IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO TAN SPOT IN DURUM WHEAT

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

By

Nelomie Nayanathara Galagedara

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Pathology

March 2018

Fargo, North Dakota

North Dakota State University
Graduate School

Title

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO TAN SPOT IN DURUM WHEAT

By

Nelomie Nayanathara Galagedara

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

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Zhaohui Liu
Chair

Dr. Justin D. Faris

Dr. Steven S. Xu

Dr. Xuehui Li

Approved:

4/10/2018
Date

Dr. Jack Rasmussen
Department Chair

ABSTRACT

Tan spot, caused by *Pyrenophora tritici-repentis* (*Ptr*), is a major foliar disease on wheat. The pathosystem involves three pairs of necrotrophic effector (NE) and host sensitivity (S) gene interactions, namely Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1*. Additionally, genetic factors conferring race-nonspecific resistance have been identified. The objectives of this study were to map tan spot resistance QTL and investigate the role of NE-S interactions in disease in durum using association and bi-parental mapping. Evaluation of a worldwide collection of durum accessions allowed identifying highly resistant nineteen lines to multiple *Ptr* races. Association mapping revealed genomic regions on chromosomes 1A, 2B and 3B significantly associated with resistance to tan spot, which likely correspond to *Tsc1*, *Tsc2* and race-nonspecific resistance. Using a bi-parental population derived from Ben and PI 41025, we found that ToxA-*Tsn1* interaction plays no significant role in disease, instead a major race-nonspecific QTL on chromosome 5A was identified.

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to my advisor Dr. Zhaohui Liu, Department of Plant Pathology, North Dakota State University for thoughtful advices, inspiring guidance, and encouragement throughout my study. I would like to give many thanks to my committee members, Drs. Justin D. Faris, Steven Xu and Xuehui Li for their valuable comments, suggestions and help in generating and analyzing data. My thanks also go to Dr. Shiaoman Chao who was originally in my research committee, but late left due to her recent retirement. I am also very grateful to my current and past lab members (Gayan Kariyawasam, Malini Jayawardana, Jingwei Guo, Yi Qu) for their continuous support, friendship and sharing of experiences in the lab and greenhouse. I give my sincere gratitude to Dr. Gongjun Shi as well for teaching me the hands-on lab techniques, guidance and support throughout this study. I also would like to extend my sincere thanks to Dr. Shalu Jain and Yuan Liu for their kind support.

In addition, I would like to express my gratitude to all faculty members, staff and fellow graduate students in the Department of Plant Pathology for their support and friendships.

Finally, I express my wholehearted gratitude to my parents, Mr. Dayaratne Galagedara, and Mrs. Soma Elvitigala, my sister Dr. Wathsala Galagedara and my brother Mr. Shehan Galagedara for their encouragement during my M.S. study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

GENERAL INTRODUCTION

Tan spot, also known as yellow leaf spot of wheat, is caused by the fungus *Pyrenophora tritici-repentis* (anamorph: *Drechslera tritici-repentis*). The disease can occur on all types of wheat including common wheat (*Triticum aestivum*) and durum (*T. turgidum*) and has a global distribution. The fungus mainly attacks leaves, particularly flag leaves of wheat, which could lead to a significant yield loss of up to 40%. The fungal infection on the spikes can cause red smudge on kernels, thus leading to a quality loss in durum. The fungal pathogen overwinters on wheat residues in the field which serves as the most important source of primary inoculum. Since 1970s, the outbreaks of wheat tan spot have been reported in many places of the world largely due to a wide adoption of reduced or no tillage farm practices, which has an intention to minimize soil erosion (Horsford 1982; Wegulo 2011).

Symptoms of tan spot mainly include necrosis or chlorosis, which is observed either individually or as a combination of the two. The manifestation of these symptoms is primarily due to the production of fungal-produced host selective toxins, now known as necrotrophic effectors (NEs), which specifically interact with the corresponding host sensitivity genes. Because the interactions of NE-host gene lead to susceptibility, the disease system has been desribed to fit an inverse gene-for-gene model. This is in contrast to the classic gene-for-gene model where the interactions of Avr gene and plant resistance genes induce resistance (Wolpert et al. 2012). To date, three NE-host gene interactions have been identified in the wheat- *P.tritici-repentis* pathosystem, including Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1* (reviewed by Ciuffetti et al. 2010; Faris et al. 2013). In many studies, the three NE-host gene interactions have been shown to be important for the disease. This suggested that the removal of host sensitivty genes can increase the level of resistance in wheat cultivars. However, some studies also showed

that importance of each NE-host interaction, particularly Ptr ToxA-*Tsn1* inteaction, in the disease development is dependent on the genetic background of wheat genotypes. In addition, quautative trait loci confering dominant race-nonspecific resistance as well as other race specific resistance have been identified (reviewed by Faris et al. 2013; Kariyawasam et al. 2016). Therefore, genetics of tan spot resistance and the role of each NE-host need to be studied in more diverse genetic background for a better utilization of host resistance. Compared to hexaploid wheat, fewer studies have been conducted in tetraploid wheat backgrounds, for example, durum. Therefore, the objectives of this study were to identify sources of resistance from durum wheat germplasm, to map quantitative trait loci for resistance to tan spot using association and bi-parental mapping, and to understand the role of each NE-host sensitivity gene interaction in disease development in durum.

LITERATURE REVIEW

The Origin of Wheat

Durum (*Triticum turgidum* L., 2n = 4x = 28, AABB genomes) and bread wheat (*T. aestivum*, 2n = 6x = 42, AABBDD genomes) are two major cultivated wheat crops, which are mainly used for making pasta and bread, respectively. Durum belongs to tetraploid wheat and is believed to be evolved from the wild emmer wheat, *T. turgidum* ssp. *dicoccoides* (Korn.) (Feldman 2001). This wild emmer wheat was first domesticated approximately 11,000 years ago to give rise to emmer wheat *T. turgidum* ssp. *dicoccum* (Schubler.) with a non-brittle rachis trait. The emmer wheat was one of the first crops domesticated in the Near East and was commonly cultivated in the ancient world. Today, the emmer wheat is still grown in many places of the world as a relict crop (Feldman 2011). These places include Morocco, Spain, the Carpathian Mountains on the border of the Czech and Slovak republics, Albania, Turkey, Switzerland, and Italy. Wild emmer wheat, which has some agronomically important traits, such as large spike and grain size, high grain and protein yield, desirable composition of storage proteins, photosynthetic yield, is considered as the immediate progenitor of tetraploid and hexaploid cultivated wheat (Alvarez et al. 2013; Chatzav et al. 2010)., *T. turgidum* ssp. *parvicoccum*, another tetraploid wheat subspecies, was believed to arise approximately 9,000 years ago in the Near East. This subspecies had naked kernels and a free threshing trait like the cultivated durum and common wheat. However, it still had the brittle rachis trait like the wild emmer wheat as well as compact spike, and small size of grain (Matsuoka 2011). This subspecies vanished nearly 2,000 years ago and was only found in archaeological excavations. It is possible that the *parvicoccum* wheat contributed its free threshing trait to durum (Matsuoka 2011). However,

most believed that wild emmer wheat is the only progenitor that undergone two independent mutations at the *Q* and *Tg* genes to form today's durum (Abu et al. 2014).

The evolution of bread wheat (*T. aestivum*, 2n = 6x =42, AABBDD genome) occurred around 8,000 years ago from the cross between a *T. turgdium* (AABB) subspecies and the diploid goatgrass *Ae. tauschii* Coss. (2n = 2x = 14, DD genome) followed by an amphiploidization event. This event first led to the formation of the hexaploid wheat *T. aestivum* ssp. *spelta* (L.) Thell. (Asian or Asian like), which was then domesticated to form the modern cultivated bread wheat (Lelley et al. 2000; Faris 2014). The most important traits associated with domestication in wheat consist of brittle rachis, tenacious glume, and free-threshing (Faris 2014).

Wheat Production in the World and the United States

World Production

Wheat is one of the staple food crops in the world, and common wheat alone accounts for 20% of the daily caloric intake for human (Faris et al. 2014). The total wheat production in 2017 was around 27,555 million bushels (USDA October 2017). The major common wheat producer in the world includes European Union, Russia, China, and US. The total durum wheat world production in 2017 was around 1,334 million bushels and the major durum wheat producers in the world are European Union, Canada, Turkey, Algeria, Morocco, and US.

US Production

Among the US field crops, wheat ranks third behind corn and soybeans in terms of planted acreage and production. The United States produces six classes of wheat, including hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), hard white (HW), soft white (SW) and durum wheat. As a major wheat producer in the world, the United States produced 2,307 million bushels of wheat in 2016/2017 market year, ranking the 5th in total wheat

production, only behind European Union, Former Soviet Union, China, and India (USDA October 2017). For durum production, US produced 55 million bushels in 2017 ranking the 6th in the world, behind European Union, Canada, Turkey, Algeria, and Morocco (https://www.igc.int/en/default.aspx). Therefore, US is an important wheat producer in the world.

In US, North Dakota typically ranks second to Kansas in total wheat production. However, North Dakota is the number one producer of hard red spring and durum wheat in the US. Nearly half of the nation's hard red spring wheat and two-thirds of the nation's durum are produced in North Dakota. Durum production is geographically concentrated to North Dakota and the surrounding area because of unique agronomic environments such as cool summer nights and long warm days. North Dakota durum is preferred in international and domestic millers for its color and strong gluten characteristics (North Dakota Wheat Commission, 2018).

The Disease

Tan spot is caused by the necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (anamorph: *Drechslera tritici-repentis*). The fungus was first described in 1823 and was occasionally reported in some parts of Europe, in US, and in Japan in 1900s as a saprophyte which could cause insignificant to severe spots on wheat crops (Hosford 1982). Then after 1940, there were several reports on the severe outbreaks of tan spot in some places. By 1970s, epidemics of tan spot were reported in many wheat-growing areas of different countries, including Canada, USA, Australia, and the southern cone of Africa (Rees et al. 1992). In Australia, tan spot has surpassed strip rust and septoria nodorum blotch to be the number one disease on wheat (Murray and Brennan 2009). Based on the disease survey by NDSU extension service, tan spot, which occurs as a leaf spotting disease complex, is the most common wheat

disease in North Dakota and Minnesota in the last several years (NDSU, extension service https://www.ag.ndsu.edu/ndipm).

The increasing importance of tan spot disease worldwide can be explained by two reasons. One is that the fungus acquired the *ToxA* gene from another wheat pathogen *Parastagonospora nodorum* likely around 1940s, which increased pathogen virulence on sensitive wheat cultivars (Friesen et al. 2006). The second reason is the wide adoption of reduced or no-till practices to reduce soil erosion. Because the fungus is stubble-born, the agriculture practices intentionally increases the primary inoculum of the pathogen (Rees and Platz 1992).

Disease Symptoms

Typical symptoms of tan spot consist of necrosis and/or chlorosis. The fungal infection can be seen initially as tan brown flecks, which can enlarge into elliptical and tan-colored necrotic lesion with a dark center. The necrotic lesions are often surrounded by yellow margins or halos depending on the genotypes. In some genotypes, extensive chlorosis can be developed across all leaf areas. These lesions could merge into bigger necrotic area on the leaves, inclining leaves to early senescence. When diseased leaves are wet, the lesions on the leaves tend to blacken in the middle because of the development of conidiophores and conidia of *P. tritici-repentis*. Similar lesions can be appeared on the leaves due to other leaf spotting pathogens such as *Mycosphaerella graminicol*a, *Parastagnospora nodorum,* and *Cochliobolus sativus*. Successful tan spot identification can be done by the investigation of the morphology of the conidia formed on the leaves. *P. tritici-repentis* forms pseudothecia, the sexual fruiting body on wheat straw during the fall and winter, which serve as primary inoculum (Wegulo 2011).

Importance of the Disease

Because the fungal infection can kill large areas of a wheat leaf affecting the photosynthesis of the leaf, tan spot can cause significant yield losses in wheat. Evaluation of yield losses caused by tan spot showed that the maximum yield reductions are recorded when older plants are affected by tan spot, such as the boot and flowering stages, as opposed to tan spot occurring on young plants (Rees and Platz 1983; Shabeer and Bockus 1988; De Wolf et al. 1998). Rees et al. (1982) demonstrated that yield losses from tan spot are mainly due to a reduction in kernel weight and a decline of number of grains per head. The other reducing factors could include number of tillers, dry matter, leaf area index and grain size. Yield losses due to tan spot can reach up to 49% in highly susceptible varieties when environmental conditions are favorable. Red smudge or pink smudge occurs when *P. tritici-repentis* infects the wheat seed during the filling stage. Pink smudge causes the discoloration of grain leading to the downgrading of wheat grains, which is usually observed in durum wheat (Schilder and Bergstorm 1994; Fernandez et al. 1994). This kernels infection can also influence seedling emergence, seedling vigor, yield, and grain quality (Davis and Bockus 1993).

