects were identified in wheat, including race specific QTLs, for example *QTsc.ndsu-1A* conferring resistance to races 1 and 3, and race non-specific QTLs, for example *QTs.fcu-1B* and *QTs.fcu-3B* conferring resistance to the four races 1, 2, 3, and 5 (Faris and Friesen, 2005; Chu et al., 2008b).

The majority of current durum and bread wheat cultivars are susceptible to tan spot. This is largely because resistance to tan spot has not been a major aim of durum and bread wheat breeding programs (Lamari et al., 2005; Singh et al., 2006a, b; Tadesse et al., 2006). As tan spot has become an increasing problem in recent years due to reduced tillage, many researchers have searched for novel sources of tan spot resistance. A high level of partial resistance to tan spot has been detected in common wheat (Mergoum et al., 2007; Rees and Platz, 1990; Singh et al., 2006a, b; Tadesse et al., 2006), synthetic hexaploid wheat (SHW) (Xu et al., 2004; Friesen et al., 2008; Morris et al., 2010), wheat-alien species derivatives (Oliver et al., 2008a), *Aegilops tauschii* accessions (Cox et al., 1992; Siedler et al., 1994), and tetraploid wheat species (Chu et al., 2008a). In contrast, durum wheat germplasm has rarely shown high levels of resistance (Xu et al., 2004; Singh et al., 2006a, b). Because transfer of disease resistance from hexaploid common wheat to tetraploid durum wheat is often complicated by the difference in ploidy level, durum wheat will benefit from identification of new sources of resistance in other tetraploid wheat.

Being a tetraploid wheat, cultivated emmer wheat (*T. turgidum* ssp. *dicoccom,* $2n = 4x = 28$, AABB) is expected to be useful for improving durum's resistance to tan spot. Lamari and Bernier (1989a) reported that 19.8% of 288 evaluated tetraploid wheat accessions, including accessions of cultivated emmer wheat, were resistant to Ptr isolate ASC1. Singh et al. (2006a) also reported that 40.7% of 91 evaluated cultivated emmer accessions were resistant to Ptr race 1. Chu et al. (2008a) evaluated 200 cultivated emmer accessions for seedling reaction to Ptr race 1 and identified 61 (30.5%) resistant accessions. The genetic basis of tan spot resistance in these cultivated emmer accessions has not been investigated and no genes or QTLs for resistance have been identified.

Association mapping is a useful strategy for identifying major genes and quantitative trait loci (QTL) in plants (Salvi and Tuberosa, 2005; Thornsberry et al., 2001; Gupta et al., 2005; Zondervan and Cardon, 2004). This strategy utilizes linkage disequilibrium among alleles at different loci to discover the targeted genes and QTL by analyzing the association between markers and characters in a natural population (Gupta et al., 2005; Breseghello and Sorrells, 2006). Association mapping has been extensively used to identify the genomic regions associated with important agronomic traits in wheat and other major crops (Gebhardt et al., 2004; Zhang et al., 2005; Breseghello and Sorrells, 2006). However, it has not been extensively used to identify the tan spot resistance loci in wheat, there being only two reports of association mapping in common wheat germplasm (Gurung et al., 2011; Kollers et al., 2014). Gurung et al. (2011) genotyped a population of 567 spring wheat landraces using 832 diversity array technology (DArT) markers and evaluated the population for reactions to Ptr races 1 and 5 in growth chambers. They identified seven DArT markers on chromosomes 2A, 2B, 2D, 4A, 5B, and 7D and three DArT markers on chromosomes 2D, 6A, and 7D, which were significantly associated

with resistance to race 1 and 5 respectively. Kollers et al. (2014) evaluated 358 European winter wheat varieties and 14 spring wheat varieties in field trials for reactions to races 1 and 5. They identified 90 markers on 21 chromosomes that were significantly associated with tan spot resistance.

It is believed that the cultivated emmer panel consisting of 180 accessions used for association mapping of the resistances to stem rust and leaf rust in Chapter 3 and Chapter 4, respectively, is a potentially useful genetic resource for identification of tan spot resistance genes present in the cultivated emmer germplasm. Therefore, the objective of this study is to detect genomic regions associated with tan spot resistance in the cultivated emmer population through genome-wide association analysis (GWAS).

Materials and Methods

Plant Materials

The same set of 180 cultivated emmer wheat accessions (Table A1) used as the panel for association mapping of the stem rust resistance in Chapter 3 was used for association mapping of the tan spot resistance in this study. These cultivated emmer wheat accessions were originally provided by USDA-ARS National Small Grains Collection (Aberdeen, ID). Their information was retrieved from USDA-ARS Germplasm Resources Information Network (http://www.ars-grin.gov/npgs/searchgrin.html).

Tan Spot Evaluation and Assessment

The 180 cultivated emmer accessions and four differential lines (Salamouni, Glenlea, 6B662, and 6B365) were evaluated with four different Ptr isolates Pti2, 86-124, 331-9, and DW5, representing four different races: 1, 2, 3 and 5, respectively. The inoculum preparation, disease inoculation and post-inoculation incubation followed the descriptions by Friesen et al.

(2003) under controlled greenhouse and growth chamber conditions. The inoculations were conducted at the two- to three-leaf stage of plants. For each isolate, the evaluation on all the 184 accessions or lines was performed with three completely randomized replications. A lesion type-based scale from 1 to 5 described by Lamari and Bernier (1989a) was adopted to score disease; the genotypes with lesion types 1, 2, 3, 4, and 5 were classified as resistant, moderately resistant, moderately resistant/moderately susceptible, susceptible, and highly susceptible, respectively, and the genotypes showing equal number of two lesion types were given an intermediate lesion type (e.g. lesion type 1 and 2 equals 1.5). All the accessions or lines were also evaluated for reactions to Ptr ToxA, following the procedure described by Xu et al. (2004).

Phenotypic Data Analysis and Association Mapping

Bartlett's test for homogeneity of error variances was performed using PROC GLM in Statistical Analysis System (SAS) version 9.3 (SAS Institute Inc., Gary, NC, U.S.A) for lesion types, in three replicates for each race. The Spearman correlation coefficient was estimated for different replicates of each race in SAS PROC CORR. The homogeneous or significantly correlated replications would be pooled together and used for further analysis (Chu et al., 2008b, 2010). Among the average lesion types for four races, Spearman correlation coefficients were also calculated for evaluating the correlation relationships among reactions to different races. The analysis of variance (ANOVA) of lesion types in three replicates for each race was performed in SAS PROC ANOVA. Based on this ANOVA and Equation 5.1, the broad-sense heritability (H^2) of tan spot resistance to each race in greenhouse was estimated (Letta et al., 2014). In Equation 5.1, σ_G^2, σ_E^2, and r represent the mean square value among accessions, the mean square value of error, and the number of replications.

$$H^2 = \sigma_G^2/(\sigma_G^2 + \sigma_E^2/r) \quad \text{(Eq. 5.1)}$$

The 180 emmer accessions had been genotyped with 9K SNP array and the genotype result was described in Chapter 3. The methods of filtering raw genotypic data, association analysis, LD block identification, and stepwise regression analysis followed the descriptions in Chapter 3. Additionally, for each significant SNP, the mean lesion types for two different alleles were calculated in SAS PROC MEANS. The additive effect of the variant allele for each significant SNP was estimated to be 50% of the difference between the mean lesion types for two different alleles (Mamidi et al., 2014). An LD heatmap was developed for specific LD blocks using an R package named LDheatmap (Shin et al., 2006).

Results

Reaction of Emmer Wheat Accessions to Tan Spot at Seedling Stage

The disease evaluation showed that the 180 cultivated emmer accessions had a wide range of variation in the reactions to the four races, ranging from high resistance (lesion type = 0.5) to high susceptibility (Lesion type = 5.0) (Figure 5.1, Table 5.1). The four differential lines had the reactions to four races that were consistent with previous studies (Lamari et al., 1995, 2003) (Table 5.4, Figure 5.1). The four differential lines, including Salamouni, Glenlea, 6B662 and 6B365, were resistant to races 1, 2, 3, and 5; races 3 and 5; races 1, 2, and 3; and races 2 and 5; respectively. And Glenlea, 6B662 and 6B365 were susceptible to races 1 and 2 with symptoms of necrosis, race 5 with symptoms of chlorosis, and races 1 and 3 with symptoms of chlorosis, respectively.

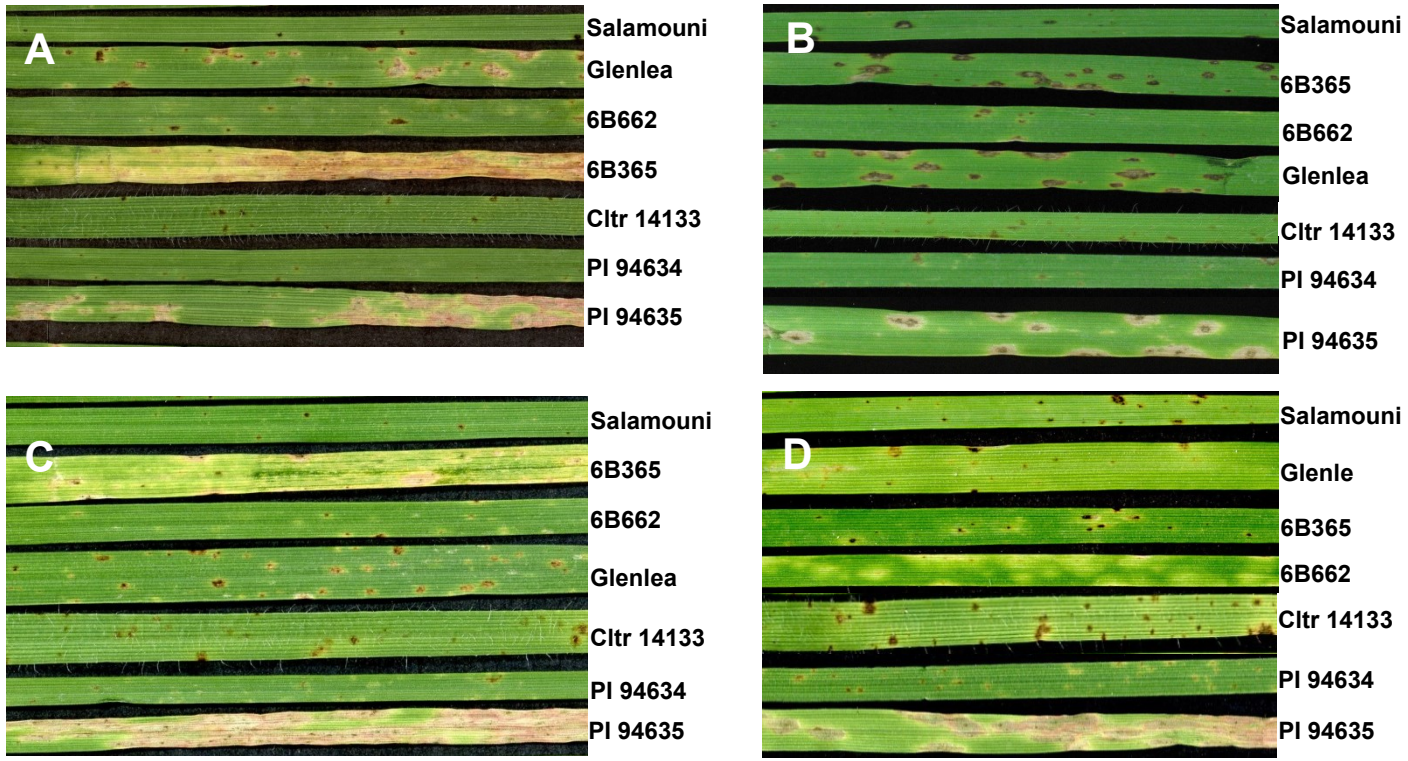

Figure 5.1. Disease reaction of two highly resistant and one highly susceptible emmer wheat accessions and four tan spot differential lines to the fungal inoculation with four *P. tritici-repentis* races. Figure A, B, C, and D represented the disease reactions of plants to race 1 (isolate Pti2), 2 (86-124), 3 (331-9), and 5 (DW5) respectively.

Table 5.1. Disease reactions of 180 emmer wheat accessions to the four *P. tritici-repentis* races.

Accession	Race 1			Race 2			Race 3			Race 5			Ptr ToxA
	Rep 1[c]	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	
CItr 7687-1	3.5	4.0	3.5	NA[d]	NA	NA	4.0	3.0	3.5	NA	3.0	3.0	0
CItr 12213-1	3.0	3.0	3.0	1.5	1.5	2.5	3.0	3.0	4.0	2.0	2.5	3.5	0
CItr 14133-1[a]	1.0	1.0	1.0	0.5	NA	1.0	1.5	2.0	1.5	1.0	1.5	2.0	0
CItr 14621-1	3.5	5.0	3.0	3.0	3.5	2.5	4.0	4.0	4.5	4.5	3.5	3.5	0
CItr 14637-1	3.0	4.0	2.5	2.5	3.0	1.0	4.0	4.0	4.5	2.5	3.0	4.0	0
CItr 14916-1	2.5	5.0	4.0	3.0	5.0	4.0	4.0	4.5	5.0	4.0	3.0	3.0	0
CItr 14917-1	4.0	4.5	3.5	2.0	2.0	3.5	4.5	3.5	4.5	3.5	3.5	3.5	0
CItr 14919-1	3.0	2.5	1.0	1.5	NA	2.5	3.5	4.0	4.5	2.5	3.0	3.0	0
PI 41024-1	1.5	2.5	2.5	2.5	2.0	1.5	2.5	3.5	3.5	NA	NA	NA	0
PI 74108-1	NA	4.5	3.5	2.0	3.5	1.5	4.5	3.5	4.5	4.0	4.0	4.5	0
PI 94616-1	4.0	5.0	4.5	NA	NA	NA	4.0	3.0	3.5	3.0	2.0	3.0	0
PI 94621-1	3.5	4.5	3.5	3.0	4.0	2.5	4.5	4.5	5.0	5.0	4.0	4.5	0
PI 94625-1	3.5	4.5	2.5	3.0	3.5	3.5	3.0	4.0	3.5	3.5	3.5	3.5	0
PI 94626-1	2.5	3.0	3.0	1.5	2.5	2.0	4.0	NA	4.5	5.0	3.0	5.0	0
PI 94627-1	4.0	4.5	3.5	2.5	3.0	3.0	4.5	3.5	NA	2.5	2.5	2.5	0
PI 94631-1	4.0	5.0	5.0	2.5	4.5	3.5	4.5	5.0	3.5	3.0	3.0	3.0	0
PI 94634-1[a]	1.5	1.0	1.0	1.0	1.0	1.0	NA	1.0	1.0	1.0	1.0	1.0	0
PI 94635-1[b]	4.0	5.0	4.0	3.0	5.0	4.0	5.0	5.0	4.5	5.0	5.0	5.0	0
PI 94638-1	3.5	4.0	3.5	5.0	3.0	4.0	4.5	4.5	4.5	3.0	3.0	3.0	0
PI 94648-1	1.5	2.0	2.5	1.0	2.0	1.0	3.5	4.0	3.5	2.5	2.0	3.5	0
PI 94656-1	1.5	2.5	2.5	2.0	1.5	1.0	4.0	3.5	3.5	2.5	NA	3.0	0
PI 94664-1	4.0	4.5	3.5	3.0	4.5	4.0	4.0	3.0	4.5	4.5	3.0	2.5	0
PI 94666-1	3.0	3.0	3.0	1.5	2.5	2.0	4.5	4.0	4.5	5.0	4.0	2.5	0
PI 94673-1	3.5	4.0	3.5	2.0	2.5	1.5	4.5	4.5	5.0	4.0	4.0	NA	0
PI 94674-1	3.0	3.0	3.0	3.0	3.0	1.5	5.0	4.0	4.0	5.0	4.0	3.5	0
PI 94675-1	NA	NA	NA	1.0	2.0	2.0	4.0	3.5	4.5	3.5	4.0	3.0	0
PI 94676-1[a]	2.5	2.0	1.0	1.0	1.0	1.0	2.5	2.5	2.5	2.0	2.0	2.5	0
PI 94738-1	3.0	3.0	2.0	2.0	2.5	1.0	4.0	3.5	4.0	4.5	3.0	3.0	0
PI 94747-1	3.5	2.5	2.5	1.0	2.5	1.0	2.5	3.0	3.5	3.5	3.0	4.0	0

Table 5.1. Disease reactions of 180 emmer wheat accessions to the four *P. tritici-repentis* races (Continued).

Accession	Race 1			Race 2			Race 3			Race 5			Ptr ToxA
	Rep 1[c]	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	
PI 101971-1	3.5	3.5	3.0	4.0	2.5	1.5	3.5	3.5	3.5	4.0	2.5	3.0	0
PI 133134-1	4.5	2.5	NA	2.5	2.5	2.0	4.5	3.5	3.5	4.0	2.5	2.5	0
PI 154582-1[b]	4.5	4.5	4.0	NA	4.0	4.0	4.0	5.0	5.0	4.5	4.5	5.0	0
PI 164578-1	3.5	3.0	3.0	2.0	2.0	1.0	NA	3.5	4.0	3.0	4.5	4.5	0
PI 168673-1	4.0	NA	2.5	NA	NA	NA	4.0	3.5	3.5	3.0	3.0	3.0	0
PI 193641-1	3.0	5.0	3.0	NA	NA	NA	4.0	2.5	3.0	5.0	3.0	3.5	0
PI 193873-1	4.0	3.0	3.0	2.0	2.5	2.0	3.5	3.5	3.5	5.0	3.0	3.5	0
PI 193879-1	2.5	3.0	2.0	2.5	2.0	1.0	4.0	2.5	3.0	1.0	2.0	2.5	0
PI 193880-1	2.5	3.5	2.5	2.5	2.0	1.0	4.0	3.5	3.0	3.0	3.0	2.5	0
PI 193882-1	3.0	4.0	3.0	2.5	2.5	1.0	4.0	3.0	4.0	3.0	3.0	4.0	0
PI 193883-1	NA	2.5	3.5	3.0	2.5	2.5	3.0	3.5	2.5	3.0	3.0	4.0	0
PI 194042-1	4.0	4.5	3.5	3.0	3.0	3.5	4.0	4.0	3.0	3.0	3.5	3.0	0
PI 194375-1	3.5	4.0	4.0	4.0	4.5	2.5	3.0	3.0	2.5	3.0	3.0	3.5	0
PI 197483-1[b]	4.0	5.0	4.0	5.0	4.5	3.5	4.5	4.5	3.5	5.0	3.5	3.5	0
PI 197485-1[b]	4.5	5.0	4.0	4.0	4.5	4.0	5.0	3.5	4.5	5.0	4.0	3.5	0
PI 217637-1	4.0	3.5	3.0	2.5	2.5	1.5	4.0	4.5	3.0	3.5	2.0	3.5	0
PI 217639-1	2.5	2.5	3.0	2.5	3.0	1.0	3.5	4.5	3.0	4.5	2.5	4.5	0
PI 217640-1	4.0	2.5	3.0	2.0	2.5	1.0	4.0	4.5	3.0	3.5	3.0	3.5	0
PI 221400-1	4.0	2.5	3.0	2.5	2.5	1.0	2.5	3.0	4.0	NA	NA	NA	0
PI 225332-1[b]	4.5	4.5	3.5	NA	NA	NA	5.0	4.5	4.0	NA	NA	NA	0
PI 244341-1	2.5	4.5	3.0	2.5	3.0	1.5	5.0	3.5	3.0	3.0	3.0	2.0	0
PI 254165-1[b]	4.5	5.0	4.5	4.0	3.5	5.0	5.0	5.0	4.0	4.0	3.0	4.0	3
PI 254167-1[b]	4.0	4.5	4.5	5.0	4.5	3.5	4.5	4.5	3.0	5.0	4.0	4.5	0
PI 254189-1	4.5	4.0	3.0	2.5	3.5	2.5	4.0	4.0	4.5	5.0	2.5	3.5	0
PI 254190-1	4.0	4.0	NA	2.5	3.0	1.0	4.5	4.0	3.5	5.0	3.0	3.5	0
PI 272533-1	4.5	4.0	2.5	2.5	1.5	1.5	4.5	4.0	3.5	4.0	2.5	4.0	0
PI 273981-1	3.5	5.0	3.0	2.5	4.0	3.0	4.0	4.5	3.5	NA	3.0	3.0	0
PI 275996-1	4.0	5.0	4.0	2.0	3.5	3.0	3.0	4.5	3.5	NA	3.5	3.0	0
PI 298582-1	4.0	4.0	3.5	2.5	3.0	2.0	3.5	4.5	4.0	4.0	2.5	3.5	0

Table 5.1. Disease reactions of 180 emmer wheat accessions to the four *P. tritici-repentis* races (Continued).

Accession	Race 1			Race 2			Race 3			Race 5			Ptr ToxA
	Rep 1[c]	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	
PI 310471-1	3.5	3.0	2.5	2.5	2.0	1.5	3.0	4.5	3.5	NA	NA	NA	0
PI 319869-1	2.5	2.5	3.0	2.5	2.5	NA	3.5	5.0	4.0	3.5	3.0	3.5	0
PI 322232-1	3.5	2.0	2.5	2.5	2.0	1.5	2.0	2.5	2.5	2.5	2.5	1.5	0
PI 324076-1	3.5	2.0	2.0	2.0	1.5	1.0	1.5	2.5	2.5	NA	2.5	3.0	0
PI 349043-1	4.0	4.0	3.0	NA	3.0	2.5	NA	NA	NA	5.0	3.5	3.0	0
PI 349046-1	2.5	4.0	3.0	2.0	3.5	1.0	2.5	3.5	4.5	5.0	4.0	3.5	0
PI 352548-1[b]	4.5	4.5	4.0	5.0	4.0	4.0	4.5	4.5	4.0	5.0	5.0	4.0	0
PI 355477-1	4.5	3.0	3.0	NA	3.5	2.0	3.5	4.0	3.5	2.5	2.5	2.5	0
PI 355507-1	4.0	4.0	4.0	4.0	4.0	2.5	3.0	4.0	4.0	3.0	3.0	3.0	0
PI 377655-1	NA	NA	NA	4.0	2.5	3.5	2.5	3.0	3.5	3.0	2.5	3.0	0
PI 377657-1	4.0	4.5	2.5	NA	3.5	1.5	NA	3.0	3.5	2.5	2.0	2.5	0
PI 384332-1	2.5	2.5	3.0	1.0	1.0	2.5	2.0	4.0	4.0	3.0	1.5	2.0	0
PI 434992-1	2.0	3.5	2.0	2.0	2.5	1.5	2.5	2.5	3.0	2.5	1.5	2.0	0
PI 480460-1	2.0	4.0	3.0	3.5	5.0	3.5	2.0	4.0	3.5	3.5	2.5	2.5	0
PI 532305-1	2.5	2.0	3.0	2.5	1.0	1.0	2.5	3.5	3.0	2.5	3.5	1.5	0
CItr 3686	3.0	3.0	3.0	3.0	3.0	2.0	3.0	NA	NA	NA	NA	NA	0
CItr 4013	3.5	4.5	3.0	NA	3.0	3.5	NA	4.0	4.0	2.5	2.0	3.5	3
CItr 7685	4.5	3.5	4.0	4.0	3.0	3.5	3.5	4.5	3.5	4.0	5.0	5.0	0
CItr 7686[b]	4.5	4.0	4.0	5.0	3.5	4.0	3.5	5.0	4.5	5.0	3.5	5.0	0
CItr 7779	3.5	3.0	3.0	2.5	2.5	1.0	4.5	4.0	4.5	4.5	3.5	2.5	0
CItr 7962	4.5	4.0	4.0	3.5	3.5	3.5	NA	NA	NA	2.5	2.5	2.0	0
CItr 14085	4.0	3.0	2.5	2.0	3.0	1.0	1.5	3.5	3.5	4.0	2.5	4.5	0
CItr 14086	3.5	3.5	2.5	2.5	3.0	2.5	2.0	2.5	3.5	4.0	2.5	4.5	0
CItr 14098	3.5	3.0	3.0	2.0	2.5	3.0	3.0	3.5	3.5	4.0	3.5	3.5	0
CItr 14639	4.0	5.0	4.5	4.5	4.0	4.0	3.5	4.0	3.5	4.0	3.5	3.0	0
CItr 14751	3.0	3.0	4.5	2.5	3.0	3.5	NA	NA	NA	4.0	3.0	3.5	0
CItr 14822	2.5	2.5	2.0	2.5	2.0	1.0	3.5	3.0	4.0	2.5	3.5	2.5	3
CItr 14834	2.0	2.5	2.0	2.5	1.5	2.0	3.5	4.5	3.0	3.0	3.5	2.0	0
CItr 14866	4.0	5.0	4.0	4.0	5.0	3.5	3.5	3.5	3.5	3.0	3.5	3.0	0

Table 5.1. Disease reactions of 180 emmer wheat accessions to the four *P. tritici-repentis* races (Continued).

Accession	Race 1			Race 2			Race 3			Race 5			Ptr ToxA
	Rep 1[c]	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	
CItr 14971	4.0	4.0	4.5	4.0	2.5	2.0	3.5	5.0	5.0	4.0	3.5	2.5	0
PI 41025	3.5	3.0	3.0	2.0	1.5	2.5	2.5	3.0	2.5	2.5	3.0	3.5	0
PI 58788	4.5	4.5	4.0	5.0	3.5	4.0	3.0	4.0	3.5	3.0	3.5	3.5	0
PI 60704[b]	4.5	4.5	4.0	5.0	4.5	3.5	3.0	4.5	4.5	5.0	3.5	4.5	0
PI 74106[b]	4.5	4.0	4.5	4.0	4.5	4.5	4.5	4.0	4.0	5.0	4.0	5.0	0
PI 94617[a]	NA	NA	NA	1.5	1.5	1.5	2.5	2.0	2.5	2.0	2.0	2.0	0
PI 94630	3.5	4.5	4.0	4.0	3.5	4.0	3.5	3.5	3.0	4.0	2.5	2.5	0
PI 94654	4.5	4.0	4.5	5.0	3.5	3.5	2.5	3.5	3.0	3.0	2.5	2.5	0
PI 94663	4.5	NA	3.0	2.5	3.0	3.0	4.5	3.5	5.0	4.0	4.0	4.5	0
PI 94665	3.5	3.0	4.0	NA	3.5	3.0	4.5	4.0	4.0	3.0	3.5	4.5	0
PI 94668	3.5	3.0	3.0	2.0	3.0	1.5	NA	4.5	4.5	5.0	4.0	3.5	0
PI 94680	4.5	3.5	3.0	2.5	3.5	3.5	2.5	3.0	3.0	3.0	2.5	4.5	0
PI 113961[b]	4.5	4.5	3.0	4.0	4.5	4.0	4.5	4.5	4.0	4.0	4.0	4.0	0
PI 168675	4.0	4.5	4.5	4.0	3.5	2.5	3.5	3.5	4.5	3.0	2.5	1.5	0
PI 190920[a]	NA	NA	NA	2.0	2.0	1.5	1.5	2.5	2.0	2.5	2.5	2.5	0
PI 190926	4.5	4.0	3.0	2.5	2.5	3.0	2.5	3.5	3.0	NA	NA	NA	0
PI 191091	3.5	3.5	3.0	2.5	3.0	3.0	NA	3.5	3.0	NA	NA	NA	0
PI 193643[b]	3.5	4.5	4.0	5.0	4.0	4.0	3.0	4.5	4.0	NA	NA	NA	0
PI 193878	4.5	4.5	4.5	5.0	3.0	3.5	4.0	4.0	4.0	4.0	3.0	3.5	0
PI 195721	4.0	4.5	4.0	5.0	5.0	4.5	3.0	3.5	4.5	4.0	3.0	3.5	0
PI 196100	4.0	4.0	4.5	4.0	3.5	3.0	2.5	3.5	3.0	4.0	2.5	3.5	0
PI 196905	4.0	3.0	3.5	NA	2.5	4.0	3.0	4.0	3.0	NA	NA	NA	0
PI 197482	4.0	3.0	3.5	3.5	3.5	4.0	4.5	4.0	3.0	3.0	3.5	3.5	0
PI 197490[b]	4.5	4.5	4.5	4.0	4.5	4.0	3.0	4.5	3.5	4.0	4.5	3.5	0
PI 221401	3.5	2.5	3.5	3.5	3.5	2.5	2.0	3.5	3.0	3.0	2.0	2.5	0
PI 226951	3.5	4.0	4.0	2.5	3.0	3.0	3.5	4.0	3.0	3.5	3.5	2.5	0
PI 248991	4.0	2.0	2.0	2.0	1.5	2.0	3.0	2.5	2.5	2.0	2.5	1.5	0
PI 254146	4.5	4.0	4.0	4.0	5.0	2.5	3.5	NA	3.0	4.0	3.0	3.0	0
PI 254163[b]	4.5	5.0	4.0	3.0	4.5	4.5	4.5	4.5	4.5	4.0	4.0	4.5	0

Table 5.1. Disease reactions of 180 emmer wheat accessions to the four *P. tritici-repentis* races (Continued).

Accession	Race 1			Race 2			Race 3			Race 5			Ptr ToxA
	Rep 1[c]	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	
PI 254188	3.0	1.5	1.5	2.0	1.0	2.5	2.5	3.5	3.0	2.5	1.0	2.0	0
PI 254193	4.5	4.0	3.0	2.0	2.5	1.0	3.0	2.5	3.5	NA	NA	NA	0
PI 272527	4.0	3.0	2.5	2.5	3.5	2.0	3.0	4.0	3.5	4.0	2.5	4.5	0
PI 273982	NA	4.5	4.0	3.0	3.5	5.0	3.5	3.0	4.0	4.0	3.5	4.0	0
PI 275997	2.0	2.5	NA	NA	NA	NA	3.0	4.0	2.5	NA	NA	NA	0
PI 275998	3.5	4.5	4.0	4.0	5.0	4.0	3.5	4.5	NA	3.5	4.0	2.5	0
PI 275999	4.0	4.0	4.5	4.0	4.5	4.0	3.5	3.5	2.5	4.5	5.0	4.0	0
PI 276000	4.0	4.0	4.5	4.0	3.0	3.0	NA	NA	NA	4.0	5.0	4.0	0
PI 276005	4.5	2.5	NA	2.5	3.5	1.0	NA	2.5	2.5	NA	NA	NA	0
PI 276006	4.5	4.0	3.5	4.0	4.0	2.5	2.5	4.5	2.5	2.5	3.0	2.5	0
PI 276007	4.5	4.0	3.0	3.0	3.5	3.0	4.0	4.0	3.0	4.0	5.0	4.5	0
PI 276012	4.0	3.5	3.5	2.5	4.0	3.0	2.5	4.0	2.5	2.5	3.5	2.5	0
PI 276014	4.0	3.0	2.5	4.0	3.5	3.5	3.5	3.5	3.0	2.5	4.5	3.5	0
PI 277670	3.5	2.0	2.0	5.0	4.5	4.0	3.0	3.5	2.0	4.0	3.5	3.0	0
PI 277671	3.5	3.0	3.0	5.0	3.5	4.0	4.0	4.0	2.0	5.0	4.0	5.0	0
PI 277677	2.5	3.5	2.5	2.5	3.0	3.0	3.0	4.5	5.0	4.0	2.5	4.5	0
PI 286061	4.0	3.0	2.5	3.0	3.5	1.5	2.5	4.0	3.0	3.0	2.5	4.0	0
PI 289603	4.0	4.0	3.0	4.0	3.5	2.5	2.0	3.5	3.5	4.0	3.0	4.0	0
PI 295065	4.5	3.5	2.5	2.0	2.5	3.0	2.5	3.0	3.5	2.0	2.5	2.0	0
PI 297830	4.0	4.0	3.5	4.0	4.0	3.5	4.0	4.0	4.0	3.5	4.5	2.5	0
PI 298543	5.0	4.5	4.0	5.0	5.0	4.0	3.5	4.0	4.0	3.0	3.0	2.0	0
PI 298548	4.5	4.5	3.5	3.0	4.0	4.0	4.0	3.5	3.5	3.5	2.5	3.0	0
PI 306536	4.5	3.0	3.0	4.0	2.5	3.0	3.0	3.0	2.5	NA	NA	NA	0
PI 330544	4.0	3.0	3.0	5.0	3.0	3.5	3.5	4.0	3.0	3.0	2.5	2.5	0
PI 349045	2.0	4.0	3.0	3.0	4.0	2.0	4.0	3.5	3.5	5.0	3.5	3.5	0
PI 350001	2.5	3.0	1.5	2.0	3.0	3.0	2.0	3.0	3.0	2.0	2.0	2.0	0
PI 352335	1.5	3.0	3.0	2.0	3.0	2.0	3.5	3.0	4.0	5.0	3.0	3.0	0
PI 352337	NA	2.5	3.5	2.5	3.5	1.5	3.0	4.5	3.0	3.0	2.5	4.0	0
PI 352338	4.5	4.0	3.0	3.5	2.5	3.0	3.0	4.0	4.0	3.5	3.0	4.5	0

Table 5.1. Disease reactions of 180 emmer wheat accessions to the four *P. tritici-repentis* races (Continued).

Accession	Race 1			Race 2			Race 3			Race 5			Ptr ToxA
	Rep 1[c]	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	
PI 352341	4.5	3.0	3.5	3.0	4.5	2.0	3.0	4.0	3.5	3.0	5.0	4.5	0
PI 352342	4.0	3.0	3.0	3.5	3.0	2.5	4.0	4.0	3.5	3.0	3.0	NA	0
PI 352358	3.5	4.5	3.0	2.5	3.5	3.0	2.5	2.5	3.0	3.0	2.5	3.5	0
PI 352365	4.0	4.0	3.0	2.5	2.5	3.0	3.5	3.5	3.0	4.0	3.0	4.5	0
PI 355460	3.5	3.5	3.0	4.0	2.5	3.0	3.0	3.0	3.0	2.5	1.5	3.5	0
PI 355461	3.5	3.5	2.5	5.0	4.5	3.5	2.5	2.5	2.5	2.5	2.5	4.5	3
PI 355470[b]	NA	NA	NA	4.0	5.0	4.5	4.0	4.0	4.0	5.0	4.0	5.0	0
PI 355475	2.0	2.5	1.0	2.5	2.0	1.5	3.0	3.5	2.5	2.0	2.0	2.5	0
PI 355483	4.5	3.5	2.0	2.5	4.0	3.0	3.0	3.5	2.5	NA	NA	NA	0
PI 355485	2.5	2.5	2.0	1.0	2.5	3.5	NA	3.5	NA	NA	NA	NA	0
PI 355486	4.5	2.5	3.0	3.0	2.5	3.0	3.5	3.0	2.0	2.0	2.0	3.0	0
PI 355489	4.0	3.5	3.0	NA	NA	NA	3.5	3.0	3.0	3.0	2.5	3.5	0
PI 355497	4.0	NA	2.0	2.0	3.0	2.5	2.5	3.5	2.0	3.0	2.0	1.5	0
PI 355505	NA	NA	NA	3.0	1.0	1.5	2.0	2.5	2.0	4.0	1.5	2.5	0
PI 361833	4.0	5.0	4.0	5.0	4.5	4.5	2.5	3.5	3.0	3.0	3.5	3.5	0
PI 362438	3.5	5.0	3.0	4.0	2.5	1.5	2.0	1.5	2.0	1.0	1.0	2.0	0
PI 362500	2.5	4.0	2.0	4.0	3.0	3.0	2.0	3.0	3.0	3.0	2.0	2.5	0
PI 362697	3.5	3.5	2.0	4.0	2.5	1.5	2.5	3.0	4.5	2.5	1.5	2.5	0
PI 374685	3.5	3.5	2.5	5.0	3.5	4.0	2.0	3.0	2.5	3.5	3.0	4.0	0
PI 377650	4.0	3.5	NA	4.0	3.0	3.0	2.5	NA	2.0	2.0	NA	3.5	0
PI 377672[a]	3.5	NA	1.5	2.0	2.0	2.0	2.0	2.0	NA	2.5	1.0	2.5	0
PI 384297	3.5	3.0	2.0	2.5	4.0	3.0	4.0	4.0	3.5	4.0	2.5	3.5	0
PI 384302	4.0	3.0	3.0	1.0	1.0	1.0	3.5	4.0	3.5	3.0	3.0	2.0	0
PI 384318	3.5	4.5	4.0	3.0	4.0	2.0	4.0	3.0	3.5	4.0	3.5	3.5	0
PI 384331	2.0	3.0	2.5	2.0	2.0	1.0	2.5	3.5	3.0	3.0	3.0	2.0	0
PI 434996	4.0	2.5	2.5	2.5	1.5	1.0	2.5	3.5	2.5	2.0	2.5	2.5	0
PI 470737	4.0	NA	3.0	5.0	3.5	3.0	4.0	5.0	4.0	5.0	4.0	4.5	0
PI 479957	NA	NA	NA	2.0	3.5	2.5	4.0	4.5	3.0	3.0	2.5	2.5	0
PI 479965	2.5	4.5	3.5	5.0	4.5	4.0	2.5	3.5	3.5	4.0	3.5	3.0	0

Table 5.1. Disease reactions of 180 emmer wheat accessions to the four *P. tritici-repentis* races (Continued).

Accession	Race 1			Race 2			Race 3			Race 5			Ptr ToxA
	Rep 1[c]	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	
PI 480307	4.0	5.0	3.0	3.0	5.0	3.0	3.0	3.0	3.5	4.0	4.0	3.5	0
PI 480312	3.5	3.5	3.0	5.0	3.0	3.5	NA	3.0	3.5	3.5	3.0	2.0	0
PI 480313[b]	4.0	4.5	4.5	4.5	4.0	3.5	4.5	3.5	3.5	4.5	4.0	3.5	0
PI 480461	2.5	3.5	3.0	4.0	4.0	4.0	3.0	3.0	3.0	4.0	3.5	2.5	0
PI 480462	3.5	3.5	3.5	4.0	4.5	4.0	4.0	4.5	3.5	4.0	4.0	2.5	0
PI 532304	3.0	2.5	3.0	4.0	2.5	2.5	2.0	3.0	2.0	2.5	3.5	2.0	0

[a]Resistant accessions to all four races. [b]Susceptible accessions to all four races. [c]Rep, Replication. [d]NA, Not available.

The analysis of variances (ANOVA) of the reactions in three replicates for each race revealed that the disease reactions of different accessions were significantly different ($p < 0.0001$) for all four races (Table 5.5). Based on the ANOVAs, the broad-sense heritability (H^2) of the tan spot resistance at the seedling stage was estimated as 0.9277, 0.9463, 0.8902, and 0.8794 for race 1 (isolate Pti2), 2 (86-124), 3 (331-9), and 5 (DW5), respectively.

The Bartlett's test of homogeneity showed that the three replicates were homogeneous for all the four races, with p values equal to 0.3001, 0.2646, 0.1089, and 0.2880 for the race 1, 2, 3, and 5 respectively. The Spearman correlation analysis showed that the reactions in three replicates were highly correlated with each other ($p < 0.0001$) for all four races. Therefore, the three replicates for each race could be pooled together for further analysis. Among 180 emmer accessions, 22, 65, 15, and 29 were highly or moderately resistant (lesion type <=2.5) to race 1, 2, 3, and 5, respectively, based on their average reactions (Figure 5.2). Across all four races, six accessions, including CItr 14133-1, PI 94634-1, PI 94676-1, PI 94617, PI 190920, and PI 377672, were highly resistant to all four races; while 17 accessions, such as PI 94635-1, were highly susceptible to all four races (Table 5.1). The disease reactions of the resistant accessions, CItr 14133-1 and PI 94634-1, and the susceptible accession PI 94635-1 are shown in Figure 5.1. Spearman correlation analysis indicated that the average reactions to four races were highly correlated with each other ($p < 0.005$) (Table 5.6). The largest and smallest correlation coefficients occurred between the reactions to race 1 and race 2, and between the reactions to race 3 and race 2, respectively.

For reactions to Ptr ToxA, only four of 180 emmer accessions were sensitive and most of them (98%) were insensitive. The insensitivity to Ptr ToxA may be the reason that the isolate 86-

124 (race 2) was the most avirulent isolate than the others because the isolate 86-124 was known to produce Ptr ToxA.

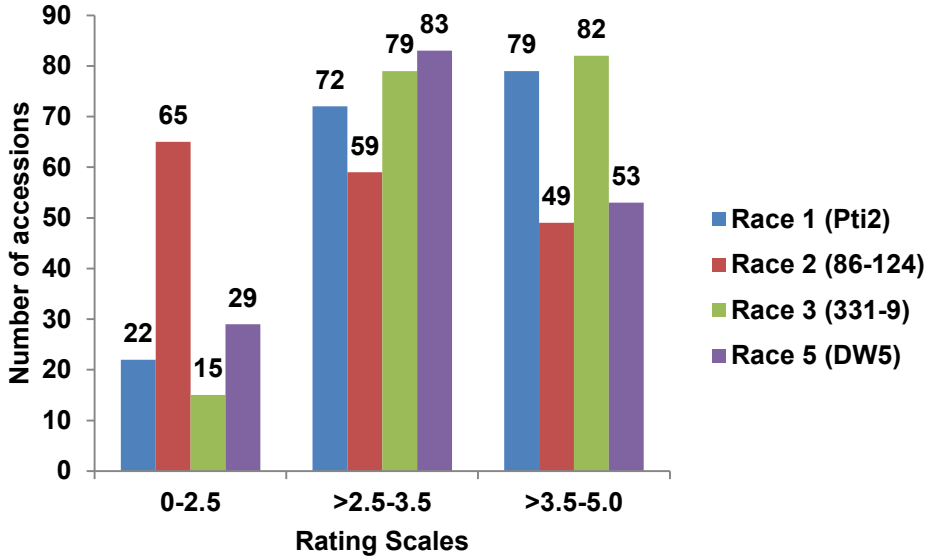

Figure 5.2. Distribution of cultivated emmer wheat accessions in each category of disease reaction based on their reactions to the fungal inoculation with four *P. tritici-repentis* races (isolates). The reaction type of 0-2.5 was considered as resistant, >2.5-3.5 as intermediate and >3.5-5.0 as susceptible. The number of accessions to each column was indicated on the top of them.

Association Analysis

To analyze four phenotypes for four races, a best linear model was first chosen for each of the 4 phenotypes based on MSD values (Table 5.7) and Q-Q plot (Figure 5.3) of eight different models. For the reactions to races 1 and 2, the best model was the MLM model "PC-BIC+K" and "K", both of which had the same MSD values because zero PC was suggested for adjusting population structure in model "PC-BIC+K". For the reactions to races 3 and 5, the best models were the GLM model 'PC50' and the MLM model 'PC25+K', respectively.

At the significant level of 0.1 percentile, the cutoff *p* values were estimated at 4.08×10^{-4}, 4.08×10^{-5}, 1.14×10^{-4}, and 8.00×10^{-5} for Ptr races 1, 2, 3, and 5, respectively. At the significant level of 1 percentile, the cutoff *p* values were estimated at 2.60×10^{-3}, 1.14×10^{-3}, 1.81×10^{-3}, and 1.35×10^{-3} for the four Ptr races, respectively. The SNP with a *p* value smaller than the cutoff *p* value identified by the best fitted model for each race was considered to be significantly associated to that race resistance. A total of 146 significant markers (1 percentile) were detected, 127, 16, and 3 of which associated with resistance to one, two, and three Ptr races, respectively (Table 5.10). Three significant markers *IWA4942*, *IWA4995*, and *IWA544* each were mapped to two different postions on two different chromosomes on 9K consensus map. Six significant markers, including *IWA793*, *IWA205*, *IWA716*, *IWA4616*, *IWA4617* and *IWA290*, had not been mapped (Table 5.10). The remaing markers were located on 14 A- and B-genome chromsomes. Their genomic positions and significant levels were shown in the Manhattan plots (Figure 5.4). Among the 146 signifcant markers, 43, 42, 42, and 41 associated with the resistance to races 1, 2, 3, and 5, respectively. Stepwise regression analysis showed that 3, 5, 4, and 5 markers had major effects on the seedling resistance to races 1, 2, 3, and 5, respectively, and they explained phenotypic variation ranging from 21.55% (race 5) to 39.23% (race 2) (Table 5.8).