Disease Cycle

The fungus *P. tritici-repentis* produces pseudothecia, the overwinter structure on infected wheat residues which are left in the field. At the beginning of growth season, the pseudothecia become mature and forcibly eject ascospores when the free water is available and the ascospores are dispersed by wind to reach plants. These ascospores are the primary source of inoculum causing initial infection in the field. After the lesions formed, the fungus can produce thousands of conidiospores within lesions. The conidia are discharged and dispersed by rain splash to upper leaves of the plant or leaves of adjacent plants. The conidia germinate and cause secondary

infection forming new lesions. In a growth season, this step can repeat multiple times leading to the increase of disease incidence and severity. Krupinsky (1992) demonstrated that conidia may play a key role in initiating a tan spot epidemic in spring wheat in the northern Great Plains. Ascospores may have a role in dispersing *P. tritici-repentis* over long distances, whereas conidia are involved in dispersal over short distance (Schilder and Bergstrom, 1992). Because *P. tritici-repentis* can also infect other gramineous grass species present in the area, these grasses can serve as alternative hosts and favor the spread of the disease.

In addition to host genotype, and virulence of the isolate, many environmental factors such as moisture, temperature, light, plant age or leaf position, independently or together, influence the amount of inoculum produced and the overall disease severity (Ciuffetti et al. 1999). Soil fertilizer may also affect disease development. It was found that when nitrogen fertilizer content and the proportion of nitrogen usage as ammonium are increased, it decreases lesion development in tan spot (Huber et al. 1987). However, Davis and Bockus (1993) argued that decrease of disease severity from N fertilizers was likely due to delay in leaf senescence but not a direct effect. The pathogen can be transported to other geographic regions though infected grains which are often observed as discolored kernels, known as red smudge.

Disease Management

Tan spot disease management can be done through in combination of appropriate cultural practices such as crop rotations or tillage treatments with fungicide application (Sutton and Vynn 1990; Bockus and Claassen 1992). Crop rotation and tillage treatment can greatly reduce the primary inoculum, thus reducing disease pressure. Use of fungicides and biological control can be used as disease management strategies as well (De Wolf et al. 1998). Protectant fungicides containing mancozeb, copper or both the ingredients have been used to control tan spot, but the

applications must be done before the infection. Systemic fungicides which contain chemicals belonging to the classes of triazoles, strobilurins and mixture of them can also be used. However, both strobilurins and triazoles are not fully systemic fungicides. They are locally systemic or have translaminar activity are best used in a protective manner (Osborne and Stein 2009). Although systemic fungicides work better than protectant ones, the application of them is recommended only under certain conditions such as wet weather, susceptible plants, and large quantity of wheat residues (Friskop and Liu 2016). The NDSU extension service has developed a small grain disease forecasting model to aid in the management of wheat tan spot and other disease through fungicide application. The web-based computer model can predict the possibility of severe disease epidemics in a specific area by integrating weather forecasting and the information on the growth stage of the plants. Although these approaches can be used to effectively manage tan spot, they are either not practical or cost effective and environmental unfriendly. Furthermore, fungicide application has a concern for fungicide resistance development rendering fungicide ineffective. Usage of genetic resistance is the most preferred way to control the disease. In some regions, due to the release of resistant cultivars of leaf and stem rusts, an increase in tan spot incidence has been reported (De Wolf et al. 1998).

The Pathogen

Pyrenophora tritici-repentis, is a member of dothideomycetes, and is the first fungus known to produce proteinaceous host selective toxins, now called necrotrophic effectors (NEs). In last two decades, significant progress has been made in the identification and characterization of NEs, cloning of the NE-encoding genes and understanding the virulence mechanism and their interactions with the host.

Taxonomy and Biology of the Pathogen

The fungus belongs to the division Ascomycota, class *Ascomycetes*/Dothideomycetes, order Pleosporales, family Pleosporaceae and genus Pyrenophora. Dothideomycetes is the biggest and most diverse class of ascomycete fungi which contains over 19,000 known species. Dothideomycetes is characteristic of producing flask-like ascocarp, called pseudothecia, which bear bitunicate asci. This fungal class contains many important plant pathogens, for example, *Parastagnospora nodorum*, *Venturia inaequalis* and *Pyrenophora teres* which cause Septoria nodorum blotch on wheat, apple scab and net blotch of barley, respectively. Besides wheat, *P. tritici-repentis* can infect other cereals, including triticale, barley, and rye, but this occurs less frequently. Other possible hosts for the fungus are grass species such as Siberian wheat grass, sand bluestem, meadow brome, sheep fescue, June grass, little bluestem, green foxtail, needle and thread, and tall wheatgrass (Ali et al. 2015).

Pathogen Virulence and Race Classification

Prior to the early 1990s, most studies on virulence of *P. tritici-repentis* were to assess the measurable differences of the disease caused by the pathogen using general parameters such as lesion size (Misra and Singh 1972; Cox and Hosford 1987) or the percentage of leaf area infected (Nagle et al. 1982; Schilder and Bergstrom 1990). It was revealed that pathogen virulence is highly variable depending on the isolates used, environmental conditions, and host genotypic backgrounds. Then, Lamari and Bernier (1989a) made a groundbreaking discovery that the symptoms of necrosis and chlorosis induced in the host by *P. tritici-repentis* infection were genetically distinct, which can be used to classify the fungal isolates. The discovery also led the researchers to develop a rating scale based on lesion type, which has been widely used in

dissecting the genetics of host pathogen interaction in this disease system since then (Lamari and Bernier 1989a, b).

Lamari and Bernier (1989a) analyzed a total of 92 *Ptr* isolates using Salamouni, Glenlea and 6B365 as differential lines. Four pathotypes were classified into including pathotype 1 (nec+ chl+) producing both necrosis and chlorosis, pathotype 2 (nec+ chl-) producing only necrosis, pathotype 3 (nec- chl+) producing only chlorosis, and pathotype 4 (nec- chl-) producing neither symptom. Salamouni was the resistant genotypes, while 'Glenlea' and '6B365' were differential lines for necrosis and chlorosis, respectively. (Lamari and Bernier 1989a).

Later, Lamari et al. (1995) recognized a new pathotype from 39 Algerian isolates because these isolates induced chlorosis on susceptible wheat lines, such as 'Katepwa', that showed resistance to the previously described pathotype 3. This work directly led to the establishment of race classification system in tan spot which contains four previously identified pathotypes as races 1 to 4 and the new pathotype as race 5. The wheat line '6B662' was added to the differential set for this race.

Using the established four differential lines, a total eight races have been described (Lamari et al. 1995). Races 2, 3 and 5 showed virulence toward differential Glenlea (necrosis), 6B365 (chlorosis) and 6B662 (chlorosis), respectively. Races 1, 6, and 7 have a combination of virulence of the above three races and cause necrosis or chlorosis on two differential lines with race 1 combining virulence of races 2 and 3, race 6 combining virulence of race 3 and 5 and race 7 combining virulence of races 2 and 5. Race 8 combines virulence of races 2, 3, and 5 (Strelkov and Lamari 2003; Faris et al. 2013).

Races 1 and 2, particularly race 1, have been found to be predominant in North America (Lamari et al. 1998; Ali et al. 2003) as well as elsewhere in the world (Larmari et al. 2005). Race

5 was originally identified from Algerian isolates, but also was found in US and Canada (Ali et al. 1999; Strelkov et al. 2002). Although virulence races 6, 7 and 8 have been identified in some areas, they have not been identified in North America (Lamari and Strelkov 2010).

It is now known that the fungus produces NEs to cause disease on wheat lines carrying corresponding host sensitivity genes. Therefore, race classification based on virulence on four differential lines correlates with that based on the necrotrophic effectors (NEs) they produce. Races 2, 3, 5 produce a single known NE: Ptr ToxA, Ptr ToxC and Ptr ToxB, respectively. Races 1, 6 and 7 produce two NEs with race 1 producing Ptr ToxA and Ptr ToxC, race 6 produce Ptr ToxC and Ptr ToxB and race 7 producing Ptr ToxA and Ptr ToxB. Race 8 produces all three NEs.

However, a number of studies recently identified isolates that do not conform the current race classification system. Ali et al. (2010) reported a set of isolates from Arkansas that did not conform to the current classification system indicating a new race. Andrie et al. (2007) reported that regardless of sharing disease phenotypes similar to known races, unknown isolates SO3 and PT82 were genotypically distinct from previously characterized races of *P. tritici-repentis*. They concluded that race classification should contain both phenotypic and genotypic analyses and subsequent expansion of the differential set.

Although *Ptr* is a homothallic fungus (Lepoint et al. 2010), genetic markers across genome and ITS sequence revealed high levels of diversity for *Ptr* populations and isolates could differ in chromosome size and/or numbers (Aboukhaddour et al. 2009 ; Lichter et al. 2002). In most cases, population genetics studies did not reveal a good correlation between genetic grouping and grouping based on race or geographic location (Singh and Hughes 2006). However, there were some studies showing that *ToxA*-containing isolates were more genetically

similar than isolates lacking ToxA (Leisova-Svobodova et al. 2010b; Lichter et al. 2002; Aboukhaddour et al. 2011). Many genetic studies indicated that isolates pathogenic on wheat are quite different from the isolates that are not pathogenic in addition to the difference in the production of known NEs, and nonpathogenic isolates tend to group together (Aboukhaddour et al. 2011; Cao et al. 2009; Lepoint et al. 2010; Lichter et al. 2002; Martinez et al.2004).

Necrotrophic Effectors

Three NE have been identified from the fungal pathogen *P. tritici-repentis*, namely Ptr ToxA, Ptr ToxB and Ptr ToxC. These NEs interact with corresponding host sensitivity genes to induce necrosis or chlorosis and promote disease. Ptr ToxA induce necrosis on Glenlea, while Ptr ToxB and Ptr ToxC explicitly induce chlorosis on 6B662 and 6B365, respectively. Thus, isolates of *P. tritici-repentis* can be also divided into eight races based on the necrotrophic effectors they produce or based on the reaction on the differential lines described above while rest of the races produce two or three combinations of these necrotrophic effectors. Out of these three NEs, the molecular nature and structure of Ptr ToxA and Ptr ToxB have been determined and mode of actions for these two NEs have also been studied (see review Ciuffetti et al. 2010).

Ptr ToxA

Ptr ToxA was the first proteinaceous NE identified from fungi and has been well-characterized. Ptr ToxA is a small protein encoded by a single-copy gene *ToxA* (Ciuffetti et al. 1997). Ptr ToxA consists of a signal peptide for secretion and a short peptide as pro-sequence (Tuori et al. 2000). After secretion, both signal peptide and pro-domain are cleaved, and a mature protein has a molecular weight at 13.2 kDa (Tuori et al. 2000). The mature Ptr ToxA has a β-barrel and a loop containing a RGD motif (Sarma et al. 2005). Ptr ToxA was shown to enter the plant cell in susceptible genotypes through unknown mechanism, then move to chloroplast where

it disrupts photosynthesis system (Manning and Ciuffetti 2005; Manning et al. 2009). Ptr ToxA is able to bind directly to plastocyanin and ToxABP1 in cholorplast (Manning et al. 2007; Tai et al. 2007). Recently, Ptr ToxA was found to bind with PR-1 protein suggesting that Ptr ToxA may counteract plant defense system (Lu et al. 2013). The binding to wheat PR-1 protein has also been detected for SnTox3, a NE produced by *P. nodorum* (Breen et al. 2016).

Ptr ToxB

Ptr ToxB is a protein with a molecular weight of 6.5 kDa (Strelkov et al. 1999, 2002). The *ToxB* gene, which encodes Ptr ToxB, was first cloned from a race 5 isolate (Martinez et al. 2001). Later, it was found that the *ToxB* gene occurs in multiple copies in race 5 isolates and the copy number is correlated with virulence of the isolate (Martinez et al. 2004; Strelkov et al. 2006; Ciuffetti et al. 2010). Multiplex polymerase chain reaction (PCR) discovered the presence of *ToxB*-like sequences in *Pyrenophora bromi*. Additionally, Southern analysis revealed *ToxB* from *P. bromi* to have a multicopy nature similar to *ToxB* from *P. tritici-repentis* (Andrie et al. 2008). The *ToxB* gene was not found in isolates classified as races 1 or 2, but a nonfunctional *ToxB* homologue, *toxb*, was identified in the races 3 and 4 isolate (Andrie et al. 2008; Aboukhaddour et al. 2013). Ptr ToxB was found to degrade chlorophyll and induce defense responses in sensitive lines (Ciuffetti et al. 2010). Fluorescence tagging study showed that Ptr ToxB remains in apoplast area instead of entering plant cell, and thus it may interact with a membrane-anchored wheat protein triggering a signaling cascade that eventually results in chlorosis (Figueroa et al. 2015). Similar to Ptr ToxA, Ptr ToxB can induce up-regulation of many plant defense genes encoding WRKY transcription factors, pathogenicity related proteins, components of phenyl propanoid pathway and jasmonic acid pathway in susceptible genotypes (Pandelova et al. 2012). Additionally, it was also shown that Reactive Oxygen Species

accumulation and decrease in chlorophyll a and b are associated with Ptr ToxB reaction (Pandelova et al. 2012; Ciuffetti et al. 2010).