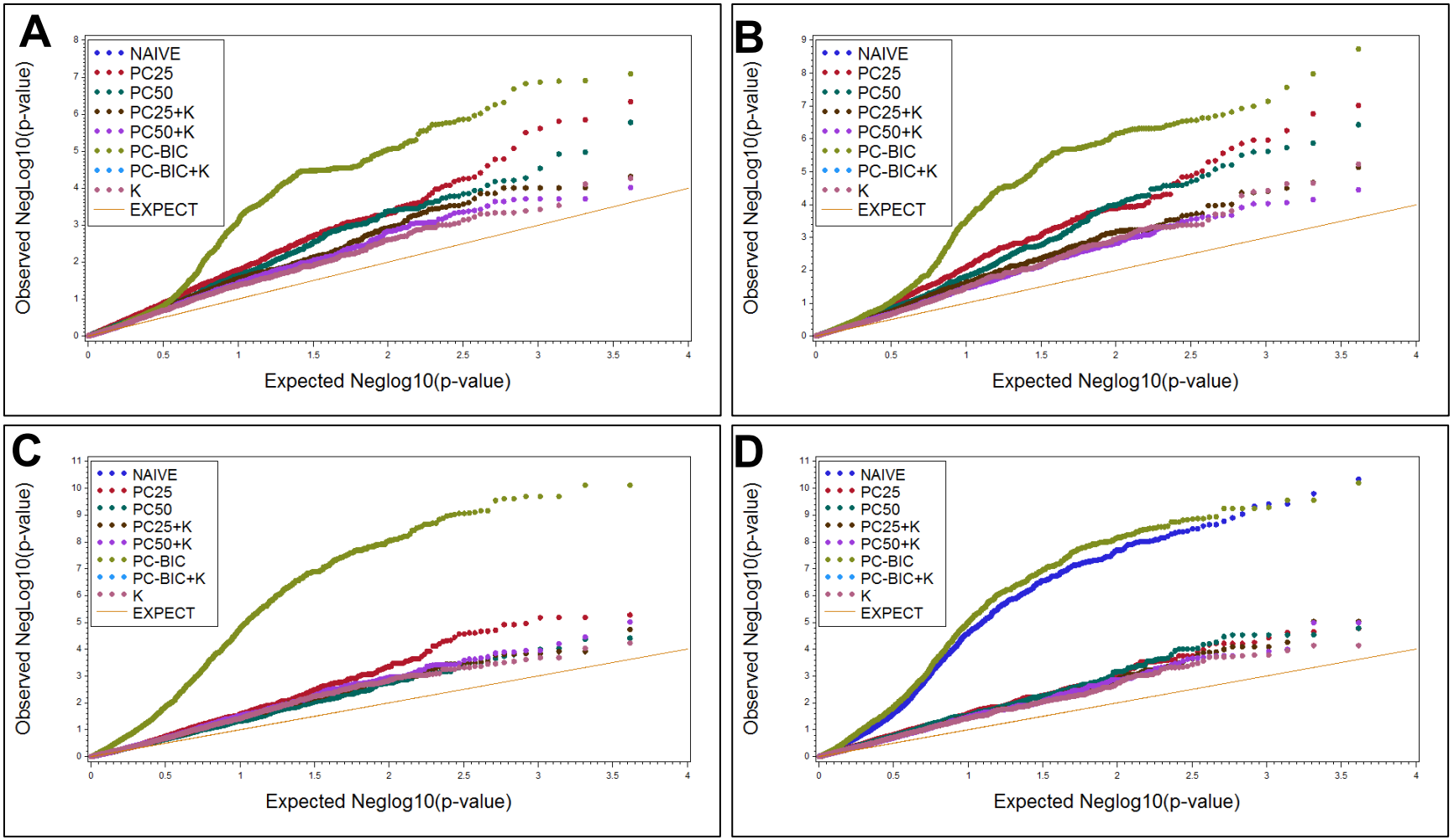

Figure 5.3. Comparison of Quantile-Quantile (Q-Q) plots for the different eight GWAS models for each of four *P. tritici-repentis* races. Figure A, B, C, and D represented the comparisons of models for race 1 (isolate Pti2), 2 (86-124), 3 (331-9), and 5 (DW5), respectively. In each figure, the X axis is the expected $-\text{Log}_{10}(p \text{ value})$, and the Y axis is the observed $-\text{Log}_{10}(p \text{ value})$. One naïve model and seven models with different methods of adjusting population structures were compared in each figure.

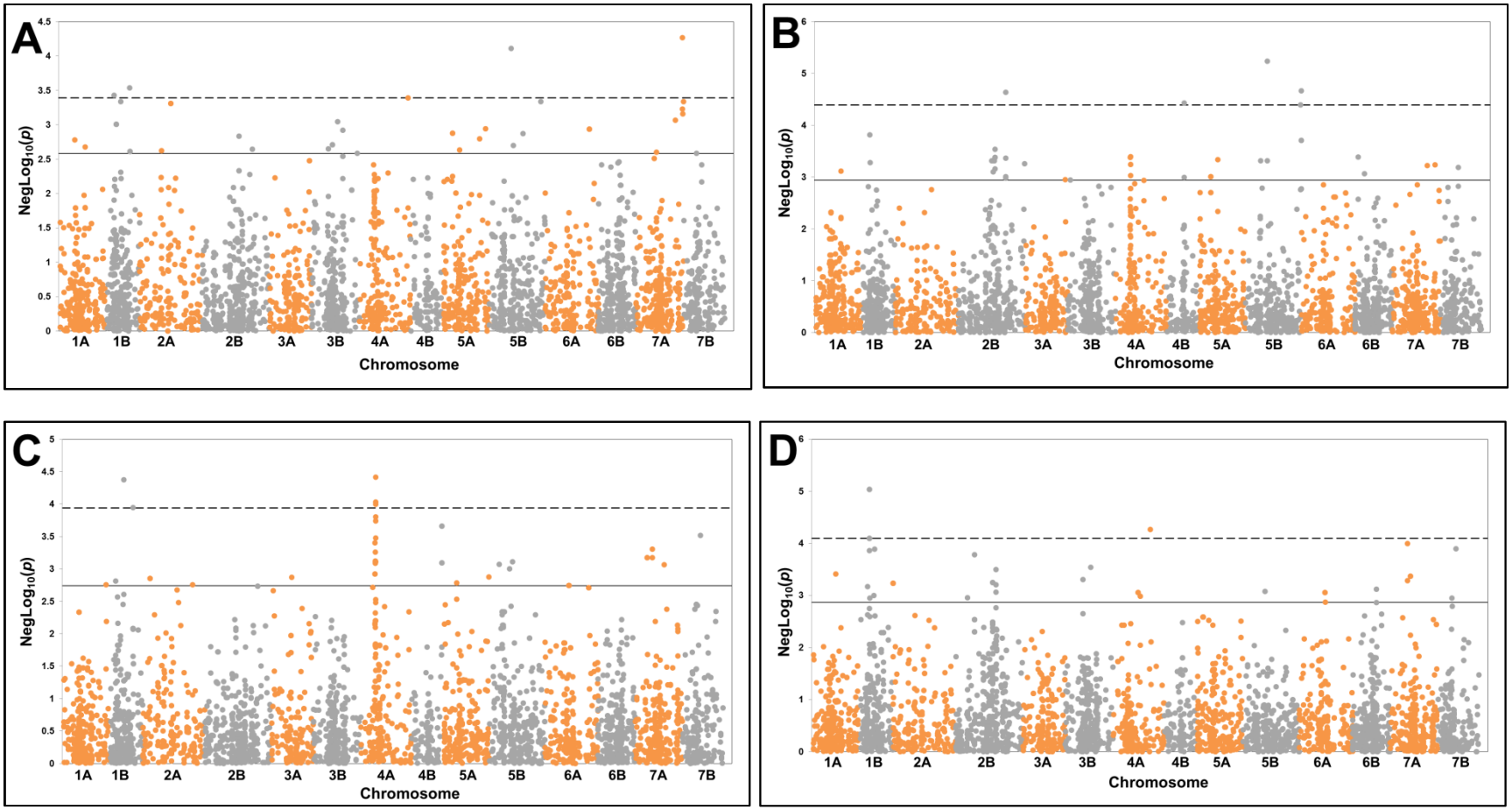

Figure 5.4. Manhattan Plots of the best models for each marker-trait association analysis of four *P. tritici-repentis* races. Figure A, B, C, and D represented the plots for races 1 (isolate Pti2), 2 (86-124), 3 (331-9), and 5 (DW5), respectively. The *x* axis shows the genetic positions of SNPs along each chromosome and y axis shows the negative $\log_{10}(p\text{-value})$ of associations. In each figure, the black solid and dashed horizontal lines indicate the significant levels of 1 percentile and 0.1 percentile, respectively. The dots above different horizontal lines represent the identified significant markers associated with disease resistance at different significant levels.

At the significant level of 0.1 percentile, five, five, five, and seven SNPs were significantly associated with resistance to Ptr races 1, 2, 3, and 5, respectively. The phenotypic variation (R^2) explained by each marker ranged from 1.19% to 16.96% (Table 5.2). For most of those 20 significant markers, each was associated with resistance to one single race except for two markers, each of which was associated with resistace to two races. Among the 20 significant markers, nine were at or near to genomic regions containing known tan spot resistance genes or QTL on chromosomes 1B (*QTs.fcu-1BS*) and 5B (*tsn1*, *QTs.fcu-5BL.1*, and *QTs.ksu-5BL*) (Table 5.23). The remaining 11 significant markers on chromosomes 1B, 2B, 4A, 4B, 5B, and 7A did not cover the genomic regions with known tan spot resistance genes or QTL based on the three genetic maps that were used. The genomic regions with those markers likely harbor novel tan spot resistance genes.

Eight LD blocks with significant SNPs (0.1 percentile) were identified on six chromosomes (Table 5.3). Among the eight LD blocks, two had their markers at the same map position and six had markers at different positions with a length of the block ranging from 0.15 cM to 6.73 cM. Based on the mapping positions of the significant markers in LD blocks, two blocks were found to be at or close to genomic regions containing previously identified QTL or tan spot resistance genes (Table 5.3). Seven LD blocks each associated with one race resistance, and the remaining sixth LD blocks on chromosome 5B associated with resistance to both races 1 and 2 (Table 5.3). Based on the result of stepwise regression analysis, two LD blocks harboring significant markers with major effects were putative major QTL, and the remaining six LD blocks were putative minor QTL. One of the two LD blocks with major effects were near to known tan spot resistance genes or QTL, and another one was at the genomic region without known resistance genes and QTL.

The fourth LD block (Table 5.3) was the largest LD block with a length of 6.73 cM, and it harbored the thrid LD block based on their genomic locations. Because the two SNPs *IWA3792* and *IWA5498* in third LD block had low LD values ($r^2 < 0.7$) with the markers in fourth LD block, these two different LD blocks appear to be located in the same genomic region. It was noticed that this genomic region harbored all the significant markers on 4A associated with race 3 resistance. This genomic region in the Manhattan plot for race 3 is enlarged in Figure 5.5B. An LD heatmap showed the levels of LD values among all the significant and non-significant markers in this region (Figure 5.5C). Because the fourth LD block may be a QTL with resistance to races 3, and no known QTL or genes were previously identified in this region, the fourth LD block could harbor a novel resistance gene to tan spot.

Stepwise regression analysis could find the significant markers with major effects and remove the significant markers with small or redundant effects. Based on the number of markers fitted into the stepwise regression, 8 to 13 allelic combinations of the significant markers were identified with major effects on all four phenotypes (Table 5.9). The average reaction of the accessions with specific allelic combination could be used for inferring the resistant or susceptible reactions of an accession (Table 5.9). For all the four phenotypes, both resistant and susceptible allelic combinations were present in the population. The allelic combinations of significant markers associated with resistance could be used for marker-assisted selection in breeding programs.

Table 5.2. Significant markers associated with tan spot resistance at the significant level of 0.1 percentile.

Race	SNP	Chr[a]	Position (cM)	$NegLog_{10}$ (p)	MAF[b]	R^2 (%)	SRI[c]	Mean for allele A	Mean for allele B	Mean difference	Additive effects	Near gene/QTL	Ref[d]
1	*IWA4806*	1B	37.19	3.43	0.30	10.77		3.87	3.26	0.61	0.31		
1	*IWA7619*	1B	101.09	3.54	0.08	3.11		4.14	3.38	0.76	0.38	*QTs.fcu-1BS*	1
1	*IWA1836*	4A	197.23	3.39	0.21	1.19		3.61	3.40	0.21	0.11		
1	*IWA4533*	5B	88.45	4.11	0.15	6.24		2.72	3.57	0.85	-0.43	*QTs.fcu-5BL.1; QTs.ksu-5BL; tsn1*	2, 3
1	*IWA6736*	7A	188.95	4.27	0.29	6.87		3.10	3.58	0.49	-0.24		
2	*IWA1708*	2B	192.19	4.63	0.18	14.34		3.11	2.07	1.04	0.52		
2	*IWA3287*	4B	71.64	4.43	0.16	16.55		2.75	3.82	1.07	-0.54		
2	*IWA4533*	5B	88.45	5.23	0.14	14.76	Yes	1.87	3.10	1.23	-0.62	*QTs.fcu-5BL.1; QTs.ksu-5BL; tsn1*	2, 3
2	*IWA2099*	5B	223.94	4.39	0.23	16.96	Yes	3.64	2.71	0.92	0.46		
2	*IWA6579*	5B	226.50	4.66	0.26	16.95		3.59	2.70	0.88	0.44		
3	*IWA5076*	1B	64.07	4.37	0.31	1.45		3.30	3.54	0.24	-0.12	*QTs.fcu-1BS*	1
3	*IWA7619*	1B	101.09	3.94	0.09	5.54	Yes	4.03	3.41	0.62	0.31	*QTs.fcu-1BS*	1
3	*IWA3792*	4A	61.12	4.42	0.20	3.29		3.41	3.70	0.29	-0.14		
3	*IWA1060*	4A	61.63	4.03	0.49	4.51		3.29	3.65	0.36	-0.18		
3	*IWA2334*	4A	61.63	4.00	0.49	4.64		3.29	3.66	0.37	-0.18		
5	*IWA6063*	1B	46.24	5.04	0.37	1.36		3.19	3.37	0.18	-0.09	*QTs.fcu-1BS*	1
5	*IWA7119*	1B	46.24	5.04	0.37	1.36		3.37	3.19	0.18	0.09	*QTs.fcu-1BS*	1
5	*IWA2222*	1B	46.24	4.10	0.33	1.67		3.19	3.38	0.19	-0.09	*QTs.fcu-1BS*	1
5	*IWA4141*	1B	46.24	4.10	0.33	1.67		3.19	3.38	0.19	-0.09	*QTs.fcu-1BS*	1
5	*IWA805*	1B	46.24	4.10	0.33	1.67		3.38	3.19	0.19	0.09	*QTs.fcu-1BS*	1
5	*IWA8047*	1B	46.24	4.10	0.33	1.67		3.38	3.19	0.19	0.09	*QTs.fcu-1BS*	1
5	*IWA8475*	4A	151.81	4.27	0.43	2.60	Yes	3.09	3.47	0.38	-0.19		

[a]Chr, Chromosome; [b]MAF, Minor allele frequency; [c]SRI, Stepwise regression included.
[d]Ref, References: 1, Faris and Friesen, 2005; 2, Chu et al., 2008b; 3, Singh et al., 2007.
[e]NA, Not available.

Table 5.3. Linkage disequilirium (LD) blocks including one or several significant markers (0.1 percentile) associated with resistances to tan spot.

LD block	Chr[a]	Pos[b](cM)	SNP	Extented length(cM)[c]	Associated race	Near gene/QTL	Ref[d]
1	1B	45.38	*IWA4849*	0.87	5	*QTs.fcu-1BS*	1
		46.24	*IWA805**, *IWA2222**, *IWA2667*, *IWA2668*, *IWA4141**, *IWA6063**, *IWA7119**, *IWA8047**				
2	2B	192.19	*IWA1708**, *IWA6054*, *IWA7615*	0	2		
3	4A	61.12	*IWA3792**, *IWA5498*	0	3		
4	4A	61.63	*IWA112*, *IWA1060**, *IWA2334**, *IWA4079*, *IWA4513*, *IWA6944*, *IWA7522*	6.73	3		
		61.12	*IWA3582*, *IWA4771*, *IWA4772*, *IWA7617*				
		60.68	*IWA3311*				
		60.24	*IWA5729*				
		59.81	*IWA1824*, *IWA2000*, *IWA3361*, *IWA6540*, *IWA6597*, *IWA7134*, *IWA7657*, *IWA7859*, *IWA8414*				
		58.92	*IWA7092*				
		58.56	*IWA115*, *IWA5309*				
		57.68	*IWA109*				
		57.24	*IWA5652*, *IWA5851*, *IWA8220*				
		56.80	*IWA6873*				
		54.89	*IWA6392*				
5	4B	70.55	*IWA1641*	1.09	2		
		71.35	*IWA7461*				
		71.64	*IWA3287**				
6	5B	88.30	*IWA7944*	0.15	1, 2	*tsn1; QTs.fcu-5BL.1; QTs.ksu-5BL*	2, 3
		88.45	***IWA4533****				
7	5B	223.94	***IWA2099****, *IWA2100*	2.56	2		
		226.50	*IWA6577*, *IWA6579**, *IWA6580*				
8	7A	188.95	*IWA6736**, *IWA7706*	4.22	1		
		189.53	*IWA7904*				
		193.18	*IWA5797*				

*Significant markers. The bold significant markers are stepwise regression model included markers.
[a]Chr, Chromosome. [b]Pos, Positions. [c]0 means that the SNPs in this LD block are at the same position. [d]Ref, Reference: 1, Faris and Friesen, 2005; 2, Chu et al., 2008b; 3, Singh et al., 2007.

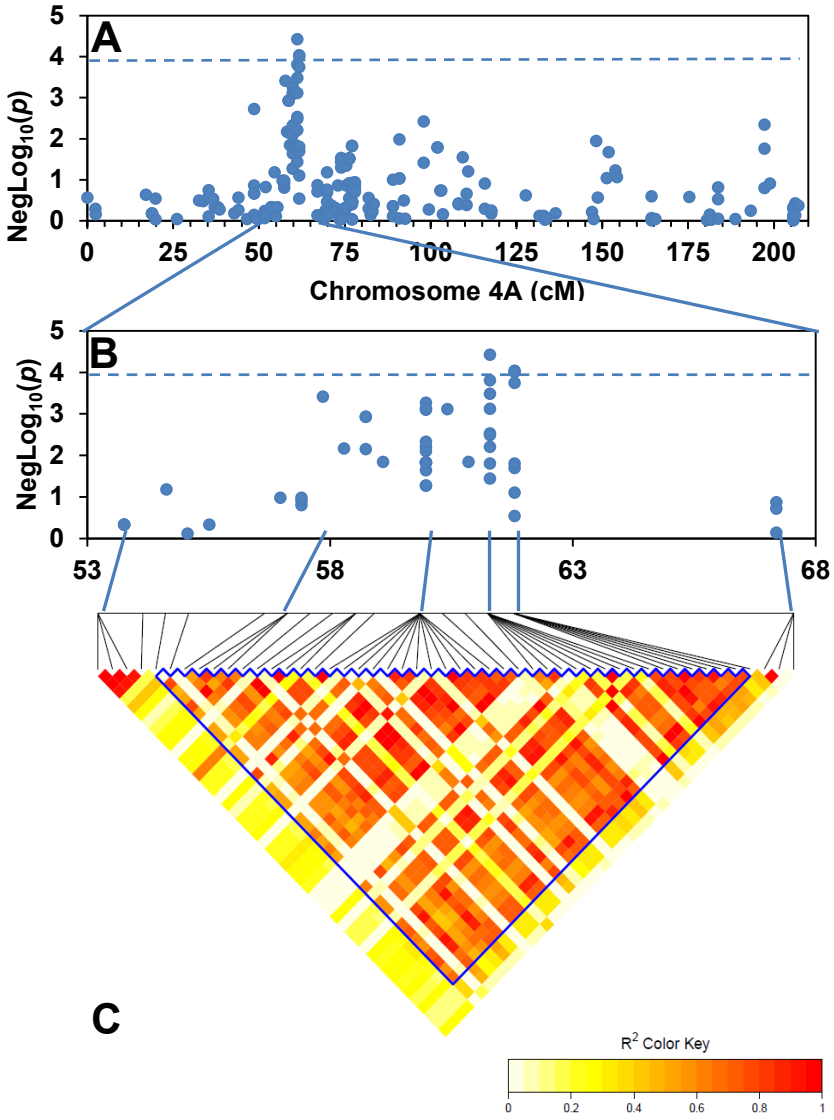

Figure 5.5. The fourth linkage disequilibrium (LD) block harboring several significant markers associated with the resistance to *P. tritici-repentis* race 3 (isolate 331-9) on chromosome 4A. The Manhattan plots in Figure A and B indicated the genetic position of this LD block. The dashed horizontal lines in the Manhattan plots indicated the threshold for significant associations between SNPs and race 3 resistance at the significant level of 0.1 percentile. The LD heatmap in Figure C indicated the pairwise LD values among the markers in the genomic region between 53 cM and 68 cM of chromosome 4A. The color of each spot in the LD heatmap was produced based on the LD value (r^2) between two markers, and the color key was also given at the bottom. In the LD heatmap the triangle region enclosed with a blue line indicated the region harboring the sixth LD block.

Discussion

In the primary gene pool of cultivated wheat, cultivated emmer is a good source of *R* genes for resistance to several important wheat diseases such as stem rust, leaf rust, and powdery mildew (see review by Harris et al., 2014). Although many cultivated emmer accessions were previously identified to be resistant to tan spot (Lamari and Bernier, 1989a; Singh et al., 2006a; Chu et al., 2008a), no tan spot resistance genes were previously reported from cultivated emmer. In this study, attempting to identify the genomic regions associated with the tan spot resistance in cultivated emmer was done through association analysis. In the panel of 180 cultivated emmer accessions evaluated in this study, 22, 65, 15, and 29 accessions had high or moderate resistance to Ptr races 1, 2, 3, and 5, respectively.

The association analysis showed that 146 significant markers were associated with the resistance. Based on the genomic positions of 20 significant markers at the significant level of 0.1 percentile, nine significant markers were found, as well as two LD blocks, located at the same or near to the genomic regions with known tan spot resistance genes or QTL, including *tsn1*, *QTs.fcu-1BS*, *QTs.fcu-5BL.1*, and *QTs.ksu-5BL*. None of previously identified genes and QTL related to tan spot resistance was derived from cultivated emmer wheat. However, Anderson et al. (1999) reported that the common wheat cultivar Hope harbored *Tsn1*. Hope was generated from the cross between emmer cultivar Yaroslav and common wheat variety Marquis (McFadden, 1930). Thus, *Tsn1* in Hope may be derived from Yaroslav emmer. The dominant allele of *Tsn1* confers wheat susceptibility to tan spot through indirectly interacting with Ptr ToxA produced by Ptr races 1 and 2 (Faris et al., 2010). In this study, the significant marker *IWA4533* on 5B was associated with races 1 and 2 resistance located near to this gene. Based on the fact that most emmer accessions in this study were insensitive to Ptr ToxA, the genomic

region with *IWA4533* is thought to carry the recessive allele (*tsn1*) of the *Tsn1* locus or any unknown tan spot resistance genes that are closely linked to *Tsn1*.

Except for the nine SNPs associated with genomic regions with known genes or QTL, the remaining 11 SNPs at the significant level of 0.1 percentile located in the genomic regions where no known tan spot resistance genes were identified on chromosomes 1B, 2B, 4A, 4B, 5B, and 7A. Based on the tetraploid consensus map developed by Maccaferri et al. (2014), it was found that most of these regions on 1BS near SSR marker *barc240*, 2BL near SSR marker *wmc592* and *barc18*, 4AS between the SSR markers *gwm1093* and *gwm165*, 4AL near the SSR markers *gwm959*, and 4BL near SSR marker *gwm251* were also identified to associate with tan spot resistance in another association mapping study on Ptr races 1 and 5 resistance in European winter wheat by Kollers et al. (2014). These genomic regions most likely harbor the novel tan spot resistance genes. The cultivated emmer accessions with high levels of resistance and favorable alleles in the marker loci at these regions will be useful materials for identification of new tan spot resistance genes through linkage mapping analysis using bi-parental mapping population.

In summary, the GWAS results from this study provide preliminary evidence towards the genomic regions associated with tan spot resistance in cultivated emmer wheat. This information is useful for identifying the tan spot resistance genes based on linkage analysis using bi-parental mapping populations. The identified LD blocks, especially for the LD blocks with major effects, will be the candidate regions for resistance genes or QTL. The allelic combinations of significant markers selected by stepwise regression could be used for identifying wheat genotypes with tan spot resistance genes located in targeted genomic regions. Therefore, this genome-wide

association study provides the first step towards identifying novel tan spot resistance genes in emmer wheat and pyramiding resistance loci from emmer wheat for MAS breeding.