Ptr ToxC

Unlike Ptr ToxA and Ptr ToxB, Ptr ToxC was characterized as a non-ionic, polar, low molecular weight molecule (Effertz et al. 2002). Due to the difficulties in purifying Ptr ToxC, the exact chemical structure of Ptr ToxC has not been determined.

Other Potential NEs

It is highly likely that *P. tritici-repentis* produces NEs in addition to the three previously described. Meinhardt et al. (2003) and Ciuffetti et al. (2003) both reported a putative Ptr ToxD from the culture filtrates of different isolates. Ptr ToxD reported by Meinhardt et al. (2003) could elicits chlorosis, while the one reported by Ciuffetti et al. 2003 elicit necrosis on specific wheat genotypes. However, the results for both Ptr ToxD has not been formally published in a scientific journal. Gamba and Lamari (1998) shown that some isolates of races 3 and 5 may produce toxic components other than Ptr ToxB and Ptr ToxC that caused necrosis instead of chlorosis in a specific durum wheat line. In addition, reports of several recessive tan spot resistance genes (Singh et al. 2006, 2008a; Tadesse et al. 2006a, b, 2008, 2010) suggesting the existence of additional host–NEs interactions in the system.

Genetics of Wheat- *P. tritici-repentis* Interaction

Inverse Gene for Gene Model and Tan Spot Sensitivity Genes

Prior to the discovery of genetic independence of chlorosis and necrosis symptoms of tan spot by Lamari and Bernier (1991), several studies have been carried out to assess the heritability of resistance to tan spot (Nagle et al. 1982; Elias et al. 1989). Even though these studies pointed out significant heritability of resistance, they also indicated that resistance was quantitatively

controlled. Since the identification of NEs and their host sensitivity genes, the wheat-*P. tritici-repentis* pathosystem has been known to fit an inverse gene-for-gene model. In this model, the interaction between necrotrophic effectors produced by the pathogen and the product of the corresponding host sensitivity gene leads to susceptibility. Therefore, susceptibility is dominant, and resistance is due to the lack of NE-host sensitivity genes interactions. For breeding, the susceptibility/sensitivity gene should be removed from the breeding materials to obtain high levels of resistance. Recently, a few NE sensitivity genes have been cloned, and were shown to resemble resistance genes (Lorang et al. 2007; Nagy et al. 2008; Faris et al. 2010). It has been hypothesized that the necrotrophic fungal pathogens can hijack plant disease resistance pathways by using the NE-S interaction (Faris et al. 2010). At the cellular level, NEs elicit a type of programmed cell death reaction in plant tissues by interacting with host susceptibility gene which benefits and supports the growth of the necrotrophic pathogen, thus leading to susceptibility.

In tan spot disease system, host sensitivity genes for Ptr ToxA, Ptr ToxB and Ptr ToxC have been identified and mapped and designated as *Tsn1*, *Tsc2* and *Tsc1*, respectively (Faris et al. 2013). Among them, *Tsn1* have been successfully isolated from the wheat genome (Faris et al. 2010), but *Tsc1* and *Tsc2* remain to be cloned. Cloning of these sensitivity genes would be very important for the understanding of the molecular basis of the disease system and would also be useful in development of functional markers for breeding programs.

Tsn1

The *Tsn1* gene was first mapped by Faris et al. (1996) by using restriction fragment length polymorphism (RFLP), which located the gene to the chromosome arm 5BL. Haen et al. (2004) described the saturation and high-resolution mapping of the *Tsn1* region in tetraploid and

hexaploid wheat populations. Lu et al. (2006) used a LDN BAC library to assemble BAC contigs of 205 and 228 kb flanking *Tsn1* and developed the PCR-based markers designated *Xfcp1* and *Xfcp2*. Faris et al. (2010) completed the assembly of a BAC contig spanning the *Tsn1* locus and delineated the *Tsn1* gene to six possible candidates. Association mapping reduced the number of candidates to four, including a gene harboring N- terminal serine/threonine protein kinase (S/TPK) and C-terminal nucleotide binding (NB), and leucine-rich repeat (LRR) domains. Comparative sequence analysis of these four genes in wild-type wheat genotypes and corresponding ethyl methanesulfonate (EMS)-induced mutants revealed that the S/TPK-NB-LRR-like gene was *Tsn1* which has a structural feature as classic disease resistance gene. The cloning of the *Tsn1* gene provided further support to the notion that necrotrophic pathogen hijack plant resistance signal pathway. The physical mapping, sequencing, and cloning of the *Tsn1* locus allowed the development of a functional marker assigned as *Xfcp623* (Faris et al. 2010). Furthermore, functional analysis showed that *Tsn1* expression was controlled by the circadian clock and light, and that the presence of ToxA will result in down-regulation of *Tsn1* (Faris et al. 2010). Nevertheless, yeast two-hybrid experiments showed that the *Tsn1* and ToxA proteins probably do not interact directly, suggesting that intermediate proteins may be involved in the recognition of ToxA by *Tsn1* (Faris et al. 2010).

Tsc1

Tsc1 confers sensitivity to Ptr ToxC which induce a chlorosis reaction. Effertz et al. (2002) map the gene to the distal end of the chromosome arm 1AS using Restriction Fragment Length Polymorphic markers in the population derived from the cross W-7984 × Opata 85. The flanking marker *XGli1* was located on the short arm of chromosome 1A, is linked to the insensitivity locus within 5.7 cM and was also identified as a major QTL for reaction to races

and 1 and 3 indicating Ptr ToxC and *Tsc1* interaction play a key role in disease development (Effertz et al. 2002). Since then, no further studies have been done on *Tsc1*.

Tsc2

Tsc2, conferring chlorosis type reaction to Ptr ToxB was first mapped by Friesen and Faris (2004) to the distal end of the chromosome arm 2BS using International Triticeae Mapping Initiative (ITMI) population. Later, saturation mapping was done by Abeysekara et al. (2010) using a Recombinant Inbred Line population derived from Salamouni × Katepwa which delimited *Tsc2* to a 3.3 cM region on 2BS by EST-based markers *XTC339813* and *XBE517745*. Furthermore, the marker *XBE444541*, which was initially detected by an RFLP, co-segregated with *Tsc2* and was converted to a PCR-based marker. *XBE444541* was demonstrated to be diagnostic for the *Tsc2.*

Role of Each Interaction in Disease

The role of NE-S interaction can be studied by conducting NE infiltration and fungal spore inoculation in the same population followed by the mapping of NE sensitivity and the QTL mapping of susceptibility to the fungal inoculations. The colocalization of QTL for susceptibility with any of sensitivity loci would indicate a significant role of the specific interaction in disease development. So far, most studies have been done with Ptr ToxA-*Tsn1* interaction using hexaploid wheat population (reviewed by Faris et al. 2013). These studies revealed that the *Tsn1*-Ptr ToxA interaction played a major role (Tomas and Bockus 1987; Lamari and Bernier 1989; Cheong et al. 2004; Singh et al. 2010), a minor role (Friesen et al. 2003; Chu et al. 2008a; Singh and Bockus 2008; Faris et al. 2012) or had no effect in tan spot susceptibility (Faris and Friesen 2005). Several disease screenings identified a number of wheat lines that are sensitive to Ptr ToxA but highly resistant to tan spot caused by races 1 and 2 indicating Ptr ToxA-*Tsn1* has no

role in disease (Noriel et al. 2011; Liu et al. 2015). Kariyawasam et al (2016) characterized the resistance mechanism in a Ptr ToxA-sensitive and disease resistant wheat cultivar 'Penawawa' and found it carries a race-nonspecific resistance QTL which could act epistatically on Ptr ToxA-*Tsn1* interaction.

Few studies have been done on tetraploid wheat to evaluate the role of *Tsn1*-PtrToxA interaction in tan spot susceptibility. Chu et al. (2010a) evaluated a tetraploid wheat doubled haploid population derived from a cross between the durum variety 'Lebsock' and accession PI 94749 of *T. turgidum* ssp. *carthlicum* for reaction to the isolates 86-124 and Pti2, which both produce Ptr ToxA. Even though the population segregated for reaction to Ptr ToxA infiltrations, sensitivity to Ptr ToxA had no effect on tan spot susceptibility and no significant QTL were detected on the *Tsn1* locus to Pti2 and 86-124 isolates. Chu et al. (2008b) tested 688 accessions of tetraploid wheat subspecies (*T. turgidum* L. subspecies *T. carthlicum*, *T. polonicum*, *T. turgidum*, *T. dicoccum*, and *T. turanicum*) with Pti2 and found that the *Tsn1*-ToxA interaction was not associated with tan spot susceptibility. From the evaluation of 172 accessions of wild emmer wheat (*T. dicoccoides*) for reaction to Ptr ToxA infiltration and fungal inoculations with the Pti2, Chu et al. (2008c) reported a weak ($R^2 = 0.03$) significant association between Ptr ToxA sensitivity and tan spot susceptibility. Virdi et al. (2016) evaluated a tetraploid population derived from Altar84× Langdon (LDN) for reaction to races 1 and 2 isolates and SnToxA-producing *P. nodorum* isolates and found that the *Tsn1* locus is not associated with susceptibility to tan spot at all, but with susceptibility to *P. nodorum*. Quantitative PCR assay of the *ToxA* gene expression showed *P. tritici-repentis* hardly express *ToxA* in durum background. Ptr ToxB- *Tsc2* and Ptr ToxC- *Tsc1* interactions have been shown to be important in disease development if they are present in the populations that were analyzed (Effertz et al. 2002; Friesen and Faris 2004;

Abeysekara et al. 2010). However, the studies for these two interactions are very limited on tetraploid wheat.

Other Major Resistance Genes

Beside the NE sensitivity gene, there are a few major recessive resistance genes having been reported from hexaploid or tetraploid wheat accessions. Tadesse et al. (2006a) identified a recessive resistance gene (*tsr3*) on the chromosome 3D from hexaploid synthetic wheat accessions XX41, XX45, XX110 using Chinese spring monosomic lines. In another study, Tadesse et al. (2006b) identified a recessive gene on 3A, which was designated as *tsr4*, conferring resistance against ASC1a using a F_2 population from the cross between Salamouni and 'Chinese Spring'. At tetraploid wheat level, Singh et al. (2006) identified the *tsr2* gene on the chromosome arm 3BL which confers recessive resistance to necrosis caused by the race 3 isolate 331-9 in a population of RI lines derived from the cross between a resistant *T. turgidum ssp. turgidum* accession (PI 352519) and the susceptible durum variety Coulter, using the same set of materials, Singh et al. (2008b) mapped another recessive gene on 3BL conferring resistance to race 5 isolate DW13. Because the gene is 8.3 cM apart from the *tsr2* gene and thus *tsr5* was designated for this gene.

Other QTLs

Many traits in plant are quantitative because they are controlled by multiple genes. These genes are called quantitative trait loci (QTL), which can be revealed by using QTL mapping. This involves the development of segregating plant population and its genetic linkage maps, phenotyping of the population, and statistical analysis of marker data and phenotypic association (Young 1996; Doerge 2002).

The first QTL mapping for wheat tan spot resistance was carried out by Faris et al. (1997) using the ITMI population (W-7984 × Opata 85) with isolates Pti2 (race 1), 86-124 (race 2) and D308 (race 3). The population segregated for the chlorosis-producing isolates Pti2 and D308 and a major QTL on 1AS (*QTsc.ndsu-1A*) and a minor QTL on chromosome 4AL were identified. Since then, various RIL or doubled haploid (DH) populations have been developed and applied to tan spot QTL mapping. Some QTLs were identified to the genomic regions that correspond to three NE insensitivity loci suggesting the NE-S interaction play a significant role in disease development in those population. However, many QTLs were mapped to the genomic locations other than know NE sensitivity loci, and the wheat chromosome arms that have tan spot resistance or susceptibility QTL identified included 1AS, 1BS, 2AS, 2BS, 2BL, 3AS, 3BS, 3BL, 4AL,5AL, 5BL, 5DL, 7BS and 7DS (Faris et al. 1997; Cheong et al. 2004; Friesen and Faris 2004; Faris and Friesen 2005; Chu et al. 2008; Singh et al. 2008; Sun et al. 2010; Li et al. 2011; Faris et al. 2012). Some of QTL identified from different studies might be the same because they were located in a very same or similar chromosome location based on the common genetic markers. For example, the 4AL QTL identified by Faris et al. (1997) might be the same as the ones identified by Friesen and Faris (2004) and Chu et al. (2008). Most of these QTL identified are effective against one specific race or two, which are considered as race specific resistance QTL.

Most of these studies employed hexaploid wheat population and only a few QTL mappings has been done in tetraploid wheat population. Virdi et al. (2016) identified *QTs.fcu-6B* for race 2 isolate, 86-124, two significant QTLs against the other race 2 isolate, L13-35 on the short arms of chromosomes 4B and 5B, designated *QTs.fcu-4B* and *QTs.fcu-5B*. For the race 5 isolate DW5, two significant QTLs were identified on chromosomes arms 2BS and 4BL and

designated as *QTs.fcu-2B* and *QTs.fcu-4B* in tetraploid AL population. Chu et al. (2010) identified five resistance QTLs on chromosome arms 3AS, 3BL, 5AL and 7BL in a tetraploid DH population when inoculated separately with two Ptr ToxA-producing isolates (Pti2 and 86-124) representing races 1 and 2.