Supplementary Tables

Table 5.4. The information on the four *P. tritici-repentis* isolates used in this experiment with their produced host-selective toxins (HSTs) and symptoms on host differentials for each race.

Isolate	Race	HSTs produced	Symptoms on host differentials			
			Salamouni	Glenlea	6B662	6B365
Pti2	1	Ptr ToxA Ptr ToxC	-	Necrosis	-	Chlorosis
86-124	2	Ptr ToxA	-	Necrosis	-	-
331-9	3	Ptr ToxC	-	-	-	Chlorosis
DW5	5	Ptr ToxB	-	-	Chlorosis	-

Source: Faris et al., 2013.

Table 5.5. The analysis of variances of the disease reactions of 180 emmer wheat accessions to each of the four *P. tritici-repentis* races.

Race	Source	DF[a]	Sum of squares	Mean square	F value	Pr > F
1	Accession	172	250.88	1.46	4.27	<.0001
	Replication	2	23.78	11.89	34.82	<.0001
	Error	330	112.68	0.34		
	Corrected Total	504	387.33			
2	Accession	172	442.15	2.57	5.87	<.0001
	Replication	2	24.40	12.20	27.88	<.0001
	Error	334	146.18	0.44		
	Corrected Total	508	612.73			
3	Accession	175	185.82	1.06	2.70	<.0001
	Replication	2	7.50	3.75	9.53	<.0001
	Error	331	130.25	0.39		
	Corrected Total	508	323.57			
5	Accession	171	230.61	1.35	2.43	<.0001
	Replication	2	14.65	7.32	13.20	<.0001
	Error	312	173.10	0.55		
	Corrected Total	485	418.36			

[a]DF, Degree of freedom.

Table 5.6. Spearman correlation coefficients (lower diagonal) and the *p*-values (upper diagonal) among the disease reactions to the four *P. tritici-repentis* races.

Race	1	2	3	5
1		<.0001	<.0001	<.0001
2	0.69078		0.0014	<.0001
3	0.42982	0.24521		<.0001
5	0.40351	0.40426	0.58306	

Table 5.7. Mean square difference (MSD) values of eight different models used to identify SNP-tan spot resistance associations for each of the four *P. tritici-repentis* races.

Model	Race 1	Race 2	Race 3	Race 5
Naïve	1.39E-02	2.83E-02	7.95E-02	6.64E-02
PC25	2.30E-02	2.13E-02	8.05E-03	1.82E-02
PC50	1.26E-02	1.63E-02	4.95E-03	1.31E-02
PC25+K	1.00E-02	1.20E-02	6.52E-03	8.74E-03
PC50+K	6.52E-03	9.00E-03	7.18E-03	8.81E-03
PC-BIC	1.39E-02	2.83E-02	7.95E-02	8.15E-02
PC-BIC+K	5.96E-03	6.49E-03	6.82E-03	9.24E-03
K	5.96E-03	6.49E-03	6.82E-03	9.24E-03

Table 5.8. Number of significant markers (1 percentile) included into stepwise regression model for each *P. tritici-repentis* race and their explained phenotypic variations together.

Race	No. of markers		Phenotypic variation (%)
	Significant	Included	
1	43	3	23.74
2	42	5	39.23
3	42	4	22.79
5	41	5	21.55

Table 5.9. Allelic combinations of stepwise included significant markers for each *P. tritici-repentis* race and their sample statistical analysis of phenotypic data.

Race	Allelic combination[a]	No. of obs[b]	Mean (Lesion type)	Std dev[c]	Min[d]	Max[e]	Reaction[f]
1	BBB	2	1.42	0.59	1.00	1.83	R
1	BAB	5	2.60	0.28	2.17	2.83	I
1	ABB	15	2.76	0.79	1.17	4.33	I
1	BBA	53	3.31	0.54	2.00	4.33	I
1	ABA	30	3.34	0.55	2.33	4.33	I
1	BAA	15	3.81	0.53	2.83	4.50	S
1	AAA	49	3.91	0.52	2.67	4.67	S
1	AAB	2	3.92	0.59	3.50	4.33	S
2	AABBB	1	1.00	.	1.00	1.00	R
2	ABAAB	2	1.13	0.53	0.75	1.50	R
2	BBBAA	1	1.83	.	1.83	1.83	R
2	ABABB	21	1.98	0.46	1.00	3.00	R
2	BBBBA	8	2.43	0.46	1.83	3.25	R
2	BBBAB	42	2.50	0.82	1.00	4.33	R
2	BABBA	1	2.83	.	2.83	2.83	I
2	BABAB	3	3.19	1.17	2.00	4.33	I
2	BBBBB	56	3.24	0.73	1.67	4.67	I
2	BABBB	35	3.77	0.57	2.50	4.83	S
2	BBAAB	1	3.83	.	3.83	3.83	S
3	BBAB	1	2.00	.	2.00	2.00	R
3	ABAA	1	2.33	.	2.33	2.33	R
3	BBAA	13	2.55	0.65	1.00	3.33	I
3	BBBB	17	2.93	0.43	2.17	4.17	I
3	BAAA	4	3.04	0.44	2.67	3.67	I
3	BBBA	115	3.56	0.52	1.67	4.67	S
3	AAAA	6	3.78	0.51	3.00	4.50	S
3	BABA	9	3.98	0.61	3.00	4.67	S
3	AABA	6	4.39	0.25	4.00	4.67	S
3	ABBA	2	4.58	0.35	4.33	4.83	S
5	AAABA	2	1.25	0.35	1.00	1.50	R
5	AABBB	1	2.17	.	2.17	2.17	R
5	AAAAB	8	2.46	0.40	1.83	3.00	R
5	AABAB	33	2.85	0.67	1.33	4.50	I
5	ABABA	21	2.87	0.58	1.83	4.00	I
5	AAABB	2	3.17	0.71	2.67	3.67	I
5	BAAAB	2	3.25	0.35	3.00	3.50	I
5	ABABB	52	3.28	0.47	2.17	4.33	I
5	ABAAB	6	3.61	0.76	2.50	4.67	S
5	ABBBB	1	3.83	.	3.83	3.83	S

Table 5.9. Allelic combinations of stepwise included significant markers for each *P. tritici-repentis* race and their sample statistical analysis of phenotypic data (Continued).

Race	Allelic combination[a]	No. of obs[b]	Mean (Lesion type)	Std dev[c]	Min[d]	Max[e]	Reaction[f]
5	BABAB	23	4.02	0.35	3.50	4.67	S
5	ABBAB	10	4.08	0.64	3.00	4.67	S
5	BBBAB	2	4.83	0.24	4.67	5.00	S

[a]A and B refer to the alleles in the 9K SNP wheat chip.
Orders of SNPs associated with race 1: *IWA4237, IWA290, IWA544*; Orders of SNPs associated with race 2: *IWA4533, IWA2099, IWA7312, IWA4124, IWA2888*; Orders of SNPs associated with race 3: *IWA7619, IWA1408, IWA5462, IWA7504*; Orders of SNPs associated with race 5: *IWA2106, IWA763, IWA8475, IWA4942, IWA4533*.
[b]Number of observations; [c]Standard deviation; [d]Min, minimum; [e]Max, Maximum; [f]R, Resistant (Lesion type <= 2.5); S, Susceptible (Lesion type > 3.5); I, Intermediate (2.5 < Lesion type <= 3.5).

Table 5.10. The *p* values of significant SNPs associated with tan spot resistance at the significant level of 1 percentile for the four Ptr races.

SNP	Chr[a]	Position	MAF[b]	Race 1	Race 2	Race 3	Race 5
IWA1991	1A	65.67	0.20	1.67E-03			
IWA2540	1A	97.55	0.31				3.89E-04
IWA3405	1A	107.73	0.42	2.11E-03	7.64E-04		
IWA1710	1A	179.03	0.49			1.76E-03	
IWA7504	1B	30.15	0.10			1.53E-03	
IWA4806	1B	37.19	0.30	**3.72E-04**[c]	1.53E-04		
IWA4402	1B	38.15	0.48				6.78E-04
IWA2222	1B	46.24	0.31	9.86E-04			**8.00E-05**
IWA4141	1B	46.24	0.31	9.86E-04			**8.00E-05**
IWA805	1B	46.24	0.31	9.86E-04			**8.00E-05**
IWA8047	1B	46.24	0.31	9.86E-04			**8.00E-05**
IWA6063	1B	46.24	0.37				**9.17E-06**
IWA7119	1B	46.24	0.37				**9.17E-06**
IWA2668	1B	46.24	0.34				1.38E-04
IWA1121	1B	47.21	0.32				1.12E-03
IWA5076	1B	64.07	0.30	4.64E-04		**4.24E-05**	1.01E-03
IWA540	1B	66.18	0.23				1.30E-04
IWA7619	1B	101.09	0.08	**2.91E-04**		**1.14E-04**	
IWA4031	1B	102.74	0.29	2.45E-03			
IWA7141	1B	102.74	0.29	2.45E-03			
IWA4995	1B/1A	38.15/67.13	0.06		5.24E-04		
IWA2425	2A	0.00	0.33				5.84E-04
IWA2426	2A	0.00	0.33				5.84E-04
IWA2427	2A	0.00	0.33				5.84E-04
IWA5462	2A	29.58	0.14			1.41E-03	
IWA7166	2A	29.58	0.14			1.41E-03	
IWA343	2A	88.27	0.40	2.39E-03			
IWA5586	2A	125.84	0.21	4.91E-04			
IWA6369	2A	125.84	0.21	4.91E-04			
IWA5978	2A	204.30	0.16			1.77E-03	
IWA5736	2B	47.22	0.20				1.11E-03
IWA763	2B	76.02	0.44				1.65E-04
IWA6438	2B	136.24	0.13		4.84E-04		
IWA7489	2B	140.04	0.13		4.84E-04		
IWA5678	2B	141.76	0.13		7.96E-04		
IWA4399	2B	145.56	0.13		4.84E-04		
IWA4880	2B	145.56	0.13		4.84E-04		
IWA310	2B	146.94	0.13		4.84E-04		
IWA7312	2B	149.36	0.15	1.46E-03	2.92E-04		
IWA5262	2B	149.36	0.15		4.16E-04		
IWA5741	2B	149.36	0.15		4.16E-04		
IWA838	2B	149.36	0.36				5.60E-04
IWA5117	2B	149.36	0.36				5.60E-04

Table 5.10. The *p* values of significant SNPs associated with tan spot resistance at the significant level of 1 percentile for the four Ptr races (Continued).

SNP	Chr[a]	Position	MAF[b]	Race 1	Race 2	Race 3	Race 5
IWA5373	2B	149.75	0.19		6.92E-04		
IWA243	2B	162.84	0.47				3.20E-04
IWA4636	2B	163.38	0.24				6.17E-04
IWA5939	2B	163.38	0.40				8.64E-04
IWA1708	2B	192.19	0.18		**2.34E-05**		
IWA6054	2B	192.19	0.19		4.31E-04		
IWA7615	2B	192.19	0.19		9.87E-04		
IWA840	2B	192.19	0.43		1.05E-03		
IWA3315	2B	270.04	0.18		5.52E-04		
IWA544	2B/2A	203.60/168.91	0.14	2.26E-03			
IWA3498	3A	83.59	0.28			1.35E-03	
IWA4259	3A	162.97	0.49		1.11E-03		
IWA758	3B	11.67	0.09		1.14E-03		
IWA6202	3B	68.71	0.23	2.25E-03			
IWA6165	3B	71.68	0.41				4.97E-04
IWA610	3B	84.55	0.49	1.96E-03			
IWA611	3B	84.55	0.49	1.96E-03			
IWA2661	3B	105.91	0.05	9.05E-04			
IWA8490	3B	127.10	0.26	1.20E-03			
IWA8203	3B	186.37	0.25	2.60E-03			
IWA4942	3B/3A	103.72/117.88	0.48				2.88E-04
IWA109	4A	57.68	0.43			3.94E-04	
IWA115	4A	58.56	0.42			1.20E-03	
IWA5309	4A	58.56	0.42			1.20E-03	
IWA7859	4A	59.81	0.46		4.18E-04	5.50E-04	
IWA8414	4A	59.81	0.44			7.53E-04	
IWA6597	4A	59.81	0.44			8.16E-04	
IWA5729	4A	60.24	0.48			7.86E-04	
IWA7617	4A	61.12	0.49		5.74E-04	3.34E-04	
IWA3582	4A	61.12	0.49		9.41E-04	7.73E-04	
IWA3792	4A	61.12	0.20			**3.84E-05**	
IWA5498	4A	61.12	0.19			1.57E-04	
IWA7522	4A	61.63	0.49		4.02E-04	1.82E-04	
IWA1060	4A	61.63	0.49			**9.31E-05**	
IWA2334	4A	61.63	0.49			**1.00E-04**	
IWA2107	4A	101.96	0.17				8.70E-04
IWA2106	4A	101.96	0.17				8.70E-04
IWA6906	4A	110.42	0.50				1.04E-03
IWA8475	4A	151.81	0.43				**5.38E-05**
IWA1836	4A	197.23	0.21	**4.08E-04**			
IWA1641	4B	70.55	0.20		1.01E-03		
IWA3287	4B	71.64	0.16		**3.72E-05**		
IWA2087	4B	124.26	0.11			2.19E-04	

Table 5.10. The *p* values of significant SNPs associated with tan spot resistance at the significant level of 1 percentile for the four Ptr races (Continued).

SNP	Chr[a]	Position	MAF[b]	Race 1	Race 2	Race 3	Race 5
IWA1798	4B	124.26	0.11			8.12E-04	
IWA4618	4B	124.54	0.11			2.19E-04	
IWA7299	4B	124.94	0.11			8.12E-04	
IWA291	5A	45.95	0.30	1.33E-03			
IWA1253	5A	45.95	0.30	1.33E-03			
IWA2548	5A	54.19	0.15		9.91E-04		
IWA5914	5A	54.19	0.15		9.91E-04		
IWA5184	5A	59.97	0.10			1.65E-03	
IWA3646	5A	75.19	0.42	2.33E-03			
IWA7529	5A	83.34	0.35		4.64E-04		
IWA4237	5A	155.73	0.44	1.60E-03			
IWA6641	5A	180.99	0.28	1.15E-03			
IWA7766	5A	192.78	0.25			1.33E-03	
IWA2610	5B	39.37	0.26			8.50E-04	
IWA7953	5B	60.72	0.13		4.84E-04		
IWA301	5B	81.32	0.26			9.91E-04	
IWA7944	5B	88.30	0.13		4.84E-04		
IWA4533	5B	88.45	0.15	**7.79E-05**	**5.87E-06**		8.36E-04
IWA1408	5B	94.46	0.14			7.75E-04	
IWA5217	5B	97.49	0.18	2.02E-03			
IWA3002	5B	137.46	0.26	1.35E-03			
IWA766	5B	208.89	0.30	4.60E-04			
IWA2099	5B	223.94	0.23		**4.08E-05**		
IWA6579	5B	226.50	0.26		**2.19E-05**		
IWA6577	5B	226.50	0.26		1.96E-04		
IWA6580	5B	226.50	0.26		1.96E-04		
IWA3526	6A	98.55	0.42			1.81E-03	
IWA2416	6A	98.98	0.42			1.81E-03	
IWA2812	6A	106.04	0.17				8.70E-04
IWA6962	6A	106.04	0.17				8.70E-04
IWA399	6A	107.29	0.49				1.35E-03
IWA3487	6A	180.19	0.06	1.16E-03			
IWA3488	6A	180.19	0.06	1.16E-03			
IWA2705	6A	180.19	0.06	1.16E-03			
IWA5943	6B	12.48	0.50		4.13E-04		
IWA2888	6B	38.51	0.06		8.63E-04		
IWA457	6B	97.18	0.28				7.51E-04
IWA1353	7A	48.90	0.29			6.74E-04	
IWA6472	7A	48.90	0.29			6.74E-04	
IWA3318	7A	69.83	0.34			6.71E-04	5.16E-04
IWA6764	7A	70.16	0.37			4.96E-04	1.00E-04
IWA4573	7A	82.34	0.18	2.52E-03			
IWA4167	7A	82.34	0.20				4.25E-04

Table 5.10. The *p* values of significant SNPs associated with tan spot resistance at the significant level of 1 percentile for the four Ptr races (Continued).

SNP	Chr[a]	Position	MAF[b]	Race 1	Race 2	Race 3	Race 5
IWA7599	7A	82.34	0.20				4.25E-04
IWA5489	7A	118.21	0.18			8.59E-04	
IWA7074	7A	140.51	0.42		6.02E-04		
IWA4438	7A	158.90	0.20	8.63E-04			
IWA4124	7A	173.34	0.29		5.77E-04		
IWA6736	7A	188.95	0.29	**5.39E-05**			
IWA7706	7A	188.95	0.30	5.93E-04			
IWA7904	7A	189.53	0.31	7.00E-04			
IWA5797	7A	193.18	0.29	4.64E-04			
IWA5103	7B	57.38	0.12				1.12E-03
IWA130	7B	73.33	0.18		6.56E-04	3.05E-04	1.27E-04
IWA716	NA	NA	0.11	7.22E-04			
IWA290	NA	NA	0.42	2.02E-03	5.31E-05		
IWA205	NA	NA	0.34			3.30E-04	2.30E-04
IWA4616	NA	NA	0.07			1.39E-03	
IWA4617	NA	NA	0.07			1.39E-03	
IWA793	NA	NA	0.18				2.25E-04

[a]Chr, Chromosome. NA, Not available. [b]MAF, Minor allele frequency. [c]The bold p-values are smaller than the cutoff p values at the significant level of 0.1 percentile.

Literature Cited

Anderson, J.A., R.J. Effertz, J.D. Faris, L.J. Francl, S.W. Meinhardt and B.S. Gill. 1999. Genetic analysis of sensitivity to a *Pyrenophora tritici-repentis* necrosis-inducing toxin in durum and common wheat. Phytopathology 89: 293-297.

Breseghello, F. and M.E. Sorrells. 2006. Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172: 1165-1177.

Chu, C.-G., T.L. Friesen, J.D. Faris and S.S. Xu. 2008a. Evaluation of seedling resistance to tan spot and *Stagonospora nodorum* blotch in tetraploid wheat. Crop Science 48: 1107-1116.

Chu, C.-G., T.L. Friesen, S.S. Xu and J.D. Faris. 2008b. Identification of novel tan spot resistance loci beyond the known host-selective toxin insensitivity genes in wheat. Theoretical and Applied Genetics 117: 873-881.

Chu, C.G., S. Chao, T.L. Friesen, J.D. Faris, S. Zhong and S.S. Xu. 2010. Identification of novel tan spot resistance QTLs using an SSR-based linkage map of tetraploid wheat. Molecular Breeding 25: 327-338.

Cox, T.S., W.J. Raupp, D.L. Wilson, B.S. Gill, S. Leath, W.W. Bockus, et al. 1992. Resistance to foliar diseases in a collection of *Triticum tauschii* germ plasm. Plant Disease 76: 1061-1064.

De Wolf, E.D., R.J. Effertz, S. Ali and L.J. Francl. 1998. Vistas of tan spot research. Canadian Journal of Plant Pathology 20: 349-370.

Effertz, R.J., S.W. Meinhardt, J.A. Anderson, J.G. Jordahl and L.J. Francl. 2002. Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. Phytopathology 92: 527-533.

Elias, E.M., R.G. Cantrell and R.M.J. Hosford. 1989. Heritability of resistance to tan spot in durum wheat and its association with other agronomic traits. Crop Science 29: 299-304.

Faris, J.D. and T.L. Friesen. 2005. Identification of quantitative trait loci for race-nonspecific resistance to tan spot in wheat. Theoretical and Applied Genetics 111: 386-392.

Faris, J.D., Z. Liu and S.S. Xu. 2013. Genetics of tan spot resistance in wheat. TAG. Theoretical and applied genetics. Theoretical and Applied Genetics 126: 2197-2217.

Faris, J.D., Z. Zhang, H. Lu, S. Lu, L. Reddy, S. Cloutier, et al. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proceedings of the National Academy of Sciences 107: 13544-13549.

Friesen, T.L., S. Ali, S. Kianian, L.J. Francl and J.B. Rasmussen. 2003. Role of host sensitivity to Ptr ToxA in development of tan spot of wheat. Phytopathology 93: 397-401.

Friesen, T.L. and J.D. Faris. 2004. Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. Theoretical and Applied Genetics 109: 464-471.

Friesen, T.L. and J.D. Faris. 2010. Characterization of the wheat-*Stagonospora nodorum* disease system: what is the molecular basis of this quantitative necrotrophic disease interaction? Canadian Journal of Plant Pathology 32: 20-28.

Friesen, T.L., Z. Zhang, P.S. Solomon, R.P. Oliver and J.D. Faris. 2008. Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. Plant Physiology 146: 682-693.

Gebhardt, C., A. Ballvora, B. Walkemeier, P. Oberhagemann and K. Schüler. 2004. Assessing genetic potential in germplasm collections of crop plants by marker-trait association: a case study for potatoes with quantitative variation of resistance to late blight and maturity type. Molecular Breeding 13: 93-102.

Gupta, P.K., S. Rustgi and P.L. Kulwal. 2005. Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Molecular Biology 57: 461-485.

Gurung, S., S. Mamidi, J.M. Bonman, E.W. Jackson, L.E. Del Río, M. Acevedo, et al. 2011. Identification of novel genomic regions associated with resistance to *Pyrenophora tritici-*

repentis races 1 and 5 in spring wheat landraces using association analysis. Theoretical and Applied Genetics 123: 1029-1041.

Hanks, S., A. Quinn and T. Hunter. 1988. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science 241: 42-52.

Harris, M.O., T.L. Friesen, S.S. Xu, M.S. Chen, D. Giron and J.J. Stuart. 2014. Pivoting from *Arabidopsis* to wheat to understand how agricultural plants integrate responses to biotic stress. Journal of Experimental Botany doi:10.1093/jxb/eru465.

Kollers, S., B. Rodemann, J. Ling, V. Korzun, E. Ebmeyer, O. Argillier, et al. 2014. Genome-wide association mapping of tan spot resistance (*Pyrenophora tritici-repentis*) in European winter wheat. Molecular Breeding: 1-9.

Krupinsky, J.M. 1982. Observations on the host range of isolates of *Pyrenophora trichostoma Triticum aestivum* and seven grasses: Elymus, North America. Canadian Journal of Plant Pathology 4: 42-46.

Lamari, L. and C.C. Bernier. 1989a. Evaluation of wheat lines and cultivars to tan spot *Pyrenophora tritici-repentis* based on lesion type. Canadian Journal of Plant Pathology 11: 49-56.

Lamari, L. and C.C. Bernier. 1989b. Toxin of *Pyrenophora tritici-repentis*: host-specificity, significance in disease, and inheritance of host reaction. Phytopathology 79: 740-744.

Lamari, L., B.D. McCallum and R.M. dePauw. 2005. Forensic pathology of Canadian bread wheat: The case of tan spot. Phytopathology 95: 144-152.

Lamari, L., R. Sayoud, M. Boulif and C.C. Bernier. 1995. Identification of a new race in *Pyrenophora tritici-repentis*: implications for the current pathotype classification system. Canadian Journal of Plant Pathology 17: 312-318.

Lamari, L., S.E. Strelkov, A. Yahyaoui, J. Orabi and R.B. Smith. 2003. The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. Phytopathology 93: 391-396.

Lehti-Shiu, M.D., C. Zou, K. Hanada and S.-H. Shiu. 2009. Evolutionary history and stress regulation of plant receptor-like kinase/pelle genes. Plant Physiology 150: 12-26.

Letta, T., P. Olivera, M. Maccaferri, Y. Jin, K. Ammar, A. Badebo, et al. 2014. Association mapping reveals novel stem rust resistance loci in durum wheat at the seedling stage. Plant Genome 7: -. doi:10.3835/plantgenome2013.08.0026.

Maccaferri, M., A. Ricci, S. Salvi, S. G. Milner, E. Noli, et al. 2014. A high-density, SNP-based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. Plant Biotechnology Journal: n/a-n/a. doi: 10.1111/pbi.12288.

Mamidi, S., R.K. Lee, J.R. Goos and P.E. McClean. 2014. Genome-wide association studies identifies seven major regions responsible for iron deficiency chlorosis in soybean (*Glycine max*). PloS One 9(9): e107469.

McFadden, E.S. 1930. A successful transfer of emmer characters to *vulgare* wheat. Journal of the American Society of Agronomy 22: 1020-1034.

Mergoum, M., P.K. Singh, S. Ali, E.M. Elias, J.A. Anderson, K.D. Glover, et al. 2007. Reaction of elite wheat genotypes from the northern Great Plains of North America to *Septoria* diseases. Plant Disease 91: 1310-1315.

Mitra, R.M., C.A. Gleason, A. Edwards, J. Hadfield, J.A. Downie, G.E.D. Oldroyd, et al. 2004. A Ca2+/calmodulin-dependent protein kinase required for symbiotic nodule development: Gene identification by transcript-based cloning. Proceedings of the National Academy of Sciences 101: 4701-4705.

Morris, J.F., B.F. Carver, R.M. Hunger and A.R. Klatt. 2010. Greenhouse assessment of seedling reaction to tan spot in synthetic hexaploid wheat. Crop Science 50: 952-959.

Nagle, B.J., R.C. Frohberg and R.M.J. Hosford. 1982. Inheritance of resistance to tan spot of wheat. In: R. M. J. Hosford, editor Tan spot of wheat and related diseases workshop. North Dakota State University, Fargo.

Oliver, R.E., X. Cai, R.-C. Wang, S.S. Xu and T.L. Friesen. 2008a. Resistance to tan spot and *Stagonospora nodorum* blotch in wheat-alien species derivatives. Plant Disease 92: 150-157.

Oliver, R.P. and P.S. Solomon. 2010. New developments in pathogenicity and virulence of necrotrophs. Current Opinion in Plant Biology 13: 415-419.

Orolaza, N.P., L. Lamari and G.M. Ballance. 1995. Evidence of a host-specific chlorosis toxin from *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat. Phytopathology 85: 1282-1287.

Qiu, Y. and D. Yu. 2009. Over-expression of the stress-induced *OsWRKY45* enhances disease resistance and drought tolerance in Arabidopsis. Environmental and Experimental Botany 65: 35-47.

Rees, R.G. and G.J. Platz. 1990. Sources of resistance to *Pyrenophora tritici-repentis* in bread wheats. Euphytica 45: 59-69.

Rushton, P.J., I.E. Somssich, P. Ringler and Q.J. Shen. 2010. WRKY transcription factors. Trends in Plant Science 15: 247-258.

Salvi, S. and R. Tuberosa. 2005. To clone or not to clone plant QTLs: present and future challenges. Trends in Plant Science 10: 297-304.

SAS Institute. 2011. The SAS system for Windows. SAS Inst., Cary, NC.

Shabeer, A. and W.W. Bockus. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. Plant Disease 72:599–602.

Shin, J.-H., S. Blay, B. McNeney and J. Graham. 2006. LDheatmap: an R function for graphical display of pairwise linkage disequilibria between single nucleotide polymorphisms. Journal of Statistical Software 16: 1-10.

Siedler, H., A. Obst, S.L.K. Hsam and F.J. Zeller. 1994. Evaluation for resistance to *Pyrenophora tritici-repentis* in *Aegilops tauschii* Coss. and synthetic hexaploid wheat amphiploids. Genetic Resources and Crop Evolution 41: 27-34.

Singh, P.K., M. Mergoum, S. Ali, T.B. Adhikari, E.M. Elias, J.A. Anderson, et al. 2006b. Evaluation of elite wheat germ plasm for resistance to tan spot. Plant Disease 90: 1320-1325.

Singh, P.K., M. Mergoum, T.B. Adhikari, T. Shah, F. Ghavami and S.F. Kianian. 2010b. Genetic and molecular analysis of wheat tan spot resistance effective against *Pyrenophora tritici-repentis* races 2 and 5. Molecular Breeding 25: 369-379.

Singh, P.K., M. Mergoum, S. Ali, T.B. Adhikari, E.M. Elias and G.R. Hughes. 2006a. Identification of new sources of resistance to tan spot, *Stagonospora nodorum* blotch, and *Septoria tritici* blotch of wheat. Crop Science 46: 2047-2053.

Singh, P.K., R.P. Singh, E. Duveiller, M. Mergoum, T.B. Adhikari and E.M. Elias. 2010a. Genetics of wheat-*Pyrenophora tritici-repentis* interactions. Euphytica 171: 1-13.

Singh, S., W.W. Bockus, I. Sharma and R.L. Bowden. 2007. A novel source of resistance in wheat to *Pyrenophora tritici-repentis* race 1. Plant Disease 92: 91-95.

Strelkov, S.E., L. Lamari and G.M. Ballance. 1999. Characterization of a host-specific protein toxin (Ptr ToxB) from *Pyrenophora tritici-repentis*. Molecular Plant-Microbe Interactions 12: 728-732.

Sun, X.-C., W. Bockus and G. Bai. 2010. Quantitative trait loci for resistance to *Pyrenophora tritici-repentis* Race 1 in a Chinese wheat. Phytopathology 100: 468-473.

Tadesse, W., S.L.K. Hsam and F.J. Zeller. 2006. Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'. Plant Breeding 125: 318-322.

Thornsberry, J.M., M.M. Goodman, J. Doebley, S. Kresovich, D. Nielsen and E.S. Buckler. 2001. *Dwarf8* polymorphisms associate with variation in flowering time. Nature Genetics 28: 286-289.

Tomás, A., G.H. Feng, G.R. Reeck, W.W. Bockus and J.E. Leach. 1990. Purification of a cultivar-specific toxin from *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. Molecular Plant-Microbe Interactions 3: 221-224.

Wu, Y. and J.-M. Zhou. 2013. Receptor-like kinases in plant innate immunity. Journal of Integrative Plant Biology 55: 1271-1286.

Xu, S.S., T.L. Friesen and A. Mujeeb-Kazi. 2004. Seedling resistance to tan spot and Stagonospora nodorum blotch in synthetic hexaploid wheats. Crop Science 44: 2238-2245.

Zhang, N., Y. Xu, M. Akash, S. McCouch and J.H. Oard. 2005. Identification of candidate markers associated with agronomic traits in rice using discriminant analysis. Theoretical and Applied Genetics 110: 721-729.

Zondervan, K.T. and L.R. Cardon. 2004. The complex interplay among factors that influence allelic association. Nature Reviews Genetics 5: 89-100.

CHAPTER 6. ASSOCIATION ANALYSIS OF SUSCEPTIBILITY TO SEPTORIA NODORUM BLOTCH IN CULTIVATED EMMER WHEAT

Abstract

Septoria nodorum blotch (SNB), caused by *Parastagonospora nodorum*, is a significant foliar disease of durum (*T. turgidum* ssp. *durum*) and common wheat (*T. aestivum*). The pathogen produces host-selective toxins (HSTs) that, when recognized by the products of corresponding host sensitivity genes, result in host-driven programmed cell death, which ultimately leads to disease susceptibility. HST-triggered susceptibility is predominant, but it is also possible that active resistance is contributing quantitatively to this trait. Cultivated emmer wheat (*T. turgidum* ssp. *dicoccum*) has been a good source of resistance genes for various wheat diseases, and may contain yet unidentified HST sensitivity genes. The objectives of this study were to evaluate 180 emmer wheat accessions, which were recently phenotyped for their reaction to *P. nodorum* and genotyped with the wheat 9K single nucleotide polymorphism (SNP) array. Association mapping was used to identify novel susceptibility genes. When the 180 emmer wheat accessions were evaluated at the seedling stage for their reaction to *P. nodorum* isolate Sn4, and 19 (11%) accessions showed moderate or high susceptibility. To identify the genomic regions associated with susceptibility, 4,134 polymorphic SNPs and 178 of the accessions were used for association analysis. Fourty-two SNP markers on 14 chromosomes were significantly associated with susceptibility. Several susceptibility loci may be due to previously identified susceptibility genes and QTL, including *Snn5*, *QSng.sfr-4BL*, *QSng.sfr-5BL*, *QSnb.ndsu-5B*, *Tsn1*, *QSnl.ihar-6AL*, *QSnb.fcu-7A*, and *QSnl.eth-7B1*, but the others may represent novel susceptibility genes that can be targeted for further characterization of the wheat-*P. nodorum* pathosystem. Nine significant markers with major effects were identified based on stepwise

regression analysis, and their allelic combinations are potentially valuable for marker-assisted selection in wheat breeding programs. Results from this study provide important knowledge and tools for genetic analysis and breeding for resistance to *P. nodorum* in wheat.

Introduction

Septoria nodorum blotch (SNB), caused by *Parastagonospora* (syn. *ana, Stagonospora; teleo, Phaeosphaeria*) *nodorum* (Berk.) Quaedvleig, Verkley & Crous, is a worldwide disease of wheat (Weber, 1922; Machacek, 1945). By attacking the leaf and glume, the pathogen threatens wheat yields, with estimated losses up to 30-50%. In the U.S., an example of this occurred in the 1960s and 1970s in the southeast (Scharen and Krupinsky, 1969; Nelson et al., 1974). In Australia, especially Western Australia, SNB causes 5% average losses (>AUD$100 million) each year (Murray and Brennan, 2009). There are high levels of genetic and genotypic diversity between and within geographic populations of *P. nodorum* because of gene flow and sexual recombination (McDonald et al., 1994; Keller et al., 1997a, b). Because of the less specificity in the wheat - *P. nodorum* pathosystem compared with other fungi (Eyal, 1999), there has not been a taxonomic system for grouping *P. nodorum* isolates or strains into physiological races as of yet.

Although SNB is a major disease in durum and bread wheat, breeding for resistance to SNB has not been a major aim of wheat breeding programs around the world. One reason for reduced efforts for this disease is that the wheat breeders often focus on a single disease that is currently causing the greatest yield losses in their region. For example, in North Dakota and nearby states, in spite of the increasing incidence of SNB in recent years, regional wheat breeders have focused their attention on resistance to Fusarium head blight (FHB; caused by *Fusarium graminearum* Schwabe), i.e., the biggest concern for farmers since the early 1990s

(McMullen et al., 1997). Another reason for ignoring SNB in wheat has been the assumption that resistance is a quantitative trait. Integrating multiple genes into a breeding program is far more difficult than incorporating a single *Resistance* gene. As a result, most of the wheat cultivars that are grown around the world are susceptible to SNB (Singh et al., 2006).

This is changing due to recent discoveries about mechanisms underlying wheat-*P. nodorum* interactions (Friesen and Faris, 2010). To search for good sources of SNB resistance, numerous studies were conducted to evaluate the reactions to *P. nodorum* (See review by Xu et al, 2004a). A high level of partial resistance to *P. nodorum* has been detected in the germplasm collections of wheat and its related species (Xu et al, 2004a, b; Singh et al., 2006; Mergoum et al., 2007). We now know that host-selective toxins (HSTs) are major virulence factors (Liu et al., 2004a; Friesen et al., 2006, 2007, 2008, 2012; Abeysekara et al., 2009; Gao et al., 2015; Oliver and Solomon, 2010; Friesen and Faris, 2010). The identification of multiple HSTs, also called necrotrophic effectors (NEs), was a significant advance in the understanding of necrotrophic pathogen-wheat interaction systems, which follow the inverse of the classical gene-for-gene relationships between a host and a biotrophic pathogen (Friesen and Faris, 2010). A compatible interaction between necrotrophic pathogens and wheat involves recognition of HSTs by the product of a host susceptibility gene, with recognition triggering a susceptible reaction of the host plant (Friesen and Faris, 2010). So far, seven proteinaceous necrosis-inducing HSTs (SnToxA, SnTox1, SnTox2, SnTox3, SnTox4, SnTox5, and SnTox6) produced by *P. nodorum* have been identified (Liu et al., 2004a; Friesen et al., 2006, 2007, 2008, 2012; Abeysekara et al., 2009; Gao et al., 2015). All seven HSTs are small proteinaceous molecules with an estimated size of 10 to 30 kD (Oliver et al., 2012). The pathogen genes that encode SnToxA, SnTox1, and

SnTox3 (*ToxA*, *Tox1*, and *Tox3*, respectively) have been cloned, and found in ca. 36%, 85%, and 59% of worldwide *P. nodorum* isolates, respectively (Liu et al., 2009, 2012).

Eight host genes (including *Tsn1*, *Snn1*, *Snn2*, *Snn3-B1*, *Snn3-D1*, *Snn4*, *Snn5*, and *Snn6*) that encode the trait that causes sensitivity to seven *P. nodorum* HSTs have been identified in wheat and related species (Friesen et al., 2012; Gao et al., 2015). For toxin SnTox3, two sensitivity genes, *Snn3-B1* and *Snn3-D1*, discovered on chromosomes 5BS and 5DS, respectively, proved to be homoeoallelic to each other (Friesen et al., 2008; Zhang et al., 2011). The other six sensitivity genes, *Tsn1*, *Snn1*, *Snn2*, *Snn4*, *Snn5*, and *Snn6*, were mapped to chromosome arms 5BL, 1BS, 2DS, 1AS, 4BL, and 6AL, respectively (Oliver et al., 2012; Friesen et al., 2012; Gao et al., 2015). Interactions between the host susceptibility genes and pathogen HSTs (SnToxA-*Tsn1*, SnTox1-*Snn1*, SnTox2-*Snn2*, SnTox3-*Snn3-B1*, SnTox3-*Snn3-D1*, SnTox4-*Snn4*, SnTox5-*Snn5*, and SnTox6-*Snn6*) explain a significant proportion of phenotypic variation in plant responses to pathogen attack (95%, 58%, 47%, 18%, 100%, 41%, 63%, and 27%, respectively) (Oliver et al., 2012; Friesen et al., 2012; Gao et al., 2015).

Besides the eight host sensitivity genes reviewed above, many QTL for seedling and adult plant resistances have been identified through bi-parental mapping (see reviews by Xu et al., 2004a). Arseniuk et al. (2004) identified a QTL on chromosome 6AL (*QSnl.ihar-6A*) that explained 36% of the phenotypic variance for disease severity at the seedling stage in double haploid (DH) populations derived from a cross between wheat cultivar ‘Alba’ and ‘Begra’. Liu et al. (2004b) identified a major QTL on 1BS and six minor QTL on 3AS, 4AL, 5AL, 4BL, 7BL, and 5DL for seedling resistance by analyzing the International Triticeae Mapping Initiative (ITMI) mapping population. Schnurbusch et al. (2003) identified two QTL (*QSng.sfr-3BS* and *QSng.sfr-4BL*) responsible for increased resistance to glume blotch using an RIL population

from the cross ‘Arina’ × ‘Forno’. Recently, Francki (2011) identified three loci conferring flag leaf resistance on chromosomes 1BS, 2AS, and 5BL using an RIL population from a cross between winter wheat genotypes ‘P92201D5’ and ‘P91193D1’ and a doubled haploid population from a cross between spring wheat EGA Blanco and ‘Millewa’. Among the QTL identified in these studies, some may correspond to the known or unidentified host genes that are sensitive to the existing or unidentified HSTs. Others may represent genes that contribute quantitatively to the active resistance to SNB.

All of the eight host sensitivity genes and QTL described above were identified based on linkage analysis using bi-parental mapping populations in the past two decades. Since 2006, association mapping has been extensively used to identify genomic regions associated with important agronomic traits in wheat (Breseghello and Sorrells, 2006; Ravel et al., 2006). However, traits associated with host susceptibility and resistance loci to SNB in wheat have rarely been the subject of association mapping. Adhikari et al. (2011) evaluated the reactions of *P. nodorum* in 567 spring wheat landraces that were genotyped by 625 DArT markers. Seven DArT markers significantly associated with *P. nodorum* resistance located on 2D, 3B, 5B, 6A, and 7A through association analysis were identified. Tommasini et al. (2007), who used association mapping to increase marker resolution in the region of *QSng.sfr-3BS* identified by Schnurbusch et al. (2003), detected a marker, SUN2-3B, that was strongly associated with resistance to glume blotch caused by *P. nodorum*.

Now the availability is increasing for high throughput marker technologies, such as 9K and 90K SNP arrays and genotype by sequence, association mapping is seen as offering great promise for gene prediction and identification in wheat. Recently, a cultivated emmer wheat panel consisting of 180 accessions was genotyped with a wheat 9K SNP array and was used for

association mapping of the resistance to stem rust, leaf rust, and tan spot (CHAPTERS 3,4,5). In this study, 180 cultivated emmer wheat accessions and genome-wide association analysis were used to evaluate the association between polymorphic SNP markers and the disease reactions to *P. nodorum* isolate Sn4 at the seedling stage.

Materials and Methods

Plant Materials

The same set of 180 cultivated emmer wheat accessions (Table A1) used as the panel for association mapping of the resistance to stem rust in Chapter 3 was used for association mapping of the SNB resistance in this study. These cultivated emmer wheat accessions were originally provided by the USDA-ARS National Small Grains Collection (Aberdeen, ID). Their information was retrieved from USDA-ARS Germplasm Resources Information Network (http://www.ars-grin.gov/npgs/searchgrin.html).

Disease Evaluation and Assessment

The 180 cultivated emmer accessions were evaluated with their reactions to *P. nodorum* isolate Sn4, which is known to be one of the most aggressive isolates of *P. nodorum* (Mebrate and cooke, 2001), and can produce several HSTs including SnToxA, SnTox1, SnTox2, and SnTox3 (Liu et al., 2009). Spring wheat line BR34 and hard red spring wheat cultivar 'Grandin' were used as the resistant and susceptible controls, respectively. The evaluation experiment was performed with three completely randomized replications. In each replication, each of the 180 cultivated emmer accessions and two checks was planted in a super-cell cone (Stuewe and Sons, Inc., Corvallis, OR, U.S.A.) with three plants of each accession per cone. The cones were placed RL98 trays (Stuewe and Sons, Inc., Corvallis, OR) consisting of 60 accessions per tray and bordered with Grandin for eliminating the border effects. For the inoculum preparation, disease

inoculation and post-inoculation incubation followed the procedures as described by Liu et al. (2004b) under controlled greenhouse and growth chamber conditions. The inoculations were conducted at the two- to three-leaf stage. The disease was scored after 7 days of inoculation, using a 0 to 5 qualitative lesion-type rating scale (Liu et al., 2004b), where 0 = highly resistant (no lesion); 1 = resistant (flecking or small dark spots); 2 = moderately resistant (dark spots with little surrounding necrosis or chlorosis); 3 = moderately susceptible (dark lesions completely surrounded by necrosis or chlorosis) ; 4 = susceptible (larger necrotic or chlorotic lesions); and 5 = highly susceptible (large coalescent lesions) (Liu et al., 2004b). Plants having equal numbers of two different lesion types were given an intermediate lesion type (e.g., lesion types 1 and 2 equals 1.5).

All lines were also evaluated for reactions to three HSTs including SnToxA, SnTox1, and SnTox3 with two replications, and the infiltration and scoring procedures followed the procedure as described by Xu et al. (2004b).

Phenotypic Data Analysis and Association Mapping

The Spearman correlation coefficient was estimated for different replicates using PROC CORR in Statistical Analysis System (SAS) version 9.3 (SAS Institute Inc., Gary, NC, U.S.A). Significantly correlated replications were pooled together and used for further analysis (Chu et al., 2008, 2010). The analysis of variance (ANOVA) of lesion types in three replicates was performed in SAS PROC ANOVA. Based on this ANOVA and Equation 5.1 in Chapter 5, the broad-sense heritability (H^2) of resistance to Sn4 was estimated (Letta et al., 2014). The 180 emmer accessions had been genotyped with 9K SNP array. And the method of filtering raw genotypic data, association analysis, LD block identification, and stepwise regression analysis followed the description in Chapter 3.

Results

Reaction of Emmer Wheat Accessions to *P. nodorum* Isolate Sn4

A wide range of variation was observed in this emmer panel for the reactions to *P. nodorum* isolate Sn4, ranging from highly resistant (lesion type = 0) to highly susceptible (Lesion type = 5.0) (Table 6.1). The analysis of variances (ANOVA) of the reactions in three replicates revealed that the disease reactions of different accessions were significantly different ($p < 0.0001$) (Table 6.4). Based on the ANOVA, the broad-sense heritability (H^2) of the SNB resistance in greenhouse was estimated as 0.917. The Spearman correlation analysis showed that the reactions in three replicates were highly correlated with each other ($p < 0.0001$) with a range of correlation coefficients (r_s) from 0.46 to 0.50. Therefore, the reaction data from the three replicates was pooled together for further analysis. Based on the average reactions, 117 (65%), 44 (24%), and 19 (11%) of the 180 emmer accessions had highly or moderately resistant (lesion type <= 2.0), intermediate (2.0 < lesion type < 3.0), and moderately or highly susceptible (lesion type >= 3.0) reactions to isolate Sn4 (Figure 6.1), respectively.

For the reactions to the three HSTs, SnToxA, SnTox1, and SnTox3, most of the 180 accessions were insensitive to all the three toxins except for the 5, 12, 11 accessions having plants that were sensitive to SnToxA, SnTox1, and SnTox3, respectively. Only two, three, and two of the accessions consistently had sensitive reactions to the three toxins, respectively, in two replicates.

Table 6.1. Disease reactions of 180 emmer wheat accessions to *P. nodorum* isolate Sn4 and three toxins.