Race Nonspecific Resistance QTL

Although most QTL identified are race-specific, a few QTL were found to confer resistance/susceptibility for multiple races, which has been referred to race-nonspecific resistance in tan spot. The race-nonspecific resistance QTL was first reported by Faris and Friesen (2005) who evaluated a wheat population derived from the cross between a Brazilian breeding line BR34 and NDSU cultivar 'Grandin' for reaction to races 1, 2, 3 and 5. The population segregated for reaction to Ptr ToxA, but *Tsn1* did not account for any QTL. Two QTL *QTs.fcu-1BS* on 1BS and *QTs.fcu-3BL* con 3BL were identified for all the races with an additive effect up to 41% in that population (Faris and Friesen 2005). Kariyawasam et al. (2016) identified a major race-nonspecific QTL on chromosome arm 3BL for resistance to all races in a recombinant inbred wheat population derived from the cross between soft white spring wheat cultivars 'Louise' and 'Penawawa'. Similarly, the QTL on 5DL and 7BS identified by Faris et al. (2012) and the QTL on 5AL by Chu et al. (2010) might also be race nonspecific because they have effect on multiple races.

Association Mapping

Principle

Bi-parental mapping has been normally used to map plant disease resistance genes or quantitative trait loci (QTL). However, as recent advancements in high-throughput genotyping technologies and statistical analysis software, genome wide association studies (GWAS) has

been increasingly used in the last 10 years as a novel approach to identify gene(s) associated with a trait. GWAS approach utilizes the analyses of linkage disequilibrium (LD) among alleles in a germplasm collection to identify significant marker-trait association. Compare to the bi-parental population mapping, association mapping (AM) take advantage of the higher frequency of recombination events that could take place during the long history of germplasm development. Another advantage of AM is that it does not require the relatively long process for bi-parental population development. Genetic materials are a key factor for a successful AM study. In wheat association mapping, landrace collections are better choice than cultivar and breeding lines for identifying marker-trait association largely due to the higher genetic diversity in landraces collection (Giraldo et al. 2016).

High Throughput Genetic Markers

DNA-based genetic markers are very important to map genes and provide the tools for plant breeders to conduct line selections, also known as marker assisted selection (MAS). In the past, several types of molecular markers have been used in the mapping and MAS, which include such as restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR). Lately, DNA marker based on single nucleotide polymorphisms (SNPs) have been developed and widely used in the genotyping of population. Because SNP-based markers are abundant in number and can be obtained in a high-throughput manner, it has mainly replaced other marker systems and become a mainstream.

SNP among the individuals generally happen at frequencies of one per ~ 100–500 bp in plant genomes, depending on the species (Choi et al. 2007; Leonforte et al. 2013). With high-throughput sequencing techniques, the SNP discovery across all the genome and the

corresponding microarray-based genotyping platform have been developed in many crops such as maize, oilseed rape, rice, soybean, *Brassica*, and alfalfa, which have been successfully used in genetic mapping (Kumar et al. 2012). Early SNPs were mainly identified from the expressed genes in the genome, particularly for plant species that have a large genome, for example, wheat. Therefore, the capacity of microarrays in a chip determined the number of SNP markers that can be obtained. The Illumina wheat Golden Gate chips containing 9,000 markers was first available for usage in wheat genetic mapping, then followed by the chips containing 90,000 markers (Akhunov et al. 2009; Cavanagh et al. 2012; Wang et al. 2015). Wang et al. (2014) used the 90k gene chip to map 46,977 SNPs in wheat genomes using eight different mapping populations, which provides an important reference for genetic mapping and AM in tetraploid and hexaploid wheat. As sharply reduced sequence cost and high-throughput capacity of next generation sequencing in the last several years, SNP markers have been commonly developed through directly sequencing the whole genome with certain depth. This SNP development method is easier and cost much less than array-based analysis (Elshire et al. 2011). This method has been referred as genotyping by sequencing (GBS). The application of GBS has been extended to population genetic studies, germplasm characterization, genetic mapping and genomic-based breeding in almost all-important crops (Poland et al. 2012). GBS usually involves the digestion of genomic DNA from different individuals with restriction enzymes, followed by library construction and high throughput genome sequencing (Huang et al. 2009, Kim et al. 2016).

Association Mapping for Tan Spot Resistance

A few studies have been conducted to use GWAS to identify tan spot resistance QTL from various germplasm panels with different marker systems. Gurung et al. (2011) screened 567 spring wheat landraces for resistance to isolates Pti2 (race 1) and DW7 (race 5) and

identified tan spot resistance QTL using 832 DArT markers. The resistance QTL were located on chromosomes 1D, 2A, 2B, 2D, 4A, 5B, and 7D for Pti2 with the genomic regions on 1D, 2B, 2D, and 7D being novel. For DW7, all QTL identified were located to the genomic regions that have not been reported previously. Patel et al. (2013) used the same set of material as Gurung et al (2011) for identifying tan spot resistance to a new race (AR Cross B10) and results revealed QTL on the chromosomes 1A, 1D, 2B, 2D, 6A, and 7A. In another GWAS study, 358 European winter wheat lines and 14 spring wheat lines which have been genotyped with 732 microsatellites were used, which not only led to the identification of all major known resistance or susceptible loci, such as *Tsn1*, *tsn2* or *tsn5*, *Tsc2* or *Tsr6* and but also some new resistant QTL on the chromosome arms 1DL, 2BL, 3BS, 3DL, and 3AL (Kollers et al. 2014). Liu et at (2015) conducted an association mapping on North American winter wheat cultivars using GBS and identified genomics regions on the chromosome arms 3BL, 3DS, 4AL, 5DL and 7DL significantly associated with resistance to tan spot. In a latest association mapping study with the 170 lines of historical bread wheat germplasm developed at CIMMYT, Singh et al. (2015) revealed significant marker associations on the chromosome arms 1AS, 1BS, 2BL, 3BL, 4AL, 5BL, 6AL (two QTL), 6BS and 7BL with genotypic data generated with 1644 molecular markers. The authors reported two QTL on chromosome arm 6AL and the QTL found on chromosome 7BL as novel regions for tan spot resistance.

Association mapping has been used in durum wheat to analyze various agronomically important traits such as grain yield, yellow pigment, root architecture, plant height and drought and salinity tolerance as well as disease resistance to Fusarium head blight, leaf rust, and stem rust (Ghavami et al. 2011; Maccaferri et al. 2010b; Letta et al. 2014). However, no studies have been done to map tan spot resistance in durum using GWAS.

Rational

Although the three NE-host gene interactions have been shown to play a role in tan spot development in many studies, the importance of Ptr ToxA-*Tsn1* inteaction seems to vary greatly depending on the wheat genetic background. In addition, the disease system has shown to invlove other qualitative genes and other race- specific and race-nonspecific resistance QTL indicating the complexity of the disease system. More studies are needed to further understand the genetic and molecular basis of the disease. This is important for better utilization of sources of resistance and development of more completely resistant cultivars. Compared to hexaploid wheat, very few studies have been conducted in durum backgrounds. Therefore, the objectives of this study are to identify sources of resistance from durum wheat germplasm, map quantitative trait loci for resistance to tan spot using association and bi-parental mapping, and to investigate the role of each NE-host sensitivity gene interaction in disease development in durum.

References

Abdurakhmonov, I. Y., and Abdukarimov, A. 2008. Application of association mapping to understanding the genetic diversity of plant germplasm resources. International Journal of Plant Genomics 2: 1-18.

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

Abeysekara, N. S., Friesen, T. L., Liu, Z., McClean, P. E., and Faris, J. D. 2010. Marker development and saturation mapping of the tan spot Ptr ToxB sensitivity locus *Tsc2* in hexaploid wheat. The Plant Genome 3:179-189.

Aboukhaddour R., Cloutier S., Ballance G. M., and Lamari L. 2009. Genome characterization of *Pyrenophora tritici-repentis* isolates reveals high plasticity and independent chromosomal location of *ToxA* and *ToxB*. Molecular Plant Pathology 10: 201–212.

Aboukhaddour R., Cloutier S., Lamari L., and Strelkov S. E. 2011. Simple sequence repeats and diversity of globally distributed populations of *Pyrenophora tritici-repentis*. Canadian Journal of Plant Pathology 33: 389–399.

Aboukhaddour, R., Turkington, T. K., and Strelkov, S. E. 2013. Race structure of *Pyrenophora triciti-repentis* (tan spot of wheat) in Alberta, Canada. Canadian Journal of Plant Pathology 35: 256- 268.

Ali, S., and Francl, L. J. 2003. Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and non-cereal grasses in the Great Plains. Plant Disease 87: 418-422.

Ali, S., and Langham, M. A. C. 2015. Reaction of five non-cereal grasses to five races and two Host Selective Toxins of *Pyrenophora tritici-repentis*. Plant Pathology Journal 31: 245–251.

Ali, S., Francl, L.J., and De Wolf, E.D. 1999. First report of *Pyrenophora tritici-repentis* race 5 from North America. Plant Disease 83:591.

Ali, S., Gurung, S., Adhikari, T. B. 2010. Identification and characterization of novel isolates of *Pyrenophora tritici-repentis* from Arkansas, Plant Disease 94:229-235.

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

Andrews, E., and Klomparens, W. 1952. Yellow spot of winter wheat in Michigan in 1951. Plant Disease Reporter 36:10-11.

Andrie, R. M., Schoch, C. L., Hedges, R., Spatafora, J. W., and Ciuffetti, L. M. 2008. Homologs of *ToxB*, a host-selective toxin gene from *Pyrenophora tritici-repentis*, are present in the genome of sister-species *Pyrenophora bromi* and other members of the Ascomycota. Fungal Genetics and Biology 45: 363-377.

Andrie, R. M., Pandelova, I., and Ciuffetti, L. M. 2007. A Combination of Phenotypic and Genotypic Characterization Strengthens *Pyrenophora tritici-repentis* Race Identification. Phytopathology 97:694-701.

Aoun, M., Breiland, M., Turner, M. K., Loladze, A., Chao, S., Xu, S. S., Ammar, K., Anderson, J. A., Kolmer., and Acevado, M. 2016. Genome-wide association mapping of leaf rust response in a durum wheat worldwide germplasm collection. The Plant Genome 9: 1-24.

Barrus, M. F. 1942. Yellow-spot disease of Wheat in New York State. Plant Disease Reporter 26:246-247.

Bockus, W. W., Claasen, M. M. 1992. Effects of crop rotation and residue management practices on severity of tan spot of winter wheat. Plant Disease 76:633–636.

Breen, S. Williams, S. J., Winterberg, B., Kobe, B., and Solomon, P. S. 2016. Wheat PR-1 proteins are targeted by necrotrophic pathogen effector proteins. Plant Journal 88:13-25.

Cao T., Kim Y. M., Kav N. N., and Strelkov S. E. 2009. A proteomic evaluation of *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat, reveals major differences between virulent and avirulent isolates. Proteomics 9: 1177–1196.

Charmet, G. 2011. Wheat domestication: lessons for the future. Comptes Rendus Biologies 334:212-220.

Cheong, J., Wallwork, H., and Williams, K. J. 2004. Identification of a major QTL for yellow leaf spot resistance in the wheat varieties Brookton and Cranbrook. Crop and Pasture Science 55:315-319.

Chu, C. G., Chao, S., Friesen, T. L., Faris, J. D., Zhong, S., and Xu, S. S. 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., Friesen, T. L., Xu, S. S., and Faris, J. D. 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.

Ciuffetti, L. M., Francl, L. J., Balance, G. M., Bockus, W. W., Lamari, L., Meinhardt, S. W., and Rasmussen, J. B. 1998. Standardization of toxin nomenclature in the *Pyrenophora tritici-repentis*/wheat interaction. Canadian Journal of Plant Pathology 20:421-424.

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

Conners, I.L. 1937. 17th Annual Report. Canadian Plant Disease. Survey (p. 5).

Conners, I. L. 1940. 20th Annual Report. Canadian Plant Disease. Survey (p. 12-14).

da Luz, W. C., and Hosford Jr, R. M. 1980. Twelve *Pyrenophora trichostoma* races for virulence to wheat in the Central Plains of North America. Phytopathology 70:1193-1196.

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

Doerge, R. W. (2002). Mapping and analysis of quantitative trait loci in experimental populations. Nature Reviews Genetics 3:43-52.

Drechsler, C. 1923. Some graminicolons species of *Helminthosporium*. I. Journal of Agricultural Research 24:641-739.

Dvořák, J., Terlizzi, P. D., Zhang, H. B., and Resta, P. 1993. The evolution of polyploid wheat identification of the A genome donor species. Genome 36:21-31.

Effertz, R. J., Anderson, J. A., and Francl, L. J. 2001. Restriction fragment length polymorphism mapping of resistance to two races of *Pyrenophora tritici-repentis* in adult and seedling wheat. Phytopathology 91:572-578.