Accessions	Sn4			SnTox A		SnTox 1		SnTox 3	
	Rep1[a]	Rep2	Rep3	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
CItr 7687-1	4.5	4.0	3.0	0	0	0	1	0	0
CItr 12213-1	2.0	3.0	2.0	0	0	0	0	0	0
CItr 14133-1	0.5	0.0	0.5	0	0	0	0	0	0
CItr 14621-1	1.0	0.5	1.5	0	0	0	0	0	0
CItr 14637-1	1.5	3.0	1.5	0	0	0	0	0	0
CItr 14916-1	1.5	3.0	2.5	0	0	0	0	0	0
CItr 14917-1	1.5	3.5	0.5	0	0	0	0	0	0
CItr 14919-1	2.5	3.5	2.5	0	0	0	0	0	0
PI 41024-1	3.0	4.0	1.5	0	0	0	0	0	0
PI 74108-1	2.0	0.5	1.5	0	0	0	0	0	0
PI 94616-1	5.0	1.0	2.0	0	0	0	0	0	0
PI 94621-1	4.0	4.5	4.0	0	0	0	0	0	0
PI 94625-1	4.5	3.5	3.0	0	0	0	0	0	NA
PI 94626-1	3.5	2.0	2.5	0	0	0	0	0	0
PI 94627-1	3.0	2.0	2.5	0	0	0	0	0	0
PI 94631-1	3.0	3.5	2.0	0	0	0	0	0	0
PI 94634-1	2.0	2.5	1.5	0	0	0	0	0	0
PI 94635-1	3.0	4.5	3.0	0	0	0	0	3	1
PI 94638-1	1.5	2.0	2.5	0	0	0	0	0	0
PI 94648-1	1.5	1.5	1.5	0	0	0	0	0	0
PI 94656-1	NA[b]	NA	1.5	0	NA	0	NA	0	NA
PI 94664-1	2.0	2.5	1.0	0	0	0	0	0	0
PI 94666-1	0.5	3.0	2.0	0	0	0	0	0	0
PI 94673-1	0.5	3.0	1.5	0	0	0	0	0	0
PI 94674-1	3.0	2.5	1.5	0	0	0	0	0	0
PI 94675-1	2.5	2.0	2.0	0	0	0	0	0	0
PI 94676-1	0.5	1.0	0.5	0	0	0	NA	0	NA
PI 94738-1	1.5	2.0	2.0	0	0	0	0	0	0
PI 94747-1	2.5	3.5	1.0	0	0	0	0	0	0
PI 101971-1	2.5	2.5	3.0	0	0	0	0	0	0
PI 133134-1	2.0	2.5	0.5	0	0	0	0	0	0
PI 154582-1	2.0	3.0	2.0	0	0	0	0	0	0
PI 164578-1	1.0	2.0	1.5	0	0	0	0	0	0
PI 168673-1	1.5	3.5	NA	0	0	0	0	0	0
PI 193641-1	4.0	4.5	4.0	0	0	3	2	0	0
PI 193873-1	0.5	2.0	2.5	0	0	0	0	0	0
PI 193879-1	0.0	3.5	1.5	0	NA	0	NA	0	NA
PI 193880-1	0.5	2.0	2.0	0	0	0	0	0	0
PI 193882-1	0.0	3.0	1.5	0	0	0	0	0	0
PI 193883-1	2.5	1.5	2.0	0	0	0	0	0	0
PI 194042-1	0.0	2.5	1.5	0	0	0	0	0	0
PI 194375-1	0.5	3.0	0.5	0	0	0	0	0	0

Table 6.1. Disease reactions of 180 emmer wheat accessions to *P. nodorum* isolate Sn4 and three toxins (Continued).

Accessions	Sn4			SnTox A		SnTox 1		SnTox 3	
	Rep1[a]	Rep2	Rep3	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
PI 197483-1	0.5	2.5	2.0	0	0	0	0	0	0
PI 197485-1	1.5	2.0	3.0	0	0	0	0	0	0
PI 217637-1	2.0	2.5	2.0	0	NA	0	NA	0	NA
PI 217639-1	2.5	2.5	1.5	0	0	0	0	0	0
PI 217640-1	1.0	3.0	2.0	0	0	0	0	0	0
PI 221400-1	3.5	4.5	4.0	0	0	3	2	0	0
PI 225332-1	3.5	3.5	3.5	0	0	1	0	0	0
PI 244341-1	1.0	2.0	1.5	0	NA	0	NA	0	0
PI 254165-1	3.0	4.0	2.5	0	0	0	0	0	0
PI 254167-1	4.0	4.0	3.0	0	0	0	0	0	0
PI 254189-1	3.5	3.5	3.0	0	0	0	NA	0	0
PI 254190-1	0.5	4.0	2.0	0	0	0	0	0	NA
PI 272533-1	1.5	2.0	2.0	0	0	0	0	0	0
PI 273981-1	0.0	2.0	2.0	0	0	0	0	0	0
PI 275996-1	1.5	2.5	2.0	0	0	0	0	0	0
PI 298582-1	2.5	2.5	1.5	0	0	0	0	0	0
PI 310471-1	0.5	2.5	1.0	0	NA	0	0	0	0
PI 319869-1	0.5	3.0	3.0	0	0	0	0	0	0
PI 322232-1	0.0	3.0	1.5	0	0	0	0	0	0
PI 324076-1	0.0	3.0	0.5	0	0	0	0	0	NA
PI 349043-1	0.0	3.0	NA	0	NA	0	NA	1	NA
PI 349046-1	0.0	3.5	2.5	0	0	0	0	0	0
PI 352548-1	2.5	3.5	2.0	0	0	0	0	1	0
PI 355477-1	2.0	2.5	1.0	0	0	0	0	0	0
PI 355507-1	2.5	1.5	1.0	0	0	1	0	1	0
PI 377655-1	3.0	1.5	2.0	0	0	0	0	0	0
PI 377657-1	NA	3.0	2.5	0	0	0	0	0	NA
PI 384332-1	0.5	2.5	0.5	0	0	0	0	0	0
PI 434992-1	0.5	0.5	1.5	0	0	0	0	0	0
PI 480460-1	0.0	1.5	0.5	0	0	0	1	0	0
PI 532305-1	0.0	2.5	1.5	0	0	1	0	0	0
CItr 3686	3.0	4.0	2.5	0	0	0	0	0	0
CItr 4013	2.5	4.0	2.5	1	3	0	0	0	0
CItr 7685	0.0	1.5	0.5	0	0	0	0	0	0
CItr 7686	0.0	3.0	1.0	0	0	0	0	0	0
CItr 7779	3.0	4.0	2.5	0	0	0	0	0	0
CItr 7962	0.0	3.0	0.5	0	0	0	0	0	0
CItr 14085	0.5	1.5	2.0	0	0	0	0	0	0
CItr 14086	0.0	2.0	1.0	0	0	0	0	0	NA
CItr 14098	0.0	2.5	0.5	0	0	0	0	0	0
CItr 14639	0.5	3.0	0.5	0	0	0	0	0	0
CItr 14751	1.5	NA	2.0	0	0	0	0	0	0

Table 6.1. Disease reactions of 180 emmer wheat accessions to *P. nodorum* isolate Sn4 and three toxins (Continued).

Accessions	Sn4			SnTox A		SnTox 1		SnTox 3	
	Rep1[a]	Rep2	Rep3	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
CItr 14822	0.0	2.0	3.0	3	0	0	0	0	0
CItr 14834	0.0	2.0	1.5	0	0	0	0	0	0
CItr 14866	2.5	3.0	2.0	0	0	0	NA	0	0
CItr 14971	2.5	4.0	3.0	0	0	0	0	0	0
PI 41025	0.0	1.5	0.5	0	0	0	0	0	0
PI 58788	0.0	3.0	1.0	0	0	0	0	0	0
PI 60704	0.0	2.5	2.5	0	0	0	0	0	0
PI 74106	4.0	3.5	2.5	0	0	0	NA	0	NA
PI 94617	0.0	0.5	0.0	0	0	0	0	0	0
PI 94630	0.0	NA	2.0	0	0	0	0	0	NA
PI 94654	3.0	2.5	2.0	0	NA	0	0	0	0
PI 94663	3.0	2.0	3.0	0	0	1	0	0	0
PI 94665	1.5	3.0	2.5	0	0	0	0	0	0
PI 94668	3.0	3.0	2.0	0	0	0	0	0	0
PI 94680	1.0	1.5	1.5	0	0	0	0	0	0
PI 113961	3.0	3.0	2.5	1	0	0	0	2	2
PI 168675	2.0	NA	1.5	0	0	0	0	0	0
PI 190920	NA	1.5	1.5	0	0	0	0	0	0
PI 190926	1.0	0.5	0.5	0	NA	0	NA	0	NA
PI 191091	0.0	1.5	1.5	0	NA	0	NA	0	NA
PI 193643	0.5	1.5	1.5	0	NA	0	NA	0	NA
PI 193878	1.5	0.5	2.5	0	0	0	0	0	0
PI 195721	2.0	3.0	1.5	0	0	0	0	0	0
PI 196100	2.0	3.0	2.5	0	0	0	0	0	0
PI 196905	2.5	3.5	3.5	2	3	0	0	0	0
PI 197482	NA	1.5	2.0	0	0	0	0	0	NA
PI 197490	0.5	2.0	1.5	0	0	0	0	0	0
PI 221401	3.0	4.5	3.0	0	0	3	2	0	0
PI 226951	1.5	2.5	0.5	0	0	0	0	0	0
PI 248991	0.0	NA	1.5	0	0	0	0	0	0
PI 254146	0.0	2.5	NA	0	0	0	0	0	0
PI 254163	4.0	3.5	2.5	0	0	1	0	1	0
PI 254188	0.0	1.0	0.5	0	0	0	0	0	0
PI 254193	0.5	1.5	0.5	0	0	0	0	0	0
PI 272527	0.5	1.5	0.5	0	0	1	0	0	0
PI 273982	2.0	3.0	1.5	0	0	0	0	0	0
PI 275997	0.0	0.5	1.5	1	0	0	0	1	1
PI 275998	0.0	1.5	2.0	0	0	0	0	0	NA
PI 275999	0.0	1.0	1.5	0	0	0	0	0	0
PI 276000	0.0	2.0	1.5	0	0	0	0	0	0
PI 276005	NA	NA	0.5	0	NA	0	NA	2	NA
PI 276006	0.0	2.0	1.0	0	0	0	0	0	1

Table 6.1. Disease reactions of 180 emmer wheat accessions to *P. nodorum* isolate Sn4 and three toxins (Continued).

Accessions	Sn4			SnTox A		SnTox 1		SnTox 3	
	Rep1[a]	Rep2	Rep3	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
PI 276007	0.0	0.5	1.0	0	0	0	NA	0	NA
PI 276012	0.0	1.5	1.0	0	0	0	0	2	NA
PI 276014	0.0	0.5	2.0	0	0	0	0	0	0
PI 277670	0.0	1.5	1.5	0	0	0	0	0	0
PI 277671	NA	0.5	NA	NA	0	NA	0	NA	NA
PI 277677	0.0	0.5	0.5	0	0	1	0	0	0
PI 286061	0.0	0.0	1.0	0	0	0	0	0	0
PI 289603	1.0	1.0	0.5	0	0	0	0	0	0
PI 295065	0.0	0.0	1.5	NA	0	NA	0	NA	0
PI 297830	1.5	3.0	2.0	0	0	0	0	0	0
PI 298543	1.5	2.0	NA	0	0	0	0	0	0
PI 298548	NA	2.5	2.0	0	NA	0	NA	NA	NA
PI 306536	1.0	2.5	2.5	0	0	0	0	0	0
PI 330544	0.0	0.0	0.5	0	0	0	0	0	0
PI 349045	3.5	2.0	2.5	0	0	0	0	0	0
PI 350001	0.5	1.5	1.0	0	0	0	0	0	0
PI 352335	1.5	4.0	2.5	0	0	0	0	1	0
PI 352337	0.5	1.5	0.5	0	0	0	0	0	0
PI 352338	0.5	0.0	0.5	0	0	0	0	0	0
PI 352341	2.0	1.5	1.5	0	NA	0	NA	0	NA
PI 352342	0.5	NA	1.0	NA	NA	NA	NA	NA	NA
PI 352358	1.5	1.0	1.0	NA	0	NA	0	NA	0
PI 352365	0.5	1.5	1.0	0	0	0	0	0	0
PI 355460	0.0	0.5	1.0	0	0	0	0	0	0
PI 355461	0.0	1.0	1.0	0	0	0	0	0	0
PI 355470	2.5	2.0	0.5	0	0	0	0	0	0
PI 355475	0.0	1.0	1.5	0	0	0	0	0	0
PI 355483	1.5	1.0	1.5	0	0	0	0	0	0
PI 355485	0.0	0.5	1.0	0	0	0	0	0	0
PI 355486	0.0	1.5	0.0	0	0	0	0	0	0
PI 355489	3.0	0.0	0.0	0	0	0	0	0	0
PI 355497	3.0	2.0	1.5	0	0	0	0	0	0
PI 355505	1.0	2.5	1.5	0	0	0	0	0	0
PI 361833	2.0	3.0	1.5	0	0	0	0	0	0
PI 362438	0.5	1.5	1.5	0	0	0	0	0	0
PI 362500	1.0	1.0	1.5	0	0	0	0	0	0
PI 362697	0.0	2.5	0.5	0	0	0	0	0	0
PI 374685	0.0	1.5	2.0	0	0	0	0	0	0
PI 377650	2.0	1.5	2.5	0	0	0	0	0	0
PI 377672	0.0	1.0	2.0	0	0	0	0	0	0
PI 384297	0.0	1.5	1.0	0	NA	0	NA	0	NA
PI 384302	0.0	2.5	0.5	0	0	0	0	0	0

Table 6.1. Disease reactions of 180 emmer wheat accessions to *P. nodorum* isolate Sn4 and three toxins (Continued).

Accessions	Sn4			SnTox A		SnTox 1		SnTox 3	
	Rep1[a]	Rep2	Rep3	Rep1	Rep2	Rep1	Rep2	Rep1	Rep2
PI 384318	0.0	2.5	2.0	0	0	0	0	0	0
PI 384331	3.5	2.0	0.5	0	0	0	0	0	0
PI 434996	0.0	2.0	2.5	0	0	0	0	0	0
PI 470737	1.5	3.0	3.0	0	0	0	0	0	0
PI 479957	0.5	2.0	0.5	0	0	0	0	0	0
PI 479965	2.0	3.0	1.0	0	0	0	0	0	0
PI 480307	2.5	2.5	2.0	NA	0	NA	0	NA	0
PI 480312	NA	3.0	NA	NA	0	NA	0	NA	NA
PI 480313	2.5	2.5	2.0	0	0	0	0	0	0
PI 480461	2.0	2.5	2.0	NA	0	NA	0	NA	0
PI 480462	2.5	3.5	2.0	0	0	0	0	0	0
PI 532304	2.5	3.0	1.0	0	0	0	0	0	0

[a]Rep, Replication. [b]NA, Not available.

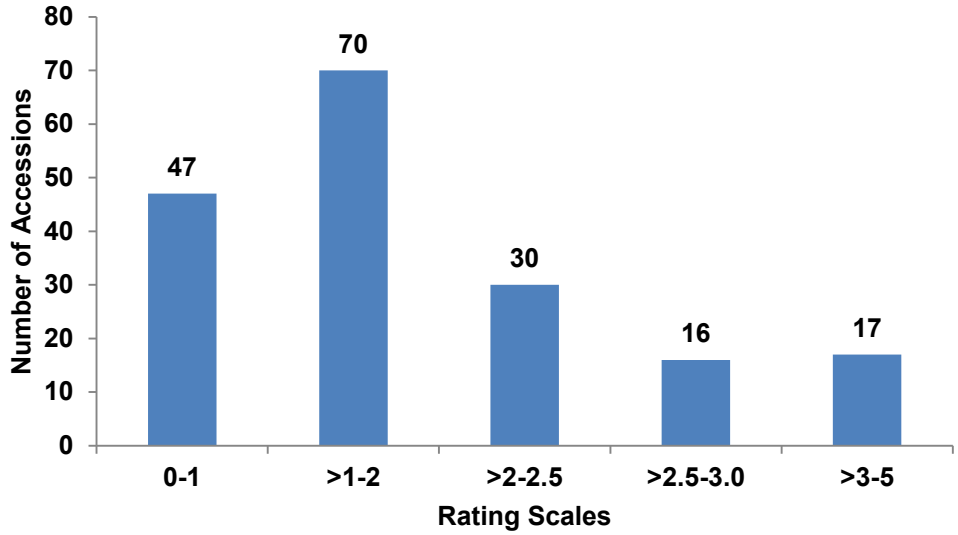

Figure 6.1. Distribution of emmer wheat accessions in each rating scales based on their reactions to the inoculation with *P. nodorum* isolate Sn4. The accessions with lesion types of 0-2.0 was considered as highly or moderately resistant; accessions with lesion types between 2.0 and 3.0 was considered to have intermediate reactions; and accessions with lesion types of >=3.0-5.0 was considered as moderately or highly susceptible. The number of accessions was indicated at the top of each column.

Association Analysis

A best linear model was first chosen based on MSD values (Table 6.5) and Q-Q plot (Figure 6.2) of eight different models. The best model was the GLM model "PC50". At the significant level of 1 percentile, the cutoff *p* values were estimated at 1.14×10^{-3}. The SNP with a *p* value smaller than the cutoff *p* value identified by the best model was considered to be significantly associated with susceptibility to *P. nodorum* isolate Sn4. A total of 42 significant markers (1 percentile) were detected (Tables 6.2). These markers were located on 14 A- and B-genome chromsomes. Their genomic positions and significant levels were shown in the Manhattan plots (Figure 6.3). The phenotypic variation (R^2) explained by each marker ranged from 0.04% to 30.42% (Table 6.2). Stepwise regression analysis showed that nine markers on seven chromosomes had major effects on Sn4 susceptibility, and they together explained phenotypic variation 71.25%.

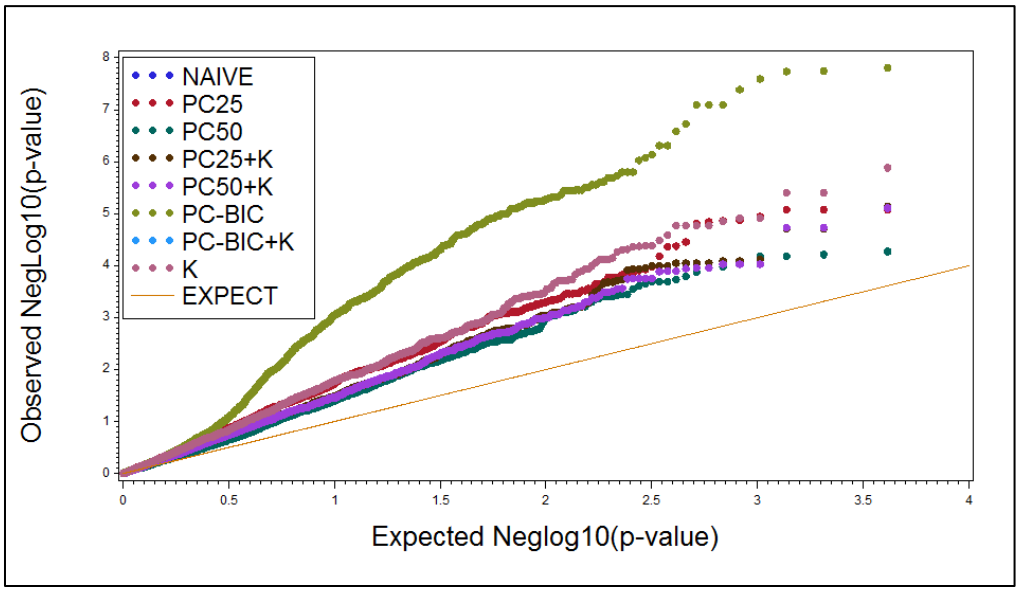

Figure 6.2. Comparison of Quantile-Quantile (Q-Q) plots for the different eight GWAS models used for identifying the significant association relationship between SNPs and the susceptibility to *P. nodorum* isolate Sn4. The X axis is the expected -Log_{10}(*p* value), and the Y axis is the observed -Log_{10}(*p* value). One naïve model and seven models with different methods of adjusting population structures were compared.

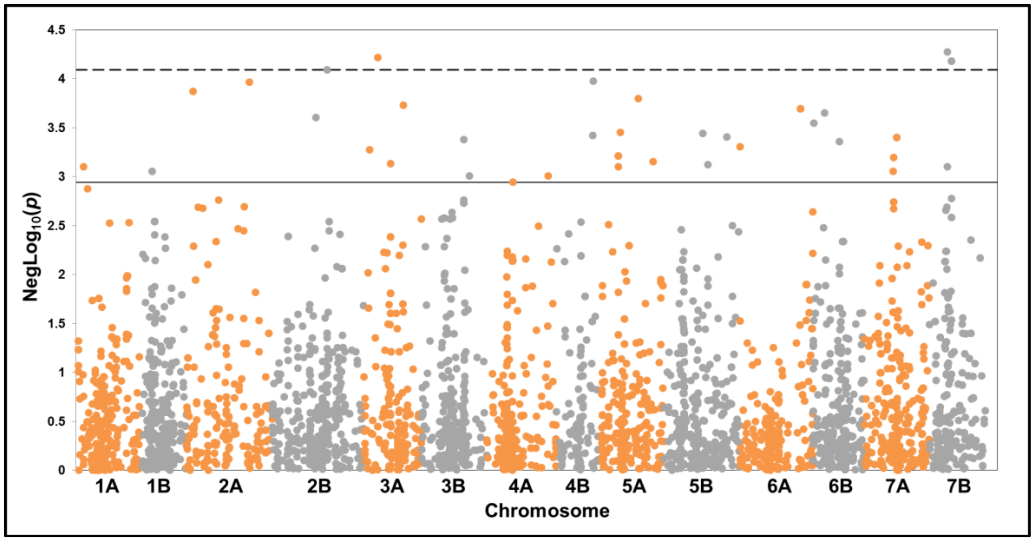

Figure 6.3. Manhattan Plots for the best model used for SNP - Sn4 susceptibility association analysis. *x* axis show the genetic positions of SNPs along each chromosome; y axis show the negative $\log_{10}$(*p*-value) of associations. The black solid and dashed horizontal lines indicate the significant levels of 1 percentile and 0.1 percentile, respectively. The dots above different horizontal lines represent the identified significant markers associated with disease resistance at different significant levels.

Among the significant markers, nine were at or near to genomic regions containing known genes or QTL on chromosomes 4B (*QSng.sfr-4BL* and *Snn5*), 5B (*Tsn1*, *QSng.sfr-5BL*, and *QSnb.ndsu-5B*), 6A (*QSnl.ihar-6AL*), 7A (*QSnb.fcu-7A*), and 7B (*QSnl.eth-7B1*) (Table 6.2). The remaining significant markers on chromosomes 1A, 1B, 2A, 2B, 3A, 3B, 4A, 5A, 5B, 6A, 6B, 7A, and 7B did not cover the genomic regions with known SNB susceptibility genes or QTL based on the three genetic maps that were used. The genomic regions with those markers likely harbor novel SNB susceptibility genes.

Table 6.2. Significant markers associated with susceptibility to *P. nodorum* isolate Sn4 at the significant level of 1 percentile.