Effertz, R. J., Meinhardt, S. W., Anderson, J. A., Jordahl, J. G., and Francl, L. J. 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., Cantrell, R.G. and Hosford, R.M. Jr. 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., Glaubitz, J. C., Sun, Qi., Poland, J. A., Kawamoto, K., Buckler, E. S., and Mitchell, S.E. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS ONE 6 e19379.

Endo, T. R., and Gill, B. S. 1996. The deletion stocks of common wheat. Journal of Heredity 87:295–307.

Faris, J. D. 2014. Wheat domestication: Key to agricultural revolutions past and future. In Genomics of plant genetic resources Springer Netherlands (p. 439-464).

Faris, J. D., Abeysekara, N. S., McClean, P. E., Xu, S. S., and Friesen, T. L. 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., and Friesen, T. L. 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., Anderson, J. A., Francl, L. J., and Jordahl, J. G. 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., Anderson, J. A., Francl, L. J., and Jordahl, J. G. 1997. RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theoretical and Applied Genetics 94:98-103.

Faris, J. D., Li, W. L., Liu, D. J., Chen, P. D., and Gill, B. S. 1999. Candidate gene analysis of quantitative disease resistance in wheat. Theoretical and Applied Genetics 98:219-225.

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

Faris, J. D., Zhang, Q., Chao, S., Zhang, Z., and Xu, S. S. 2014. Analysis of agronomic and domestication traits in a durum× cultivated emmer wheat population using a high-density single nucleotide polymorphism-based linkage map. Theoretical and Applied Genetics 127:2333-2348.

Faris, J. D., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J. P., Meinhardt, S. W., Rasmussen, J. B., Oliver, R. P., and Friesen, T. L. 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., and Levy, A. A. 2012. Genome evolution due to allopolyploidization in wheat. Genetics 192:763-774.

Fernandez, M. R., Clarke, J. M., and DePauw, R. M. 1994. Response of durum wheat kernels and leaves at different growth stages to *Pyrenophora tritici-repentis*. Plant Disease 78: 597-600.

Fernandez, M. R., Clarke, J. M., and DePauw, R. M. 1994. Response of durum wheat kernels and leaves at different growth stages to *Pyrenophora tritici-repentis*. Plant Disease 78: 597-600.

Figueroa, M., Manning, V. A., Pandelova, I., and Ciuffetti, L. M. 2015. Persistence of the Host-Selective Toxin Ptr ToxB in the Apoplast. Molecular Plant-Microbe Interactions 28:1082-1090.

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

Friesen, T. L. and Faris, J. D. 2012. Characterization of plant–fungal interactions involving necrotrophic effector-producing plant pathogens. In: Plant Fungal Pathogens: Methods and Protocols, Methods in Molecular Biology, Vol. 835 (Bolton, M. D. and Thomma, B. P. H. J., eds), pp. 191–207. New York: Humana Press.

Friesen, T. L., and Faris, J. D. 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., Stukenbrock, E. H., Liu, Z., Meinhardt, S., Ling, H., Faris, J. D., Rasmussen, J. B., Solomon, P. S., McDonald, B. A. and Oliver, R. P. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. Nature Genetics 38:953-956.

Gamba, F. M., and Lamari, L. 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.

Gamba, F. M., Lamari, L., and Brülé-Babel, A. L. 1998. Inheritance of race-specific necrotic and chlorotic reactions induced by *Pyrenophora tritici-repentis* in hexaploid wheats. Canadian Journal of Plant Pathology 20:401-407.

Ghavami, F., Elias, M.E., Mamidi, S., Ansari, O., Sargolzaei, M., Adhikari, T., Mergoum, M., and Kianian, S.F. 2011. Mixed model association mapping for Fusarium head blight resistance in Tunisian-derived durum wheat populations. G3: Genes, Genomes, Genetics 3:209–218.

Gilchrist, S.L., Fuentes, S.F. and De la Isla de Bauer, M. de L. 1984. Determinacion de funentes de resistencia contra *Helminthosporium tritici-repentis* bajo conditiones de campo e invernadero. Agrociencia 56:95-105.

Gurung, S., Mamidi, S., Bonman, J. M., Jackson, E. W., Del Rio, L. E., Acevedo, M., Mergoum, M. and Adhikari, T. B. 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.

Haen, K. M., Lu, H. J., Friesen, T. L., and Faris, J. D. 2004. Genomic targeting and high-resolution mapping of the *Tsn1* gene in wheat. Crop Science 44:951–962.

Hosford, R. M Jr.1982. Tan spot-developing knowledge 1902-1981, virulent races and differentials, methodology, rating systems, other leaf diseases, literature. In: Hosford, RM Jr (ed) Tan spot of wheat and related diseases workshop. North Dakota Agricultural Experiment Station, Fargo. (p. 1-24).

Hosford, R.M. Jr., C.R. Larez, and J.J. Hammond. 1987. Interaction of wet period and temperature on *Pyrenophora tritici-repentis* infection and development in wheats of differing resistance. Phytopathology 77:1021-1027.

Huber, D.M., Lee, T.S., Ross, M.A., and Abney, T.S. 1987. Amelioration of tan spot-infected wheat with nitrogen. Plant Disease 71:49–50.

Ito, S. 1930. On some new ascigerous stages of the species of *Helminthosporium* parasitic on cereals. Proceedings of the Japan Academy Tokyo 6:352-355.

Johnson, A.J. 1942. *Helminthosporium tritici-vulgaris* on wheat in Maryland. Plant Disease. Reporter 26:246–247.

Kollers, S., Rodemann, B., Ling, J., Korzun, V., Ebmeyer, E., Argillier, O., Hinze, M., Plieske, J., Kulosa, D., Ganal, M. W., and Röder, M. S. 2014. Genome-wide association mapping of tan spot resistance (*Pyrenophora tritici-repentis*) in European winter wheat. Molecular Breeding 34:363- 371.

Lamari, L., & Bernier, C. C. 1989. 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 Bernier, C. C. 1989a. Virulence of isolates of *Pyrenophora tritici-repentis* on 11 wheat cultivars and cytology of the differential host reactions. Canadian Journal of Plant Pathology 11:284-290.

Lamari, L., and Bernier, C. C. 1989b. 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 Strelkov, S. E. 2010. Minireview/Minisynthèse The wheat/*Pyrenophora tritici-repentis* interaction: progress towards an understanding of tan spot disease. Canadian Journal of Plant Pathology 32:4-10.

Lamari, L., Bernier, C. C., and Smith, R. B. 1991. Wheat genotypes that develop both tan necrosis and extensive chlorosis in response to isolates of *Pyrenophora tritici-repentis*. Plant Disease 75:121-122.

Lamari, L., Gilbert, J., and Tekauz, A. 1998. Race differentiation in *Pyrenophora tritici-repentis* and survey of physiologic variation in western Canada. Canadian Journal of Plant Pathology 20:396–400.

Lamari, L., Sayoud, R., Boulif, M., and Bernier, C. C. 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., Strelkov, S. E., Yahyaoui, A., Orabi, J., and Smith, R. B. 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.

Lamari, L., Strelkov, S.E., Yahyoui, A., Amedov, M., Saidov, M., Djunusova, M., and Koichibayen, M. 2005. Virulence of *Pyrenophora tritici-repentis* in the countries of the Silk Road. Canadian Journal of Plant Pathology 27:383–388.

Lee, T.S., and F.J. Gough. 1984. Inheritance of Septoria leaf blotch (*S. tritici*) and Pyrenophora tan spot (*P. tritici-repentis*) resistance in *Triticum aestivum* cv. Carifen 12. Plant Disease 68:848-851.

Leisova L., Hanzalova A., and Kucera L. 2008. Genetic diversity of *Pyrenophora tritici-repentis* isolates as revealed by AFLP analysis. Journal of Plant Pathology. 90: 233–245.

Leisova-Svobodova L., Hanzalova A., and Kucera L. 2010a. Expansion and variability of the *Ptr ToxA* gene in populations of *Pyrenophora tritici-repentis* and *Pyrenophora teres*. Journal of Plant Pathology 92: 729–735.

Leisova-Svobodova L., Hanzalova A., and Kucera L. 2010b. The variability of a *Pyrenophora tritici-repentis* population as revealed by inter-retrotransposon amplified polymorphism with regard to the *Ptr ToxA* gene. Czech Mycology. 61: 125–128.

Lelley, T., Stachel, M., Grausgruber, H., and Vollmann, J. 2000. Analysis of relationships between *Aegilops tauschii* and the D genome of wheat utilizing microsatellites. Genome 43:661-668.

Lepoint P., Renard M.-E., Legreve A., Duveiller E., and Maraite H. 2010. Genetic diversity of the mating type and toxin production genes in *Pyrenophora tritici-repentis*. Phytopathology 100: 474–483.

Letta, T., Olivera, P., Maccaferri, M., Jin, Y., Ammar, K., Badebo, A., Saliva, S., Nolia, E., Crossa, J., and Tuberosa, R. 2014. Association mapping reveals novel stem rust resistance loci in durum wheat at the seedling stage. Plant Genome 7: 1-13.

Li, H. B., Yan, W., Liu, G. R., Wen, S. M., and Liu, C. J. 2011. Identification and validation of quantitative trait loci conferring tan spot resistance in the bread wheat variety Ernie. Theoretical and Applied Genetics 122:395-403.

Lichter A., Gaventa J. M., and Ciuffetti L. M. 2002. Chromosome-based molecular characterization of pathogenic and non-pathogenic wheat isolates of *Pyrenophora tritici-repentis*. Fungal Genet. Biol. 37: 180–189.

Liu, S., Zhang, X., Pumphrey, M. O., Stack, R. W., Gill, B. S., and Anderson, J. A. 2006. Complex microcolinearity among wheat, rice, and barley revealed by fine mapping of the

genomic region harboring a major QTL for resistance to Fusarium head blight in wheat. Functional and Integrative Genomics 6:83-89.

Liu, Z. H., Faris, J. D., Meinhardt, S. W., Ali, S., Rasmussen, J. B., and Friesen, T. L. 2004. 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., Zhang, Z., Faris, J. D., Oliver, R. P., Syme, R., McDonald, M. C., McDonald, B. A., Solomon, P. S., Lu, S., Shelver, W. L., Xu, S. and Friesen, T. L. 2012. The cysteine rich necrotrophic effector SnTox1 produced by *Stagonospora nodorum* triggers susceptibility of wheat lines harboring *Snn1*.Public Library of Science Pathology 8: e1002467.

Lorang, J. M., Sweat, T. A., and Wolpert, T. J. 2007. Plant disease susceptibility conferred by a "resistance" gene. PNAS 104: 14861-14866.

Loughman, R., and Deverall, B. J. 1986. Infection of resistant and susceptible cultivars of wheat by *Pyrenophora tritici-repentis*. Plant Pathology 35:443-50.

Lu, B. R., and Ellstrand, N. 2014. World food security and the tribe Triticeae (Poaceae): Genetic resources of cultivated, wild, and weedy taxa for crop improvement. Journal of Systematics and Evolution 52:661-666.

Lu, H. J., and Faris, J. D. 2006. Macro- and microcolinearity between the genomic region of wheat chromosome 5B containing the *Tsn1* gene and the rice genome. Functional Integrative Genomics 6:90–103.

Lu, H. J., Fellers, J. P., Friesen, T. L., Meinhardt, S.W., and Faris, J. D. 2006. Genomic analysis and marker development for the *Tsn1* locus in wheat using bin-mapped ESTs and flanking BAC contigs. Theoretical and Applied Genetics 112:1132–1142.

Lu, S., Faris, J. D., Sherwood, R., and Edwards, M. C. 2013. Dimerization and protease resistance: new insight into the function of PR-1. Journal of Plant Physiology 170:105-110.

Luo, M. C., Yang, Z. L., You, F. M., Kawahara, T., Waines, J. G., and Dvorak, J. 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.

Luz, W. C. da. 1995. Evaluation of wheat cultivars for resistance to tan spot. Fitopatologia Brasileira 20:444-448.

Maccaferri, M., Sanguineti, M.C., Mantovani, P., Demontis, A., Massi, A., Ammar, K., Kolmer, J.A., Czembor, J.H., Ezrati, S., and Tuberosa, R. 2010b. Association mapping of leaf rust response in durum wheat. Molecular Breeding 26:189–228.

Manning, V. A., and Ciuffetti, L. M. 2005. Localization of Ptr ToxA produced by *Pyrenophora tritici-repentis* reveals protein import into wheat mesophyll cells. The Plant Cell 17: 3203-3212.

Manning, V. A., Chu, A. L., Steeves, J. E., Wolpert, T. J., and Ciuffetti, L. M. 2009. A host-selective toxin of *Pyrenophora tritici-repentis*, Ptr ToxA, induces photosystem changes and reactive oxygen species accumulation in sensitive wheat. Molecular Plant-Microbe Interactions 22: 665-676.

Manning, V. A., Hardison, L.K. and Ciuffetti, L. M. 2007. Ptr ToxA interacts with a chloroplast-localized protein. Molecular Plant-Microbe Interactions 20: 168–177.