SNP[a]	Chr[b]	Position (cM)	NegLog$_{10}$(p)	MAF[c]	R^2 (%)	SRI[d]	Near gene/QTL	Ref[e]
IWA3393	7B	54.08	4.27	0.21	30.18		*QSnl.eth-7B1*	6
IWA7552	3A	42.37	4.21	0.07	4.69			
IWA3852	7B	65.56	4.18	0.43	2.50			
IWA3854	7B	65.56	4.18	0.43	2.50			
IWA6169	2B	163.38	4.09	0.06	3.09			
IWA2031	4B	110.59	3.98	0.23	1.15		*QSng.sfr-4BL*; *Snn5*	1, 2
IWA5855	2A	186.14	3.96	0.06	4.88			
IWA4441	2A	18.65	3.87	0.26	10.08	Yes		
IWA7579	5A	119.74	3.80	0.42	2.90			
IWA445	3A	119.09	3.73	0.24	26.60	Yes		
IWA2705	6A	180.19	3.69	0.06	19.74	Yes	*QSnl.ihar-6AL*	4
IWA3487	6A	180.19	3.69	0.06	19.74		*QSnl.ihar-6AL*	4
IWA3488	6A	180.19	3.69	0.06	19.74		*QSnl.ihar-6AL*	4
IWA5282	6B	34.34	3.65	0.06	8.32			
IWA6778	2B	130.36	3.60	0.11	11.37			
IWA4997	6B	2.58	3.55	0.16	0.09			
IWA6881	5A	65.79	3.45	0.42	0.04	Yes		
IWA951	5B	115.34	3.44	0.39	4.27		*QSng.sfr-5BL*; *QSnb.ndsu-5B*; *Tsn1*	1, 3
IWA5358	4B	108.15	3.42	0.40	3.27		*QSng.sfr-4BL*; *Snn5*	1, 2
IWA7340	5B	187.90	3.40	0.21	2.70			
IWA4529	7A	97.25	3.40	0.08	3.17		*QSnb.fcu-7A*	5
IWA6829	7A	97.25	3.40	0.08	3.17		*QSnb.fcu-7A*	5
IWA8490	3B	127.10	3.38	0.26	25.92			
IWA1839	6B	78.05	3.36	0.23	26.32			
IWA8336	6A	0.94	3.31	0.06	4.32			
IWA4257	3A	18.76	3.27	0.49	0.17			
IWA7130	5A	59.97	3.21	0.27	30.42	Yes		
IWA7129	5A	59.97	3.21	0.27	30.42			
IWA4063	7A	87.49	3.20	0.24	2.76			

Table 6.2. Significant markers associated with susceptibility to *P. nodorum* isolate Sn4 at the significant level of 1 percentile (Continued).

SNP[a]	Chr[b]	Position (cM)	NegLog$_{10}$(p)	MAF[c]	R^2 (%)	SRI[d]	Near gene/QTL	Ref[e]
IWA2282	5A	162.91	3.15	0.08	26.46	Yes		
IWA7319	3A	81.44	3.13	0.28	1.11			
IWA4829	5B	130.39	3.12	0.16	6.84			
IWA7191	1A	21.74	3.10	0.31	1.85	Yes		
IWA8254	7B	54.08	3.10	0.21	27.53			
IWA5107	5A	60.32	3.10	0.39	10.36			
IWA139	1B	38.15	3.05	0.10	1.95			
IWA5895	7A	85.51	3.05	0.31	22.04	Yes		
IWA3943	NA	NA	3.05	0.28	21.80			
IWA348	NA	NA	3.03	0.15	2.93			
IWA6774	4A	183.69	3.01	0.07	8.68			
IWA1094	3B	142.78	3.00	0.44	3.36			
IWA101	4A	77.84	2.94	0.44	1.70	Yes		

[a]The bold significant SNPs are also significant at the level of 0.1 percentile. [b]Chr, Chromosome; NA, Not available; [c]MAF, Minor allele frequency; [d]SRI, Stepwise regression included. [e]Ref, References: 1, Schnurbusch et al., 2003; 2, Friesen et al., 2012; 3, Gonzalez-Hernandez et al., 2009; 4, Arseniuk et al., 2004; 5, Abeysekara et al., 2012; 6, Aguilar et al., 2005.

Five LD blocks with significant SNPs were identified and located on four chromosomes (3A, 6A, 7A, and 7B) (Table 6.3). Among the five LD blocks, three had their markers at the same map position and two had markers at different positions with block lengths of 1.02 cM and 1.39 cM, respectively. Based on the mapped positions of the significant markers in LD blocks, three blocks were found to be at or close to genomic regions containing previously identified genes or QTL (Table 5.3). Based on the result of stepwise regression analysis, the second LD block harboring a significant marker with major effects was a putative major QTL, and the remaining four were putative minor QTL. The LD block with major effects was near to a known QTL, *QSnl.ihar-6AL* on chromosome 6A.

Table 6.3. Linkage disequilibrium (LD) blocks including one or several significant markers associated with the susceptibility to *P. nodorum* isolate Sn4.

LD block	Chr[a]	Pos[b](cM)	SNP	Extended length(cM)[c]	Near gene/QTL	Ref[d]
1	3B	127.10	*IWA8490**, *IWA5982*	0		
2	6A	180.19	***IWA2705****, *IWA3487**, *IWA3488**	0	*QSnl.ihar-6AL*	1
3	7A	97.25	*IWA4529**, *IWA6829**	0	*QSnb.fcu-7A*	2
4	7B	54.08	*IWA3393**, *IWA8254*	1.39	*QSnl.eth-7B1*	3
		55.47	*IWA375*			
5	7B	64.54	*IWA8469*	1.02		
		65.56	*IWA3852**, *IWA3854**			

*Significant markers. The bold significant markers are stepwise regression model included markers.
[a]Chr, Chromosome. [b]Pos, Positions. [c]0 means that the SNPs in this LD block are at the same position. [d]Ref, Reference: 1, Arseniuk et al., 2004; 2, Abeysekara et al., 2012; 3, Aguilar et al., 2005.

Based on the number of markers fit into the stepwise regression, 30 allelic combinations of the nine significant markers with major effects on SNB susceptibility were identified (Table 6.6). The average reaction of the accessions with specific allelic combination could be used for inferring the resistance or susceptibility of an accession (Table 6.6). Seventeen, seven, and six allelic combinations have averaged highly or moderately resistant, intermediate, and highly or moderately susceptible reactions to SNB, respectively. The combinations of stepwise regression

analysis included significant markers could be used for marker-assisted selection in breeding programs.

Discussion

Cultivated emmer wheat is a good source of *R* genes for resistance to several biotrophic pathogens that are causal agents for several major diseases such as stem rust, leaf rust, and powdery mildew in wheat. A number of major *R* genes such as *Sr2, Sr9d, Sr9e, Sr13, Sr14,* and *Sr17* for stem rust, *Lr14a* and *Lr27* for leaf rust, and *Pm5a* for powdery mildew were identified in cultivated emmer and transferred into common wheat and/or durum wheat (McIntosh et al., 2013). Although several studies have shown that a high level of resistance to *P. nodorum* is present in many cultivated emmer accessions (Xu et al, 2004a; Singh et al., 2006), the responsible resistance genes have not been identified from cultivated emmer germplasm. In the cultivated emmer panel used in this study, a majority of accessions (65%) had high and moderate resistance to *P. nodorum* isolate Sn4; they may be a useful resource for identifying resistance genes for SNB. This cultivated emmer panel had 19 (11%) accessions with moderately susceptible or susceptible reactions to Sn4. Because most of the accessions in this panel were insensitive to the three HSTs (SnToxA, SnTox1, and SnTox3) produced by Sn4, the susceptibility in most of the 19 emmer accessions should not be caused by the interactions SnToxA-*Tsn1*, SnTox1-*Snn1*, and SnTox3-*Snn3*. Some of Sn4-susceptible accessions that are insensitive to the three HSTs may carry uncharacterized susceptible genes.

Association analysis has shown that 42 SNP markers on 14 chromosomes were significantly associated with SNB susceptibility. Based on the genomic positions of the 42 significant markers, nine significant markers and three LD blocks were found to be at the same position or near to the genomic regions with known SNB susceptibility genes or QTL, including

Tsn1, *Snn5*, *QSng.sfr-4BL*, *QSng.sfr-5BL*, *QSnb.ndsu-5B*, *QSnl.ihar-6AL*, *QSnb.fcu-7A*, and *QSnl.eth-7B1* (Table 6.2, Table 6.3). None of the previously identified genes and QTL related to SNB susceptibility was derived from cultivated emmer wheat. However, it was reported that the common wheat cultivar Hope harbored a *Tsn1* locus (Anderson et al., 1999). A cross between emmer cultivar Yaroslav and common wheat variety Marquis generated Hope (McFadden, 1930). Thus, the *Tsn1* in Hope may be derived from Yaroslav emmer and other emmer accessions could also harbor *Tsn1*. The dominant allele of *Tsn1* confers wheat susceptibility to SNB through indirectly interacting with SnToxA (Faris et al., 2010). In this study, the significant marker *IWA951* on 5B associated with Sn4 susceptibility located near this gene. Based on the fact that most emmer accessions in this study were insensitive to SnToxA, the genomic regions with *IWA951* should carry the recessive allele (*tsn1*) of the locus *Tsn1* or any unknown SNB resistance genes that are closely linked to *Tsn1*. Except for *Tsn1*, no evidence shows that other known genes and QTL were originally derived from cultivated emmer, so the genomic regions containing the significant markers and LD block near these genes are unlikely to harbor these known genes. It is possible that these genomic regions may contain unknown genes near to these known genes or QTL.

Except for the nine SNPs associated with genomic regions with known genes or QTL, the remaining 33 SNPs located at the genomic regions where no known SNB susceptibility genes were identified on chromosomes 1A, 1B, 2A, 2B, 3A, 3B, 4A, 5A, 5B, 6A, 6B, 7A, and 7B. The regions on 3BL and 5BL were also identified to associate with quantitative resistance to SNB in another association mapping study of resistance to SNB isolate Sn2000 in spring wheat landraces by Adhikari et al. (2011). These genomic regions most likely harbor the novel SNB susceptibility/resistance genes. The cultivated emmer accessions with high levels of

susceptibility and associated alleles in the marker loci at these regions may be useful materials for identification of new HST-susceptibility gene interaction system through linkage mapping analysis using bi-parental mapping populations.

In summary, the results from GWAS in this study provide preliminary evidence of the genomic regions associated with SNB susceptibility or resistance in cultivated emmer wheat. Although identification of SNB susceptibility and resistance genes requires linkage analysis using bi-parental mapping populations, the results from this study provide guidance for searching for genomic regions harboring target genes. The identified LD blocks, especially for the LD block with major effects, will be at the candidate regions. The allelic combinations of significant markers selected by stepwise regression can be used for identifying cultivated emmer accessions with SNB susceptibility or resistance genes located in targeted genomic regions. Therefore, this genome-wide association study provides the first step towards identifying novel HST-susceptibility gene interaction systems in emmer wheat and pyramiding resistance loci from emmer wheat for MAS breeding. The knowledge gained from this study may help increasing the genetic diversity for resistance to SNB in modern durum and bread wheat germplasm.

Supplementary Tables

Table 6.4. Analysis of variance of the disease reactions of 180 emmer wheat accessions to *P. nodorum* isolate Sn4.

Source	DF[a]	Sum of squares	Mean square	F value	Pr > F
Accession	179	406.89	2.27	3.68	<.0001
Replication	2	68.50	34.25	55.51	<.0001
Error	337	207.96	0.62		
Corrected Total	518	683.35			

[a]DF, Degree of freedom.

Table 6.5. Mean square difference (MSD) values of eight GWAS models used for identifying significant associations between SNPs and susceptibility to *P. nodorum* isolate Sn4.

Model	Sn4
Naïve	2.75E-02
PC25	2.07E-02
PC50	5.02E-03
PC25+K	1.09E-02
PC50+K	1.05E-02
PC-BIC	2.75E-02
PC-BIC+K	1.91E-02
K	1.91E-02

Table 6.6. Allelic combinations of stepwise included significant markers (1 percentile) associated with susceptibility to *P. nodorum* isolate Sn4 and their sample statistical analysis of phenotypic data.

Allelic combination[a]	No. of obs[b]	Mean (Lesion type)	Std dev[c]	Min[d]	Max[e]	Reaction[f]
ABBBABBAB	2	0.33	0.24	0.17	0.50	R
BBBBABBBB	1	0.33	.	0.33	0.33	R
ABBAABAAB	1	0.67	.	0.67	0.67	R
BBBAABBBB	16	0.78	0.35	0.17	1.33	R
BBBAABABB	20	0.90	0.38	0.33	1.67	R
BBBBBBBBB	2	0.92	0.12	0.83	1.00	R
ABBBBBBAB	5	1.00	0.12	0.83	1.17	R
BABAABABB	3	1.22	0.82	0.67	2.17	R
BABBABBBB	2	1.25	0.59	0.83	1.67	R
BBBBBBABB	3	1.44	0.84	0.67	2.33	R
ABBABBBAB	1	1.50	.	1.50	1.50	R
BBBAABBBA	1	1.67	.	1.67	1.67	R
BBBBBBBBA	52	1.75	0.52	0.67	3.00	R
ABBBBBAAB	4	1.85	0.69	1.17	2.75	R
BBBBBBABA	21	1.94	0.55	0.75	2.83	R
AABBBBBAB	7	1.98	0.51	1.17	2.67	R
BBAAABBBB	1	2.00	.	2.00	2.00	R
BABBBBBAB	1	2.17	.	2.17	2.17	I
AABABBBAB	11	2.26	0.52	1.33	3.00	I
ABBABBBAA	1	2.33	.	2.33	2.33	I
BBBABBBAB	1	2.50	.	2.50	2.50	I
BABBBBBBA	1	2.67	.	2.67	2.67	I
ABAABBBAA	1	2.83	.	2.83	2.83	I
AABABABAB	7	2.86	0.60	1.67	3.33	I
BBBBBBBAA	1	3.17	.	3.17	3.17	S
BBBBBBBAB	1	3.17	.	3.17	3.17	S
BAAAAABAB	1	3.50	.	3.50	3.50	S
AAAABABAB	7	3.62	0.33	3.17	4.17	S
BABABBAAB	2	3.75	0.35	3.50	4.00	S
BABABBAAA	1	4.17	.	4.17	4.17	S

[a]A and B refer to the alleles in the 9K SNP wheat chip.
Orders of SNPs associated with Sn4 susceptibility: *IWA4441, IWA445, IWA2705, IWA6881, IWA7130, IWA2282, IWA7191, IWA5895, IWA101*.
[b]Number of observations; [c]Standard deviation; [d]Min, minimum; [e]Max, Maximum;
[f]R, highly or moderately resistant (lesion type <= 2.0); I, Intermediate reaction (2.0 < lesion type < 3.0); S, highly or moderately susceptible (lesion type >= 3.0).

Literature Cited

Abeysekara, N.S., J.D. Faris, S. Chao, P.E. McClean and T.L. Friesen. 2012. Whole-genome QTL analysis of *Stagonospora nodorum* blotch resistance and validation of the SnTox4–Snn4 interaction in hexaploid wheat. Phytopathology 102: 94-104.

Abeysekara, N.S., T.L. Friesen, B. Keller and J.D. Faris. 2009. Identification and characterization of a novel host-toxin interaction in the wheat-*Stagonospora nodorum* pathosystem. Theoretical and Applied Genetics 120: 117-126.

Adhikari, T.B., E.W. Jackson, S. Gurung, J.M. Hansen and J.M. Bonman. 2011. Association mapping of quantitative resistance to *Phaeosphaeria nodorum* in spring wheat landraces from the USDA National Small Grains Collection. Phytopathology 101: 1301-1310.

Aguilar, V., P. Stamp, M. Winzeler, H. Winzeler, G. Schachermayr, B. Keller, et al. 2005. Inheritance of field resistance to *Stagonospora nodorum* leaf and glume blotch and correlations with other morphological traits in hexaploid wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 111: 325-336.

Anderson, J.A., R.J. Effertz, J.D. Faris, L.J. Francl, S.W. Meinhardt and B.S. Gill. 1999. Genetic analysis of sensitivity to a *Pyrenophora tritici-repentis* necrosis-inducing toxin in durum and common wheat. Phytopathology 89: 293-297.

Arseniuk, E., P. Czembor, A. Czaplicki, Q. Song, P. Cregan, D. Hoffman, et al. 2004. QTL controlling partial resistance to Stagonospora nodorum leaf blotch in winter wheat cultivar Alba. Euphytica 137: 225-231.

Breseghello, F. and M.E. Sorrells. 2006. Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172: 1165-1177.

Cheung, M.-Y., N.-Y. Zeng, S.-W. Tong, F. Wing-Yen Li, K.-J. Zhao, Q. Zhang, et al. 2007. Expression of a RING-HC protein from rice improves resistance to *Pseudomonas syringae* pv. *tomato* DC3000 in transgenic *Arabidopsis thaliana*. Journal of Experimental Botany 58: 4147-4159.

Chu, C.G., S. Chao, T.L. Friesen, J.D. Faris, S. Zhong and S.S. Xu. 2010. Identification of novel tan spot resistance QTLs using an SSR-based linkage map of tetraploid wheat. Molecular Breeding 25: 327-338.

Chu, C.G., T.L. Friesen, S.S. Xu and J.D. Faris. 2008. Identification of novel tan spot resistance loci beyond the known host-selective toxin insensitivity genes in wheat. Theoretical and Applied Genetics 117: 873-881.

Eyal, Z. 1999. Breeding for resistance to *Septoria* and *Stagonospora* diseases of wheat. In: J. A. Lucas, P. Bowyer and A. M. Anderson, editors, *Septoria* on cereals: a study of pathosystems IACR Long Ashton Research Station, Bristol, UK. p. 332-344.

Faris, J.D., Z. Zhang, H. Lu, S. Lu, L. Reddy, S. Cloutier, et al. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proceedings of the National Academy of Sciences 107: 13544-13549.

Francki, M.G., M. Shankar, E. Walker, R. Loughman, H. Golzar and H. Ohm. 2011. New quantitative trait loci in wheat for flag leaf resistance to Stagonospora nodorum blotch. Phytopathology 101: 1278-1284.

Friesen, T.L., C. Chu, S.S. Xu and J.D. Faris. 2012. SnTox5–*Snn5*: a novel *Stagonospora nodorum* effector–wheat gene interaction and its relationship with the SnToxA–*Tsn1* and SnTox3–*Snn3–B1* interactions. Molecular Plant Pathology 13: 1101-1109.

Friesen, T.L. and J.D. Faris. 2010. Characterization of the wheat-*Stagonospora nodorum* disease system: what is the molecular basis of this quantitative necrotrophic disease interaction? Canadian Journal of Plant Pathology 32: 20-28.

Friesen, T.L., S.W. Meinhardt and J.D. Faris. 2007. The *Stagonospora nodorum*-wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. The Plant Journal 51: 681-692.

Friesen, T.L., E.H. Stukenbrock, Z. Liu, S. Meinhardt, H. Ling, J.D. Faris, et al. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. Nature Genetics 38: 953-956.

Friesen, T.L., Z. Zhang, P.S. Solomon, R.P. Oliver and J.D. Faris. 2008. Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. Plant Physiology 146: 682-693.

Gao, Y., J.D. Faris, Z. Liu, Y. Kim, R.A. Syme, et al. 2015. Identification and characterization of the SnTox6-*Snn6* interaction in the *Parastagonospora nodorum* - wheat pathosystem. Molecular Plant-Microbe Interactions. DOI: 10.1094/MPMI-12-14-0396-R.

Glickman, M.H. and A. Ciechanover. 2002. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiological Reviews 82: 373-428.

Gonzalez-Hernandez, J.L., P.K. Singh, M. Mergoum, T.B. Adhikari, S.F. Kianian, S. Simsek, et al. 2009. A quantitative trait locus on chromosome 5B controls resistance of *Triticum turgidum* (L.) var. *diccocoides* to Stagonospora nodorum blotch. Euphytica 166: 199-206.

Goritschnig, S., Y. Zhang and X. Li. 2007. The ubiquitin pathway is required for innate immunity in Arabidopsis. The Plant Journal 49: 540-551.

Hopkins, M.T., Y. Lampi, T.-W. Wang, Z. Liu and J.E. Thompson. 2008. Eukaryotic translation initiation factor 5A is involved in pathogen-induced cell death and development of disease symptoms in Arabidopsis. Plant Physiology 148: 479-489.

Keller, S.M., J.M. McDermott, R.E. Pettway, M.S. Wolfe and B.A. McDonald. 1997a. Gene flow and sexual reproduction in the wheat glume blotch pathogen *Phaeosphaeria nodorum* (anamorph *Stagonospora nodorum*). Phytopathology 87: 353-358.

Keller, S.M., M.S. Wolfe, J.M. McDermott and B.A. McDonald. 1997b. High genetic similarity among populations of *Phaeosphaeria nodorum* across wheat cultivars and regions in Switzerland. Phytopathology 87: 1134-1139.

Letta, T., P. Olivera, M. Maccaferri, Y. Jin, K. Ammar, A. Badebo, et al. 2014. Association mapping reveals novel stem rust resistance loci in durum wheat at the seedling stage. Plant Genome 7: -. doi:10.3835/plantgenome2013.08.0026.

Liu, Z., J.D. Faris, R.P. Oliver, K.-C. Tan, P.S. Solomon, M.C. McDonald, et al. 2009. SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. PLoS Pathogens 5: e1000581.

Liu, Z., Z. Zhang, J.D. Faris, R.P. Oliver, R. Syme, M.C. McDonald, et al. 2012. The cysteine rich necrotrophic effector SnTox1 produced by *Stagonospora nodorum* triggers susceptibility of wheat lines harboring *Snn1*. PLoS Pathogens 8: e1002467.

Liu, Z.H., J.D. Faris, S.W. Meinhardt, S. Ali, J.B. Rasmussen and T.L. Friesen. 2004a. Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially purified host-selective toxin produced by *Stagonospora nodorum*. Phytopathology 94: 1056-1060.

Liu, Z.H., T.L. Friesen, J.B. Rasmussen, S. Ali, S.W. Meinhardt and J.D. Faris. 2004b. Quantitative trait loci analysis and mapping of seedling resistance to Stagonospora nodorum leaf blotch in wheat. Phytopathology 94: 1061-1067.

Ma, K., J. Xiao, X. Li, Q. Zhang and X. Lian. 2009. Sequence and expression analysis of the C3HC4-type RING finger gene family in rice. Gene 444: 33-45.

Machacek, J.E. 1945. The prevalence of *Septoria* on cereal seed in Canada. Phytopathology 35: 51-53.

Mamidi, S., R.K. Lee, J.R. Goos and P.E. McClean. 2014. Genome-wide association studies identifies seven major regions responsible for iron deficiency chlorosis in soybean (*Glycine max*). PloS One 9(9): e107469.

McDonald, B.A., J. Miles, L.R. Nelson and R.E. Pettway. 1994. Genetic variability in nuclear DNA in field populations of *Stagonospora nodorum*. Phytopathology 84: 250-255.

McFadden, E.S. 1930. A successful transfer of emmer characters to *vulgare* wheat. Journal of the American Society of Agronomy 22: 1020-1034.

McIntosh, R.A., Y. Yamazaki, J. Dubcovsky, J. Rogers, C. Morris, R. Appels, et al. 2013. Catalogue of gene symbols for wheat. 12th International Wheat Genetics Symposium, Yokohama, Japan

McMullen, M.P., R. Jones and D. Gallenberg. 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Disease 81:1340-1348.