Maraite, H. and Weyns, J. 1982. Observations d'une epidemic de *Pyrenophora tritici-repentis* (Died.) Drechs. Sur froment en Belgique. Premiers donnees sur le cycle de development,

le pouvoir pathogene et la sensibilte aux fongicides. Med. Fac. Landbouww. Rijskuniv 47:913-923.

Martinez, J. P., Oesch, N. W., and Ciuffetti, L. M. 2004. Characterization of the multiple copy host-selective toxin gene, ToxB, in pathogenic and nonpathogenic isolates of *Pyrenophora tritici-repentis*. Molecular Plant Microbe Interactions 22: 665–676.

Matsuoka, Y. 2011. Evolution of Polyploid Triticum Wheats under Cultivation: The Role of domestication, natural hybridization and allopolyploid speciation in their diversification. Plant & Cell Physiology 52: 750–764.

Friskop, A., and Liu, Z. 2016. Fungal Leaf Spot Diseases of Wheat: Tan Spot, Stagonospora nodorum blotch and Septoria tritici blotch. North Dakota State University Extension Service. (p. 1249).

Mehta, Y. A., and Almadia, A. M. R. 1977. Nota Sobre maturacco de *Pyrenophora trichostoma*. Summa Phytopathology 1:283-288.

Meinhardt, S. W., Ali, S., Ling, H., and Francl, L. J. 2003. A new race of *Pyrenophora tritici-repentis* that produces a putative host-selective toxin. In: Rasmussen, J. B., Friesen, T. L., Ali, S. (eds) Proceedings of the fourth international wheat tan spot and spot blotch workshop. North Dakota Agricultural Experiment Station, Fargo (p.117-119).

Misra, A. P., and Singh, R. A. 1972. Pathogenic differences amongst three isolates of *Helminthosporium tritici-repentis* and the performance of wheat varieties against them. Indian Phytopathology 25:350-353.

Mitra, M. 1934. A leaf spot disease of wheat caused by *Helminthosporium tritici-repentis* Died. Indian Journal of Agricultural Science 4:692-700.

Moreno M. V., Stenglein S. A., Balatti P. A., Perello A. E., 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.

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

Nagle, B.J., Frohberg, R. C. and Hosford, R. M. Jr. 1982 Inheritance of resistance to tan spot of wheat. Pages 40-45 in R.M. Hosford, Jr. ed., Tan Spot of Wheat and Related Diseases Workshop. North Dakota Agricultural Experiment Station, Fargo.

Nagy, E. D., and Bennetzen, J. L. 2008. Pathogen corruption and site-directed recombination at a plant disease resistance gene cluster. Genome Research 18:1918–1923.

Nisikado, Y. 1928. Preliminary notes on yellow leaf spot diseases of wheat caused by *Helminthosporium tritici-vulgaris* Nisikado. Institute of Agricultural Biology 4:103-109.

Osborne, L., and Stein, J. 2009. Wheat fungicide recommendations (p. 952).

Pandelova, I., Betts, M. F., Manning, V. A., Wilhelm, L. J., Mockler, T. C., Ciuffetti, L. M. 2009. Transcriptional reprogramming induced by Ptr ToxA in wheat provides insights into the mechanisms of plant susceptibility. Molecular Plant 2: 1067–1083.

Pandelova, I., Figueroa, M., Wilhelm, L. J., Manning, V. A., Mankaney, A. N., Mockler, T. C., and Ciuffetti, L. M. 2012. Host-selective toxins of *Pyrenophora tritici-repentis* induce common responses associated with host susceptibility. PLoS one 7:1-16.

Patel, J. S., Mamidi, S., Bonman, J. M., and Adhikari, T. B. 2013. Identification of QTL in spring wheat associated with resistance to a novel isolate of *Pyrenophora tritici-repentis*. Crop Science 53:842-852.

Raymond, P.J., W. W. Bockus, and B.L. Norman. 1985. Tan spot of winter wheat: procedures to determine host response. Phytopathology 75:686-690.

Rees, R. G., & Platz, G. J. 1992. Tan spot and its control: some Australian experiences.

Rees, R. G., and Platz, G. J. 1979. The occurrence and control of yellow spot of wheat in north-eastern Australia. Animal Production Science 19:369-372.

Rees, R. G., Platz, G. J. 1983. Effects of yellow spot on wheat: comparison of epidemics at different stages of crop development. Australian Journal of Agriculture Research 34:39–46.

Rees, R. G., Platz, G.J., Mayer, R. J. 1982. Yield losses in wheat from yellow spot: comparison of estimates derived from single tillers and plots. Australian Journal of Agriculture Research 33:899–908.

Riede, C.R., Francl, L.J., Anderson, J. A., Jordahl, J.G. and Meinhardt, S.W. 1996. Additional sources of resistance to tan spot of wheat. Crop Science 36:771-777.

Sarma, G. N., Manning, V.A., Ciuffetti, L. M., and Karplus, P. A. 2005. Structure of Ptr ToxA: an RGD-containing host-selective toxin from *Pyrenophora tritici-repentis*. Plant Cell 17: 3190–3202.

Schilder, A. M. C., and Bergstrom, G. C. 1994. Infection of wheat seed by *Pyrenophora tritici-repentis*. Canadian Journal of Plant Pathology 72:510-519.

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

Shoemaker, R.A. 1962. Drechslera Ito. Canadian Journal of Botany 40:809-836.

Singh P. K., and Hughes G. R. 2006. Genetic similarity among isolates of *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. J. Phytopathol. 154: 178–184.

Singh, P. K., Crossa, J., Duveiller, E., Singh, R. P., and Djurle, A. 2015. Association mapping for resistance to tan spot induced by *Pyrenophora tritici-repentis* race 1 in CIMMYTs historical bread wheat set. Euphytica 1-11.

Singh, P. K., Gonzalez-Hernandez, J. L., Mergoum, M., Ali, S., Adhikari, T. B., Kianian, S. F., Elias, E. M. and Hughes, G. R. 2006. 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., Mergoum, M., Gonzalez-Hernandez, J. L., Ali, S., Adhikari, T. B., Kianian, S. F. Elias, E. M. and Hughes, G. R. 2008b. Genetics and molecular mapping of resistance to necrosis inducing race 5 of *Pyrenophora tritici-repentis* in tetraploid wheat. Molecular Breeding 21:293-304.

Singh, S., Bockus, W. W., Sharma, I., and Bowden, R. L. 2008a. A novel source of resistance in wheat to *Pyrenophora tritici-repentis* race 1. Plant Disease 92:91-95.

Soreng, R. J., Peterson, P. M., Romaschenko, K., Davidse, G., Zuloaga, F. O., Judziewicz, E. J., Filgueiras, T. S., Davis, J. I., and Morrone, O. 2015. A worldwide phylogenetic classification of the Poaceae (Gramineae). Journal of Systematics and Evolution 53:117-137.

Sprague, R. 1950. Diseases of Cereals and Grasses in North America. Ronald Press Co., New York.

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

Strelkov, S. E., Kowatsch, R. F., Ballance, G. M., and Lamari, L. 2006. Characterization of the ToxB gene from North African and Canadian isolates of *Pyrenophora tritici-repentis*. Physiological and Molecular Plant Pathology 67:164-170.

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

Strelkov, S. E., Lamari, L., Sayoud, R., and Smith, R. B. 2002. Comparative virulence of chlorosis-inducing races of *Pyrenophora tritici-repentis*. Canadian Journal of Plant Pathology 24:29-35.

Sun, X. C., Bockus, W., and Bai, G. 2010. Quantitative trait loci for resistance to *Pyrenophora tritici-repentis* race 1 in a Chinese wheat. Phytopathology 100:468-473.

Sutton, J. C., and Vyn, T. J. 1990. Crop sequences and tillage practices in relation to diseases of winter wheat in Ontario. Canadian Journal of Plant Pathology 12:358-368.

Sykes, E.E., and Bernier, C. C. 1991. Qualitative inheritance of tan spot resistance in hexaploid, tetraploid, and diploid wheat, Canadian Journal of Plant Pathology 13:38-44.

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

Tadesse, W., Hsam, S. L. K., and Zeller, F. J. 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.

Tai, Y. S., Bragg, J. 2007. Dual applications of a virus vector for studies of wheat-fungal interactions. Biotechnology 6: 288–291.

Tekauz, A. (1976). Distribution, severity, and relative importance of leaf spot diseases of wheat in western Canada in 1974'. Survey 56:36.

Tekauz, A., Samborski, D. J., Rourke, D.S. R., and Iverson, A. T. 1983. Disease of winter wheat in Manitoba in 1983. Manitoba Agronomists Proceedings 63-68.

Tuori, R. P., Wolpert, T. J., and Ciuffetti, L. M. 2000. Heterologous expression of functional Ptr ToxA. Molecular plant-microbe interactions 13:456-464.

Valder, P. G. and Shaw, D. E. 1952. Yellow spot disease of wheat in Austrailia. Proceedings of the Linnean Society of New South Wales 77:1337-1340.

Virdi, S. K., Liu, Z., Overlander, M. E., Zhang, Z., Xu, S. S., Friesen, T. L., and Faris, J. D. 2016. New insights into the roles of host gene-necrotrophic effector interactions in governing susceptibility of durum wheat to tan spot and Septoria nodorum blotch. G3 6:4139-4150.

Walton, J. D. 1996. Host-selective toxins: agents of compatibility. The Plant Cell 8:1723.

Wegulo, S. N. 2011. Tan Spot of Cereals. The Plant Health Instructor.

Weise, M. V. 1987. Compendium of wheat diseases, 2nd ed. APS, St. Paul.

Wolpert, T. J., Dunkle, L. D., Ciuffetti, L. M. 2002. Host-selective toxins and avirulence determinants: what's in a name? Annual Review of Phytopathology 40:251-285.

Young, N. D. 1996. QTL mapping and quantitative disease resistance in plants. Annual Review of Phytopathology 34: 479-501.

PAPER 1: GENOME WIDE ASSOCIATION MAPPING OF RESISTANCE TO TAN SPOT IN DURUM WHEAT

Abstract

Genome wide association study (GWAS) has become an important approach in the last decade to dissecting genetic basis of any given trait, particularly for a quantitative trait. Tan spot, caused by *Pyrenophora tritici-repentis*, is a major foliar disease on all cultivated wheat crops. Resistance to tan spot is a complex, which include insensitivity to necrotrophic effectors, unknown recessive resistance, and race-specific or –nonspecific resistance QTL. Several studies have used GWAS to identify QTL associated with tan spot resistance in hexaploid wheat, but not in durum. The objectives of this study were to identify sources of resistance to multiple races and to locate QTL for tan spot resistance in a core durum collection using GWAS. Disease evaluations showed that nineteen lines were highly resistant to all races tested. A total of 37,285 SNP markers from genotype-by-sequencing were employed in GWAS. As expected, sensitivity to Ptr ToxA and Ptr ToxB was mapped to the chromosome arms 5BL and 2BS, respectively. A major QTL was identified to 2BS for the race 5 isolate DW5, the same location as that for sensitivity to Ptr ToxB. However, there was no QTL for races 1 and 2 detected on the same location as that for sensitivity to Ptr ToxA. Two QTLs, one on 1AS and the other on 1BS, were identified for race 1, one of which likely corresponded to the *Tsc1* locus. A genomic region on 3BL was identified for three races, which is likely the race-nonspecific resistance QTL identified previously. Our work suggests the interactions of Ptr ToxC-*Tsc1* and Ptr ToxB-*Tsc2* and race-nonspecific resistance are important for tan spot system in durum.

Introduction

Tan spot is a destructive foliar disease that can occur on both bread wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD genomes) and durum (*T. turgidum* L. ssp. *durum*, 2n = 4x = 28, AABB genomes). The disease is caused by the fungus *Pyrenophora tritici-repentis* (*Ptr*) (anamoph: *Drechslera tritici-repentis*). The pathogen forms pseudothecia on wheat stubbles as an overwinter structure. When the new growth season comes, ascospores are released from the pseudothecia on wheat stubbles acting as a primary source of inoculum to infect young wheat seedlings (Singh et al. 2011). The infections often result in formation of necrotic lesions with a distinct tan center and sometimes being surrounded by a yellow halo (Lamari and Bernier 1989). Within these disease lesions, conidia can be produced and dispersed leading to the spreading of the disease to the upper leaves or other healthy plants. Under favorable weather conditions and on susceptible cultivars, conidium infection can repeat multiple times to cause high disease incidence and serious damage to plant. Since 1970s, the adoption of reduced or no-till practices has been widely adopted, which has led to the increased level of primary inoculum at local and global scales, thus leding to establishment of tan spot as an important disease of wheat in nearly all parts of wheat-growing areas in the world (Hosford 1971; Rees and Platz 1992). The yield losses due to tan spot can be up to 50% on highly susceptible cultivars under favorable conditions (Rees et al. 1982). Apart from yield losses, the disease can cause reduction of grain quality by the formation of red or pink smudge due to the fungal infection (Ferdinandez et al. 1997). The red smudge is of more concern in durum than in common wheat (Ferdinandez et al. 1997).

The disease system has been well known to involve fungal produced necrotrophic effectors (NEs) that interact with the corresponding host sensitivity genes to cause disease. Up to

now, three NE-host sensitivity gene interactions have been identified in wheat-*Ptr* pathosystem, including Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1* (Faris et al. 1997; Abeysekara et al. 2010; Faris et al. 2013). The host sensitivity genes *Tsn1*, *Tsc2* and *Tsc1* have been mapped to the wheat chromosome arms 5BL, 2BS and 1AS, respectively (Faris et al. 1996; Faris et al. 2010; Friesen and Faris 2004; Abeysekara et al. 2009; Effertz et al. 2001). Among them, the *Tsn1* gene has been cloned and it has a structure similar to the typical resistance gene (Faris et al. 2010).

In addition to these three NEs- host sensitivity gene interactions, genetic studies have identified additional four recessive tan spot resistance genes, including *tsr2* on the chromosome arm 3BL (Singh et al. 2006), *tsr3* on 3DL (Tadesse et al. 2006a), *tsr4* on 3AL (Tadesse et al. 2006b) and *tsr5* on chromosome arm 3BL (Singh et al. 2008). Gamba and Lamari (1998) reported that race 3 and race 5 isolates, which induce chlorosis on hexaploid wheat lines, were able to induce necrosis on some tetraploid wheat genotypes. Both the *tsr2* and *tsr5* genes were identified in durum wheat background to confer recessive resistance to necrosis caused by races 3 and 5, respectively (Singh et al. 2006, 2008). These suggested additional NE- host sensitivity gene interactions are presented in durum wheat to induce necrosis.

Since 1990s, many studies have been conducted in diverse bi-parental wheat populations to identify quantitative trait locus (QTL) conferring resistance or susceptibility to tan spot. Some QTL were located to the same loci of three host sensitivity genes, which strongly indicates that these NE-host sensitivity gene interactions are important for tan spot disease in these wheat populations. However, QTL on other chromosomes or genomic regions were also reported (see review by Faris et al. 2013). Most noticeably, several genomic regions were found conferring resistance to multiple races, which has been referred to race-nonspecific resistance QTL (Faris and Friesen 2005; Kariyawasam et al. 2016). The race-nonspecific resistance QTL reported in

Kariyawasam et al. (2016) could have an epistatic effect on necrosis induced by Ptr ToxA-*Tsn1* interaction.

Genome wide association study (GWAS) has become an important approach to identify genomic region associated with a quantitative trait in plant. GWAS is based on the concept of gene linkage disequilibrium (LD) and examines the association between a specific trait and a set of genetic markers across the whole genome in a given germplasm collection. Significant marker trait association (MTA) can been detected by using special computer software, for example TASSEL (Bradbury et al. 2007). Compared to traditional bi-parental mapping, GWAS has a few advantages, including 1) having better precision to localize genes conferring a quantitative trait due to a higher rate of recombination among loci; 2) no time need to develop segregating populations as well as no limits for sample size; 3) more QTL detection in one study due to diverse collection used.

In recent years, GWAS approach has been applied to the identification of the genomic regions associated with tan spot resistance. Gurung et al. (2011) conducted the first GWAS of tan spot resistance by using a spring wheat landrace collection and DArT markers across the genome. In this study, several genomic regions on the chromosomes 1D, 2A, 2B, 2D, 4A, 5B and 7D were identified for the race 1 isolate Pti2 and genomic region on 1D, 2B, 2D, and 7D for DW7. Since then, GWAS has been performed for different races on various wheat panels, including a new race on spring common wheat landraces (Patel et al. 2013), unknown races on an European winter wheat collection (Koller et al. 2014), multiple races on a North America winter wheat cultivars and breeding lines (Liu et al. 2015). Some genomic regions identified in these GWAS corresponded to NE sensitivity loci and previously reported QTL, while others may be novel. However, no GWAS has been performed for tan spot resistance in durum wheat.

In this work, we conducted tan spot disease evaluations and GWAS of tan spot resistance in a world durum wheat collection, which was assembled in USDA-ARS Triticeae-Coordinated Agricultural Project (T-CAP). This durum panel has been used in GWAS of wheat leaf rust resistance (Aoun et al. 2016). The objectives of this research were to identify sources of resistance to tan spot from this panel and locate quantitative trait loci for resistance to tan spot using GWAS.

Materials and Methods

Plant Materials

The T-CAP durum panel consisted of 497 durum wheat accessions that were collected from 67 different countries in the world across almost all continents. These materials were at different improvement status including landraces, cultivars, breeding and cultivated lines (ambiguous improvement status). This panel has been used in a study funded by USDA-ARS T-CAP (Aoun et al. 2016). All the 497 accessions were included in disease evaluations and NE infiltration. For genotyping-by-sequencing and the final GWAS, 371 lines were used, which included 171 landraces, 57 breeding lines, 44 cultivars, and 99 "cultivated". Disease evaluations with different races also include four commonly used tan spot differential lines, which are 'Salamouni' (universal resistant), 'Glenlea' (sensitive to Ptr ToxA), '6B365' (sensitive to Ptr ToxC) and '6B662' (sensitive to Ptr ToxB). Additionally, a Brazilian 'BR34' and the NDSU breeding line 'ND495' were included as resistant and susceptible checks. The NDSU winter wheat cultivar 'Jerry' was used in the planting as protecting boarder for disease inoculation experiments.

Plant Genotyping

This durum wheat collection was previously genotyped through the T-CAP using the Illumina iSelect 9K wheat array at the USDA–ARS genotyping laboratory in Fargo, ND (Cavanagh et al. 2013). The SNP markers obtained from 9k gene array was employed in GWAS for wheat leaf rust resistance (Aoun et al. 2016). However, in this study, genotyping-by-sequencing was performed on 371 lines for generating more SNP markers for GWAS.

For DNA isolation, plant materials were planted in a small cone with two seeds in one cone and leaf tissue from each line was collected at one-week old seedlings. DNA was isolated with the Wizard Genomic DNA Purification Kit (A1125; Promega) per the manufacturer's instructions and quantified with a Quant-iT PicoGreen dsDNA assay kit (P7589; Thermo Fisher Scientific). Genotyping-by-Sequencing (GBS) library was prepared using the method described in (Poland et al. 2012). Briefly, 100 ng of each DNA was digested with *PstI* and *MseI*, and then ligated to a barcoded adapter unique to each sample and a common adapter. Equal volumes of the ligated products were pooled and purified with the QIAquick PCR purification kit (28104; QIAGEN) for PCR amplification. For the PCR amplification, 50 ng of template DNA was mixed with NEB 2X Taq Master Mix and two primers (5 nmol each) in a 200 µL of total volume and amplified on a thermocycler for 18 cycles with 10 sec of denaturation at 98 ºC, followed by 30 sec of annealing at 65ºC, and finally 30 sec extension at 72ºC. The PCR product was then cleaned using a QIAquick PCR purification kit. One GBS library was constructed for the 371 durum accessions and quantified with BioAnalyzer. The GBS library was sequenced on four lanes of Illumina HiSeq2500 at the Genomic Sequencing and Analysis Facility at the University of Texas Southwestern Medical Center at Dallas, Texas. All sequences were submitted to the National Center for Biotechnology Information (NCBI) Short Read Archive.

Single nucleotide polymorphism (SNP) discovery and genotype calling was performed using the TASSEL-GBS pipeline (Glaubitz et al. 2014) with the *Triticum aestivum* IWGSC1.0 +POPSEQ genome assembly (Ensembl.org release 29) as reference genome (https://www.wheatgenome.org/Tools-and-Resources/Sequences) (Mayer et al. 2014; Chapman et al. 2015). SNP markers were filtered with an individual read depth greater than 1, minor allele frequency greater than 0.01, and missing data less than 50%.

Disease Evaluations

The disease evaluation experiments were carried out in the greenhouse and growth chamber under controlled conditions. Super cell cone-tainers purchased from Stuewe and Sons, Inc., Corvallis,OR were used in planting all the durum lines, differential lines, and checks. The cone-tainers were filled with Sunshine SB100 soil (Sun Grro Horticulture, Bellevue, WA) and then placed in RL98 racks. After planting, a pinch of the fertilizer Osmocote Plus 15-19-12 (Scotts Sierra Horticultural Product Company, Maysville, OH) were applied to each cone-tainer. The border cones of each rack were planted with 'Jerry' to reduce edge effect, which leave a total 60 cone-tainers in each rack to plant durum lines. Three seeds of each durum and differential line and checks were planted in one cone-tainer for each inoculation and the inoculation for each isolate was repeated at least three times with randomized complete block design (RCBD). The plants were grown in greenhouse room with a temperature ranging from 20-25 $^{\circ}$C and were inoculated with fungal spores at 14 days (two-three leaf stage) after planting.

The durum panel was evaluated for tan spot individually with four *Ptr* isolates Pti2, 86-124, 331-9 and DW5, which represented races 1, 2, 3 and 5, respectively. In term of known NEs they produce, Pti2 produces Ptr ToxA and Ptr ToxC, 86-124 produces only Ptr ToxA, 331-9 produces only Ptr ToxC, and DW5 produces only Ptr ToxB.

Fungal spore suspension was prepared based on the standard procedure described by Lamari and Bernier (1989). Prior to inoculation, the concentration of the spore suspension was adjusted approximately to 2000-3000 spores per ml. Two drops of Tween 20 (a surfactant reagent) per 100 ml were added to spore suspension. The seedling plants at two to three leaf stage were inoculated in a closed and air-conditioned room by using an air-pressured spray gun. The inoculated plants were placed right away into mist chambers with 100% relative humidity at the temperature of 21˚C and continuous lighting for 24 hours. After that, the plants were moved into a growth chamber with 12-hour photoperiod and temperature of 21˚C for disease development. At 7th day after the inoculation, second leave of plants were rated for tan spot disease using a 1-5 rating scale based on lesion types with 1 being highly resistant and 5 being highly susceptible (Lamari and Bernier 1989a). For plants which have two lesion types, an intermediate score between the two-lesion types was given.

Necrotrophic Effector Infiltration

The same set of panels was also evaluated for reaction to Ptr ToxA and Ptr ToxB. The plantings were similar to those in disease inoculation, but only two seeds were planted for each line in a container and all the containers in the RL98 racks were used for durum lines (no boarder was used). Two plants were evaluated for each line and each NE and the experiment was repeated twice. Ptr ToxA and Ptr ToxB were prepared by culturing the relevant genetically engineered yeast strains in yeast peptone dextrose (YPD) liquid medium and then the culture filtrates were harvested (Liu et al. 2009). Approximate 25 µl of this harvested culture filtrates were injected in to fully expanded secondary leaf using a 1 ml syringe with the needle removed. The area of infiltration was marked using a permanent marker. Then these infiltrated plants were placed in a growth chamber with temperature of 21˚C for 3 - 5 days and the responses were rated

using a 0-3 rating scale which was developed by Friesen and Faris (2012) in which 0 being insensitive and 1-3 being sensitive. The lines having inconsistent reactions were treated as missing data.

Statistical Analysis and Association Mapping

Statistical analysis was carried out using SAS 9.4 software with corresponding command codes (SAS Institute 2012). The disease reaction data of each isolate was initially tested for normal distribution using Shapiro-Wilk in the PROC UNIVARIATE procedure (SAS Institute 2012). Homogeneity of variance among replicates of each isolate was then evaluated using Bartlett's χ^2 test if the data had a normal distribution (Snedecor and Cochran 1989), or Levene's test if it did not (Levene 1960) at $P < 0.05$ significant level. Disease reaction data from homogenized experiments for each isolate were combined and the average was computed, which were used for association mapping. Correlation analyses between NE sensitivity and disease reactions to the races producing the corresponding NE were conducted by using Pearson correlation coefficient in SAS 9.4 software (SAS Institute 2012).

Four regression models were used to analyze marker trait associations using TASSEL 5.2.38. They were (i) naive model which does not account for kinship and population structure, (ii) kinship, (iii) kinship+ population structure (first two PCs), and (iv) kinship+ population structure (first five PCs). The Kinship and the population structure analysis were incorporated in to the final analysis to make sure that only genetically significant associations were discovered in the GWAS instead of false associations resulting from population structure or familial relatedness. For each model, the SNP markers were ranked from smallest to largest *P*-values. The best model for each tan spot race was selected by calculating the mean squared difference

(MSD) between observed and expected *P*-values (Aoun et al., 2016). The MSD was calculated using the following formula:

$$MSD= [pi- (i/n)]^{2} /n$$

Where n = number of markers, i = the rank number from 1 to n, and pi is the probability value of the ith-ranked *P*-value. Significant markers associated with tan spot resistance were selected only from the model with the lowest MSD value.