Mebrate, S. and B.M. Cooke. 2001. Response of wheat cultivars to infection by *Stagonospora nodorum* isolates/mixture on detached and intact seedling leaves. Euphytica 122: 263-268.

Mergoum, M., P.K. Singh, S. Ali, E.M. Elias, J.A. Anderson, K.D. Glover, et al. 2007. Reaction of elite wheat genotypes from the northern Great Plains of North America to *Septoria* diseases. Plant Disease 91: 1310-1315.

Murray, G.M. and J.P. Brennan. 2009. Estimating disease losses to the Australian wheat industry. Australasian Plant Pathology 38: 558-570.

Nelson, L.R., D.D. Morey and A.R. Brown. 1974. Wheat cultivar responses to severe glume blotch in Georgia. Plant Disease Reporter 58: 21-23.

Oliver, R.P., T.L. Friesen, J.D. Faris and P.S. Solomon. 2012. *Stagonospora nodorum*: from pathology to genomics and host resistance. Annual Review of Phytopathology 50: 23-43.

Oliver, R.P. and P.S. Solomon. 2010. New developments in pathogenicity and virulence of necrotrophs. Current Opinion In Plant Biology 13: 415-419.

Ravel, C., S. Praud, A. Murigneux, L. Linossier, M. Dardevet, F. Balfourier, et al. 2006. Identification of *Glu-B1-1* as a candidate gene for the quantity of high-molecular-weight glutenin in bread wheat (*Triticum aestivum* L.) by means of an association study. Theoretical and Applied Genetics 112: 738-743.

Robaglia, C. and C. Caranta. 2006. Translation initiation factors: a weak link in plant RNA virus infection. Trends in Plant Science 11: 40-45.

SAS Institute. 2011. The SAS system for Windows. SAS Inst., Cary, NC.

Scharen, A.L. and J.M. Krupinsky. 1969. Effect of *Septoria nodorum* infection on CO_2 absorption and yield of wheat. Phytopathology 59: 1228-1301.

Schnurbusch, T., S. Paillard, D. Fossati, M. Messmer, G. Schachermayr, M. Winzeler, et al. 2003. Detection of QTLs for Stagonospora glume blotch resistance in Swiss winter wheat. Theoretical and Applied Genetics 107: 1226-1234.

Singh, P.K., M. Mergoum, S. Ali, T.B. Adhikari, E.M. Elias and G.R. Hughes. 2006. Identification of new sources of resistance to tan spot, *Stagonospora nodorum* blotch, and *Septoria tritici* blotch of wheat. Crop Science 46: 2047-2053.

Tommasini, L., T. Schnurbusch, D. Fossati, F. Mascher and B. Keller. 2007. Association mapping of *Stagonospora nodorum* blotch resistance in modern European winter wheat varieties. Theoretical and Applied Genetics 115: 697-708.

Wang, Y.-S., L.-Y. Pi, X. Chen, P.K. Chakrabarty, J. Jiang, A.L. De Leon, et al. 2006. Rice XA21 binding protein 3 is a ubiquitin ligase required for full *Xa21*-mediated disease resistance. The Plant Cell 18: 3635-3646.

Weber, G.F. 1922. *Septoria* diseases of cereals. II. *Septoria* diseases of Wheat. Phytopathology 12: 537-585.

Xu, S.S., Friesen, T.L., and Cai, X. 2004a. Sources and genetic control of resistance to Stagonospora nodorum blotch in wheat. In: Recent Research Development in Genetics and Breeding 1:449-469. Research Signpost, Kerala, India.

Xu, S.S., T.L. Friesen and A. Mujeeb-Kazi. 2004b. Seedling resistance to tan spot and Stagonospora nodorum blotch in synthetic hexaploid wheats. Crop Science. 44: 2238-2245.

Zhang, Z., T.L. Friesen, S.S. Xu, G. Shi, Z. Liu, J.B. Rasmussen, et al. 2011. Two putatively homoeologous wheat genes mediate recognition of SnTox3 to confer effector-triggered susceptibility to *Stagonospora nodorum*. The Plant Journal 65: 27-38.

APPENDIX

Table A1. Origin and accession types of the *Triticum turgidum* ssp. *dicoccum* panel used in this study.

Accession name	Origin	Type	Latitude (degree)	Longitude (degree)	Elevation (meters)	Alternative ID
CItr12213-1	India	Uncertain	25.00	86.00	NA[a]	Garden; CI 12213
CItr14085	Unknown	Uncertain	NA	NA	NA	CI 14085
CItr14086	Unknown	Uncertain	NA	NA	NA	CI 14086
CItr14098	Ethiopia	Landrace	8.55	38.87	2128	ELS 6304-57; CI 14098
CItr14133-1	United States	Wild material	34.00	-112.00	NA	CI 14133
CItr14621-1	Ethiopia	Landrace	8.90	38.97	2780	ELS 6304-72; CI 14621
CItr14637-1	Ethiopia	Landrace	9.38	41.60	1982	ELS 6404-78-1; CI 14637
CItr14639	Ethiopia	Landrace	9.38	41.60	1982	ELS 6404-78-2; CI 14639
CItr14751	Ethiopia	Landrace	9.77	38.73	2818	ELS 6404-108-5; CI 14751
CItr14822	Eritrea	Landrace	NA	NA	2141	ELS 6404-128-6; ICARDA-IG-45393; CI 14822
CItr14834	Ethiopia	Landrace	13.13	39.55	2050	ELS 6404-132; CI 14834
CItr14866	Ethiopia	Landrace	10.35	37.73	2403	ELS 6404-142-1; CI 14866
CItr14916-1	Ethiopia	Landrace	8.87	38.78	2068	ELS 6404-165-1; CI 14916
CItr14917-1	Ethiopia	Landrace	8.87	38.78	2068	ELS 6404-165-2; CI 14917
CItr14919-1	Unknown	Uncertain	NA	NA	NA	CI 14919
CItr14971	Unknown	Uncertain	NA	NA	NA	CI 14971
CItr3686	United States	Uncertain	46.00	-94.00	NA	Vernal Emmer; CI 3686
CItr4013	India	Cultivar	22.75	77.72	303	Khapli; CI 4013
CItr7685	Russian Federation	Landrace	NA	NA	NA	CI 7685
CItr7686	Russian Federation	Landrace	NA	NA	NA	CI 7686
CItr7687-1	Russian Federation	Landrace	NA	NA	NA	CI 7687
CItr7779	Ethiopia	Landrace	9.03	38.70	2402	340; CI 7779
CItr7962	Ethiopia	Landrace	11.50	40.00	2745	493; CI 7962
PI101971-1	India	Landrace	19.50	75.00	NA	Khapli; ICARDA-IG-88761
PI113961	Georgia	Wild material	NA	NA	NA	28170
PI133134-1	Peru	Uncertain	-6.33	-80.00	NA	Lambayaque; ICARDA-IG-88762
PI154582-1	Taiwan	Uncertain	NA	NA	NA	NA
PI164578-1	India	Landrace	11.40	76.70	2135	9000; ICARDA-IG-88763
PI168673-1	United States	Cultivar	45.00	-90.00	NA	Vernal; CItr 1524; CI 1524
PI168675	Ethiopia	Landrace	NA	NA	NA	16; CItr 8641; CI 8641
PI190920	Portugal	Uncertain	NA	NA	NA	2323A
PI190926	Belgium	Uncertain	NA	NA	NA	2475
PI191091	Spain	Landrace	43.10	-5.80	484	Escanda de Malvedo; 2153
PI193641-1	Ethiopia	Landrace	9.03	38.70	2402	8555

Table A1. Origin and accession types of the *Triticum turgidum* ssp. *dicoccum* panel used in this study (Continued).

Accession name	Origin	Type	Latitude (degree)	Longitude (degree)	Elevation (meters)	Alternative ID
PI193643	Ethiopia	Landrace	9.03	38.70	2402	8591
PI193873-1	Ethiopia	Landrace	9.03	38.70	2402	8818
PI193878	Ethiopia	Landrace	9.03	38.70	2402	8929
PI193879-1	Ethiopia	Landrace	9.03	38.70	2402	8930
PI193880-1	Ethiopia	Landrace	9.03	38.70	2402	8931
PI193882-1	Ethiopia	Landrace	9.03	38.70	2402	8933
PI193883-1	Ethiopia	Landrace	9.03	38.70	2402	8934
PI194042-1	Ethiopia	Landrace	9.03	38.70	2402	8865
PI194375-1	Ethiopia	Landrace	7.67	36.83	1716	9076
PI195721	Ethiopia	Landrace	11.18	40.02	1670	9876
PI196100	Ethiopia	Landrace	11.13	39.63	2495	9907
PI196905	Ethiopia	Landrace	8.73	38.98	1910	10090; ICARDA-IG-85431
PI197482	Ethiopia	Landrace	9.32	42.12	1935	10175
PI197483-1	Ethiopia	Landrace	9.32	42.12	1935	10176
PI197485-1	Ethiopia	Landrace	9.32	42.12	1935	10178
PI197490	Ethiopia	Landrace	9.32	42.12	1935	10183
PI217637-1	India	Landrace	11.35	76.82	1525	13825; ICARDA-IG-45358
PI217639-1	India	Landrace	11.35	76.82	1525	13879
PI217640-1	India	Landrace	11.35	76.82	1525	13882
PI221400-1	Serbia	Uncertain	NA	NA	NA	Farum
PI221401	Serbia	Uncertain	NA	NA	NA	Fictesemicanum
PI225332-1	Iran	Landrace	NA	NA	NA	146; ICARDA-IG-45359; Volgens
PI226951	Ethiopia	Landrace	9.03	38.70	2402	404
PI244341-1	Ethiopia	Landrace	NA	NA	NA	Jimma; ICARDA-IG-45316
PI248991	India	Landrace	17.33	77.90	638	K126; Joad; ICARDA-IG-45305
PI254146	Ethiopia	Landrace	NA	NA	NA	NA
PI254163	Iran	Landrace	NA	NA	NA	223-a-2
PI254165-1	Iran	Landrace	NA	NA	NA	223-a-5
PI254167-1	Iran	Landrace	NA	NA	NA	223-a-7
PI254188	Former Soviet Union	Landrace	NA	NA	NA	NA
PI254189-1	Georgia	Landrace	NA	NA	NA	35900
PI254190-1	Russian Federation	Landrace	42.00	47.00	NA	233
PI254193	Spain	Landrace	41.58	-1.00	NA	2475
PI272527	Hungary	Breeding material	47.42	19.33	NA	I-1-3428

Table A1. Origin and accession types of the *Triticum turgidum* ssp. *dicoccum* panel used in this study (Continued).

Accession name	Origin	Type	Latitude (degree)	Longitude (degree)	Elevation (meters)	Alternative ID
PI272533-1	Hungary	Breeding material	47.42	19.33	NA	I-1-3427; ICARDA-IG-45426
PI273981-1	Ethiopia	Landrace	8.93	38.77	2186	1940
PI273982	Ethiopia	Landrace	9.03	43.02	2501	2098
PI275996-1	Spain	Landrace	42.18	0.33	540	76
PI275997	Spain	Landrace	43.38	-6.07	56	89
PI275998	Spain	Landrace	43.07	-5.77	672	92
PI275999	Spain	Landrace	43.07	-5.77	672	93
PI276000	Spain	Landrace	43.07	-5.77	672	94
PI276005	Spain	Landrace	43.10	-6.25	1012	101
PI276006	Spain	Uncertain	43.37	-5.83	NA	102
PI276007	Spain	Uncertain	43.37	-5.83	NA	103
PI276012	Spain	Uncertain	43.37	-5.83	NA	111
PI276014	Spain	Landrace	43.07	-5.77	672	118
PI277670	Spain	Landrace	43.10	-5.80	484	Escandia de Malvedo; 2153
PI277671	Spain	Landrace	42.75	-1.67	NA	Escandia; 2155
PI277677	Spain	Uncertain	NA	NA	NA	Rufum; 2475
PI286061	Poland	Uncertain	NA	NA	NA	Rufum
PI289603	United Kingdom	Uncertain	NA	NA	NA	160; ICARDA-IG-45419
PI295065	Bulgaria	Uncertain	NA	NA	NA	Schwarzer Bartspelz
PI297830	Ethiopia	Landrace	7.02	39.98	2900	ELS 6404-9-B
PI298543	Ethiopia	Landrace	12.78	39.53	2806	ELS 6304-34; 16
PI298548	Ethiopia	Landrace	10.35	37.73	2470	ELS 6404-11; 94
PI298582-1	Ethiopia	Landrace	8.27	39.28	1860	ELS 6404-50; 404
PI306536	Romania	Uncertain	NA	NA	NA	2890
PI310471-1	India	Uncertain	28.75	77.25	NA	Khapli; E-56
PI319869-1	Turkey	Landrace	NA	NA	NA	2
PI322232-1	India	Cultivar	NA	NA	NA	NP 201
PI324076-1	India	Cultivar	NA	NA	NA	NP 200; New Pusa 200; ICARDA-IG-118256
PI330544	United Kingdom	Uncertain	NA	NA	NA	Stratum; 180
PI349043-1	Georgia	Landrace	NA	NA	NA	WIR 6388; ICARDA-IG-45360
PI349045	Russian Federation	Landrace	43.00	47.00	NA	WIR 23917
PI349046-1	Georgia	Landrace	NA	NA	NA	WIR 43848; ICARDA-IG-45401
PI350001	Serbia	Landrace	43.47	19.70	643	596-III/16
PI352335	United States	Uncertain	NA	NA	NA	Vernal; ICARDA-IG-45362; ICARDA-IG-86136; T-487
PI352337	Spain	Uncertain	43.37	-5.83	NA	Asturie L2; T-520

Table A1. Origin and accession types of the *Triticum turgidum* ssp. *dicoccum* panel used in this study (Continued).

Accession name	Origin	Type	Latitude (degree)	Longitude (degree)	Elevation (meters)	Alternative ID
PI352338	Spain	Uncertain	43.37	-5.83	NA	Asturie L6; T-521
PI352341	Spain	Uncertain	43.37	-5.83	NA	Asturie 4B; T-525
PI352342	Spain	Uncertain	43.37	-5.83	NA	Asturie 4D; T-526
PI352358	France	Cultivar	NA	NA	NA	Amidonnier; T-1604
PI352365	Germany	Uncertain	NA	NA	NA	T-2376; ICARDA-IG-86141
PI352548-1	Ethiopia	Uncertain	NA	NA	NA	T-1520
PI355460	Switzerland	Landrace	NA	NA	NA	Emmer 52; ICARDA-IG-45364
PI355461	Germany	Uncertain	NA	NA	NA	T 110
PI355470	Germany	Uncertain	NA	NA	NA	T 2924
PI355475	Germany	Uncertain	NA	NA	NA	68Z99.24; ICARDA-IG-45438
PI355477-1	Canada	Uncertain	55.00	-97.00	NA	Khapli
PI355483	Spain	Uncertain	43.37	-5.83	NA	T 563
PI355485	Spain	Uncertain	43.37	-5.83	NA	T 567
PI355486	Spain	Uncertain	43.37	-5.83	NA	69Z5.45
PI355489	France	Uncertain	NA	NA	NA	69Z5.48
PI355497	Former Soviet Union	Uncertain	NA	NA	NA	69Z5.57
PI355505	Ancient Palestine	Uncertain	NA	NA	NA	69Z5.65; ICARDA-IG-45433
PI355507-1	Turkey	Uncertain	41.01	34.04	830	T 2352
PI361833	Denmark	Uncertain	NA	NA	NA	Adjaz; DN-2314
PI362438	Serbia	Landrace	43.47	19.70	643	III/14-X8
PI362500	Serbia	Landrace	43.58	22.25	225	IV/17-X8
PI362697	Montenegro	Landrace	43.47	19.10	975	VIII/27-X9
PI374685	Bosnia and Herzegovina	Landrace	43.52	18.67	678	223/71
PI377650	Former Yugoslavia	Uncertain	NA	NA	NA	939
PI377655-1	Former Yugoslavia	Uncertain	NA	NA	NA	953
PI377657-1	Former Yugoslavia	Uncertain	NA	NA	NA	973
PI377672	Former Yugoslavia	Uncertain	NA	NA	NA	985
PI384297	Ethiopia	Landrace	13.53	39.57	2440	GAW 33-2
PI384302	Ethiopia	Landrace	13.53	39.57	2440	GAW 33-8
PI384318	Ethiopia	Landrace	9.07	38.48	2430	GAW 47-7
PI384331	Ethiopia	Landrace	13.53	39.57	2440	GAW 33-9
PI384332-1	Ethiopia	Landrace	13.53	39.57	2440	GAW 33-10
PI41024-1	Russian Federation	Landrace	53.00	50.00	NA	417; Farrum; CI 4572; CItr 4572
PI41025	Russian Federation	Landrace	53.00	50.00	NA	859; Rufum; CI 4573; CItr 4573
PI434992-1	Montenegro	Landrace	42.80	18.93	628	62

Table A1. Origin and accession types of the *Triticum turgidum* ssp. *dicoccum* panel used in this study (Continued).

Accession name	Origin	Type	Latitude (degree)	Longitude (degree)	Elevation (meters)	Alternative ID
PI434996	Montenegro	Landrace	43.32	19.43	1098	66
PI470737	Turkey	Landrace	40.77	43.28	1590	Kaplica; 79TK98-506; JAH 0879
PI479957	Ethiopia	Landrace	9.20	38.60	2440	MG 31050
PI479965	Ethiopia	Landrace	9.20	38.60	2440	MG 31058
PI480307	Ethiopia	Landrace	9.50	38.88	2570	MG 31536
PI480312	Ethiopia	Landrace	9.50	38.88	2570	MG 31541
PI480313	Ethiopia	Landrace	9.50	38.88	2570	MG 31542
PI480460-1	Ethiopia	Landrace	9.12	38.38	2620	MG 31714
PI480461	Ethiopia	Landrace	9.12	38.38	2620	MG 31715
PI480462	Ethiopia	Landrace	9.12	38.38	2620	MG 31716
PI532304	Oman	Landrace	23.33	57.33	700	Alas; 7369
PI532305-1	Oman	Landrace	23.83	56.33	500	Alas; 7378
PI58788	Ethiopia	Landrace	8.60	39.12	1766	311; ICARDA-IG-45321; ICARDA-IG-88877; CItr 7814; CI 7814
PI60704	Ethiopia	Landrace	NA	NA	NA	16; CItr 7494; AUS 13091; CI 7494
PI74106	Iran	Landrace	NA	NA	NA	35896; CItr 9309; CI 9309
PI74108-1	Georgia	Landrace	NA	NA	NA	35900; CItr 9311; CI 9311
PI94616-1	Russian Federation	Landrace	NA	NA	NA	232
PI94617	Russian Federation	Landrace	42.00	47.00	NA	233
PI94621-1	Armenia	Landrace	NA	NA	NA	238; ICARDA-IG-45380
PI94625-1	Iran	Landrace	NA	NA	NA	242; ICARDA-IG-45330; ICARDA-IG-88725
PI94626-1	Turkey	Landrace	NA	NA	NA	243
PI94627-1	Asia Minor	Landrace	NA	NA	NA	244
PI94630	Ethiopia	Landrace	NA	NA	NA	247; ICARDA-IG-88729
PI94631-1	Ethiopia	Landrace	NA	NA	NA	248
PI94634-1	Morocco	Landrace	NA	NA	NA	251; ICARDA-IG-45317; ICARDA-IG-88730; AUS 13140
PI94635-1	Iran	Landrace	NA	NA	NA	252; ICARDA-IG-45331; ICARDA-IG-88731
PI94638-1	Iran	Landrace	NA	NA	NA	255; ICARDA-IG-45381
PI94648-1	Italy	Landrace	NA	NA	NA	265; ICARDA-IG-45436
PI94654	Bulgaria	Landrace	NA	NA	NA	271; ICARDA-IG-45332; ICARDA-IG-88739
PI94656-1	Serbia	Landrace	44.83	20.50	71	273
PI94663	Germany	Landrace	NA	NA	NA	280; ICARDA-IG-45350; ICARDA-IG-45414; ICARDA-IG-88746
PI94664-1	Saudi Arabia	Uncertain	NA	NA	NA	Early Spelt; 282
PI94665	Ethiopia	Landrace	NA	NA	NA	286; ICARDA-IG-45385; ICARDA-IG-88747
PI94666-1	Russian Federation	Landrace	42.00	47.00	NA	292

Table A1. Origin and accession types of the *Triticum turgidum* ssp. *dicoccum* panel used in this study (Continued).

Accession name	Origin	Type	Latitude (degree)	Longitude (degree)	Elevation (meters)	Alternative ID
PI94668	Russian Federation	Landrace	42.00	47.00	NA	294
PI94673-1	Armenia	Landrace	NA	NA	NA	298; ICARDA-IG-88751
PI94674-1	Georgia	Landrace	NA	NA	NA	301; ICARDA-IG-45388; ICARDA-IG-88752
PI94675-1	Georgia	Landrace	NA	NA	NA	302; ICARDA-IG-45415; ICARDA-IG-88753
PI94676-1	Russian Federation	Landrace	45.30	40.90	116	342
PI94680	Germany	Landrace	NA	NA	NA	372
PI94738-1	Ukraine	Breeding material	NA	NA	NA	284
PI94747-1	Georgia	Landrace	41.72	44.78	441	301; ICARDA-IG-88760

[a]NA, Not Available.

Table A2. Summary of the statistic models used in marker-trait association analysis.

Model	Equation[a]	Model type[b]	PC matrix[c]	Kinship matrix
Naïve	**y=Xβ+Sα+e**	Simple	NO	NO
PC25	**y=Xβ+Pv+Sα+e**	GLM	YES	NO
PC50	**y=Xβ+Pv+Sα+e**	GLM	YES	NO
PC25+K	**y=Xβ+Pv+Sα+Iu+e**	MLM	YES	YES
PC50+K	**y=Xβ+Pv+Sα+Iu+e**	MLM	YES	YES
PC-BIC	**y=Xβ+Pv+Sα+e**	GLM	YES	NO
PC-BIC+K	**y=Xβ+Pv+Sα+Iu+e**	MLM	YES	YES
K	**y=Xβ+Sα+Iu+e**	MLM	NO	YES

[a] **y**, a vector of phenotypic values; **X, S** and **I,** identity matrices; **β**, a vector of fixed effects except the effects from markers and population structure; **P,** principle component matrix or PC matrix; **v,** a vector of fixed effects from population structure; ***α,*** a vector of fixed effects from each marker; **u,** a vector of random effects regarding to recent ancestry, Var(**u**)=2**KVg, K** is the kinship matrix, **Vg** is the genetic variance; **e** is a vector of residual effects, Var(**e**)=**IV**$_{\mathbf{R}}$, **I** is an identity matrix, $\mathbf{V_R}$ is the residual variance.

[b]Simple, GLM, and MLM represent simple regression model, general linear model, and mixed linear model, respectively.

[c] 'Yes' represents the matrix is included into the model; 'NO' represents the matrix is not included into the model.