The association mapping was conducted using the TASSEL 5.2.38 software with the corresponding best models (Bradbury et al. 2007). Missing values were imputed with LD-KNNi method (Money et al. 2015) implemented in TASSEL v5.2 (Bradbury et al. 2007). Principal component analysis (PCA) was conducted with the 13,263 SNPs (Since 13,263 SNPs had a call rate greater than 80% out of total 68382 markers) to assess population structure using R command *prcomp*. Based on the elbow shape of the Scree plot (Figure 3), the first two PCs were used for model-based cluster analysis with R package *mclust* and first two PCs and five PCs were incorporated in the models as covariates to capture population structure. Centered_IBS kinship (K) matrix was calculated using the TASSEL 5.2.38 software (Bradbury et al. 2007). GWAS was conducted for each trait with four statistical models: naïve, K, and PK models. The best model was determined based on MSD values for each trait. Linkage disequilibrium (LD) was estimated as r^2 between pairs of SNPs using TASSEL v5.2 (Bradbury et al. 2007). The *P*-values of the selected model were later adjusted by using R version 3.4.2 software according to Ben-Hochberg method and the corresponding false discovery rate (FDR) values were obtained. Marker–trait associations were finally considered significant at FDR ≤ 0.1.

Results

Reaction of Durum Lines to Tan Spot

The durum lines in this panel varied from highly resistant to high susceptible in reaction to tan spot caused by all the races and the distributions of durum lines in each category of disease scale were shown in Figure 1. For all the races, the majority of durum lines distribute in the lesion type of 3.1 to 4.0 indicating a susceptible reaction and only a few lines showed to be highly resistant. The average disease means of the entire panel for Pti2, 86-124, 331-9 and DW5 were 3.45, 3.27, 3.82 and, 3.12 respectively. Among the four isolates, the race 3 isolate 331-9 was more virulent on these durum lines because there were more number of lines having susceptible reaction, while DW5 were the least virulent with more number of lines toward resistance (Fig. 1). Normality test revealed that disease reaction of the durum panel for all four isolates does not fit to a normal distribution ($P < 0.0001$-0.0005).

Although the majority of durum lines were susceptible, there were nineteen lines highly resistant with disease means low than 2.5 to all the races (Table 1). After inoculation, these lines mainly developed pin-sized lesions on the leaves like the resistant check BR34 (Fig. 2). The disease mean and country origin for these lines were provided in Table 1.

Reaction of Durum Lines to Ptr ToxA and Ptr ToxB

For Ptr ToxA, there were 258 sensitive lines and 160 insensitive lines. For Ptr ToxB, there were 181 sensitive lines and 278 insensitive lines. For Ptr ToxA, the correlation co-efficient (r) were 0.1841, 0.2910, 0.09047 and 0.03719 for Pti2, 86-124, 331-9 and DW5 respectively. The correlation co-efficient (r) for Pti2 and 86-124 were significant at $p<0.01$ and $p <0.001$ (Table 2).

Table 1. The disease means and country origin of highly resistant lines in the durum panel

Line[a]	Country Origin	Improvement Status[b]	Pti2[c]	86-124[c]	331-9[c]	DW5[c]
Cltr17337	India	Landrace	1.75	2.50	2.58	1.50
PI32156	Turkey	Landrace	1.75	1.50	1.40	1.00
PI43247	Nigeria	Cultivated	2.25	1.50	1.70	1.50
PI57556	Tunisia	Landrace	2.00	2.50	2.50	2.00
PI182113	Unknown	Cultivated	2.50	2.33	2.10	2.00
PI191645	United States	Cultivar	2.00	1.88	1.92	1.75
PI191654	Sweden	Cultivated	1.50	1.17	1.17	1.25
PI191958	Hungary	Breeding	1.38	1.25	1.50	1.13
PI192640	Australia	Breeding	1.33	1.13	1.80	1.38
PI274670	Algeria	Landrace	1.88	1.75	2.08	1.75
PI274678	Israel	Landrace	2.50	1.17	1.50	1.25
PI519567	Iraq	Landrace	2.00	1.83	2.50	1.17
PI519750	Yemen	Cultivated	2.00	2.50	2.42	1.50
PI519759	Italy	Cultivar	2.00	1.63	1.83	1.50
PI520044	India	Cultivated	2.13	1.75	2.33	1.88
PI520392	Egypt	Landrace	1.75	1.17	1.30	1.33
PI520518	Italy	Cultivated	2.50	2.38	2.50	2.25
PI537310	Portugal	Landrace	2.13	1.75	2.58	1.50
PI565264	Egypt	Landrace	2.25	1.75	1.75	2.38

[a] Highly resistant TCAP durum lines multiple isolates of *Pyrenophora tritici-repentis*.
[b] Improvement status of each line
[c] *Pyrenophora tritici-repentis* pathogen isolates: Pti2, 86-124, 331-9, and DW5 which represent races 1, 2, 3 and 5 respectively.

Table 2. Correlation between the reactions to necrotrophic effectors and disease reactions caused by different isolates of *Pyrenophora tritici-repentis* in the durum panel.

Isolates /nec. effector [a]	Pti2	86-124	331-9	DW5
Ptr ToxA	0.1841***	0.2910***	0.09047^{NS}	0.03719^{NS}
Ptr Tox B	-	-	-	0.51705***

[a] The durum panel was evaluated with four *P. tritici-repentis* isolates: Pti2, 86-124, 331-9, and DW5 which represent races 1, 2, 3 and 5 respectively, and with two necrotrophic effectors: Ptr ToxA and Ptr ToxB. Significance level was indicated by NS (non-significant), ** (Significant at $p<0.01$), *** (Significant at $p<0.001$) or – (no calculation was done for the two traits).

Marker Properties and Linkage Disequilibrium Analysis

A total of 68,382 SNP markers with a minor allele frequency greater than 1% and a call rate greater than 50% were obtained; 13,263 of which had a call rate greater than 80% were used for PCA. LD was measured as r^2 between a pair of markers. Means of LD decay were 0.61 between markers with distance <0.1 Mb and 0.37 with distance <0.2 Mb (Figure 4). Given sub-genome A and B size of ~12 Gb (Mayer et al. 2014; Chapman et al. 2015), marker density is about 0.2 Mb per marker and over 37% of genetic variation could be captured by the 68,382 SNPs in this study.

Population Structure and Line Relatedness

The first two PCs explained 10.7% and 5.5% of the total variation, respectively. Model-based cluster analysis suggested there were nine subgroups, and accessions with different improvement status were intermixed (Figure 5). The relatedness was estimated using Centered_IBS Kinship matrix (Supplementary Table S1). Because T-CAP panel contains subgrouping and lines which are not related, GWAS should be considered for both population structure and line relatedness to decrease the probability of false-positive associated markers.

For each races and NE, we calculated the MSD for different AM models (Table 3). Based on MSD values, the model of kinship+ population structure (first two PCs) fits best for disease data of race 5 and Ptr ToxB sensitivity. The model of Kinship+ population structure (first five PCs) fits best for the disease data of race 1. The model of Kinship only fits best for disease data of races 2 and 3 and Ptr ToxA sensitivity (Table 3).

Table 3. Mean square difference (MSD) among different models for each trait.

Trait	Naïve	Kinship	2PCs+Kinship [a]	5PCs+Kinship[b]
Race 1 (Pti2)	0.102036	6.56×10^{-5}	4.45×10^{-5}	$\underline{3.32\times10^{-5}}$
Race 2 (86-124)	0.105897	<u>0.000121</u>	0.000197	0.000174
Race 3 (331-9)	0.064551	$\underline{7.79\times10^{-5}}$	0.000116	0.000174
Race 5 (DW5)	0.060288	3.07×10^{-6}	$\underline{2.51\times10^{-6}}$	1.31×10^{-5}
Ptr ToxA	0.067192	<u>0.000122</u>	0.000142	0.000129
Ptr ToxB	0.067489	0.001015	<u>0.000848</u>	0.001757

[a] 2PCs indicates population structure matrix (Q matrix) based on the first two principal components
[b]5PCs indicates population structure matrix (Q matrix) based on the first five principal components
Underlined numbers show the lowest MSD value and the best model for each trait. The best model was used to investigate associations between single-nucleotide polymorphic markers and tan spot disease response.

Association Mapping for Different Races and NEs

Ptr ToxA

Thirty-four marker positions of loci were significant for Ptr ToxA which spanned on chromosome 5B from S5B_545794002 bp to S5B_653785165 bp and their R^2 values ranged from 0.05 to 0.58. The SNP marker S5B_546810215 on 5B had the highest LOD and R^2 values of 15 and 0.30 (Figure 6). There were markers on other chromosomes having a FDR slightly above cutoff lines.

Ptr ToxB

Fifty-seven marker positions of loci were significant for Ptr ToxB which spanned on chromosome arm 2BS from 22783770 bp to 31597610 bp and their R^2 values ranged from 0.04 to 0.30. The SNP marker S2B_24100467 on 2B had the highest LOD and R^2 values of 31 and

0.58, respectively (Figure 7). Similar to Ptr ToxA, a few markers on other chromosomes had a FDR slightly above cutoff lines and were likely due to background noise.

Pti2 (Race 1)

The SNPs markers significantly associated with tan spot caused by Pti2 were located to two genomic regions, one on the chromosome arm 1AS from 2988007 bp to 6424367 bp positions and the other on chromosome 1B from 4859254 to 6049233 bp positions (Fig. 8, Table 4). The R^2 values and LOD values for these SNP markers ranged from 0.04 to 0.11, and from 1.03 to 5.06, respectively. The SNP marker S1A_5025450 on 1A and S1B_6049220 had the highest LOD and R^2 values (Table 4).

86-124 (Race 2)

The SNPs markers significantly associated with tan spot caused by 86-124 were located on the chromosome arm 3B from 466618145 bp to 474316625 bp positions (Fig. 9, Table 5). The R^2 values and LOD values for these SNP markers ranged from 0.06 to 0.07, and from 1.46 to 1.98, respectively. The SNP marker S3B_474316625 on 3B had the highest LOD and R^2 values (Table 5).

331-9 (Race 3)

The SNPs markers significantly associated with tan spot caused by 331-9 were located on the chromosome arm 3B from 466618145 bp to 474316625 bp positions (Fig. 10, Table 6). The R^2 values and LOD values for these SNP markers ranged from 0.06 to 0.11, and from 1.33 to 4.56, respectively. The SNP marker S3B_474316625 on 3B had the highest LOD and R^2 values (Table 6).

DW5 (Race 5)

The SNPs markers significantly associated with tan spot caused by DW5 were located to three genomic regions, one of them on the chromosome arm 2B from 24099403 bp to 24356781 bp positions, on chromosome 3B 474316625 bp position and the other on chromosome 5B 707540820 bp to 708182335 bp positions (Fig. 11, Table 7). The R^2 values and LOD values for these SNP markers ranged from 0.05 to 0.11, and from 1.04 to 4.73, respectively. The SNP marker S2B_24099403 on 2B, S3B_474316625 on 3B and S5B_707540820 on 5B had the highest LOD and R^2 values (Table 7).

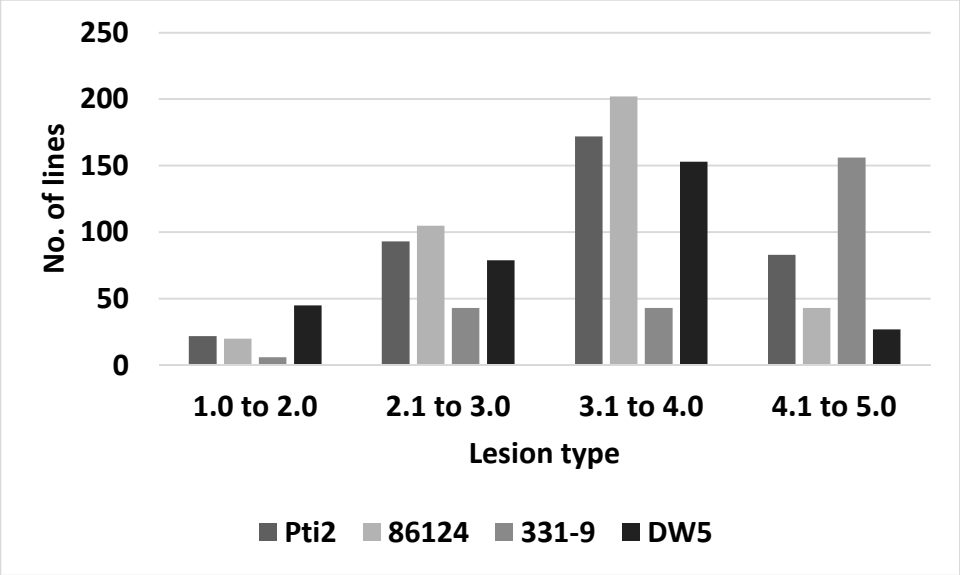

Figure 1. Histogram of disease reaction of the T-CAP panel to individual isolate. All durum lines in the T-CAP panel were evaluated with four *Pyrenophora tritici-repentis* isolates representing different races including Pti2 (race 1), 86-124 (race 2), 331-9 (race 3) and DW5 (race 5). Disease was evaluated using a 1-5 rating scale with 1 highly resistant and 5 highly susceptible (ref?). The x-axis is the lesion type based on disease scale, and y-axis is the number of lines.

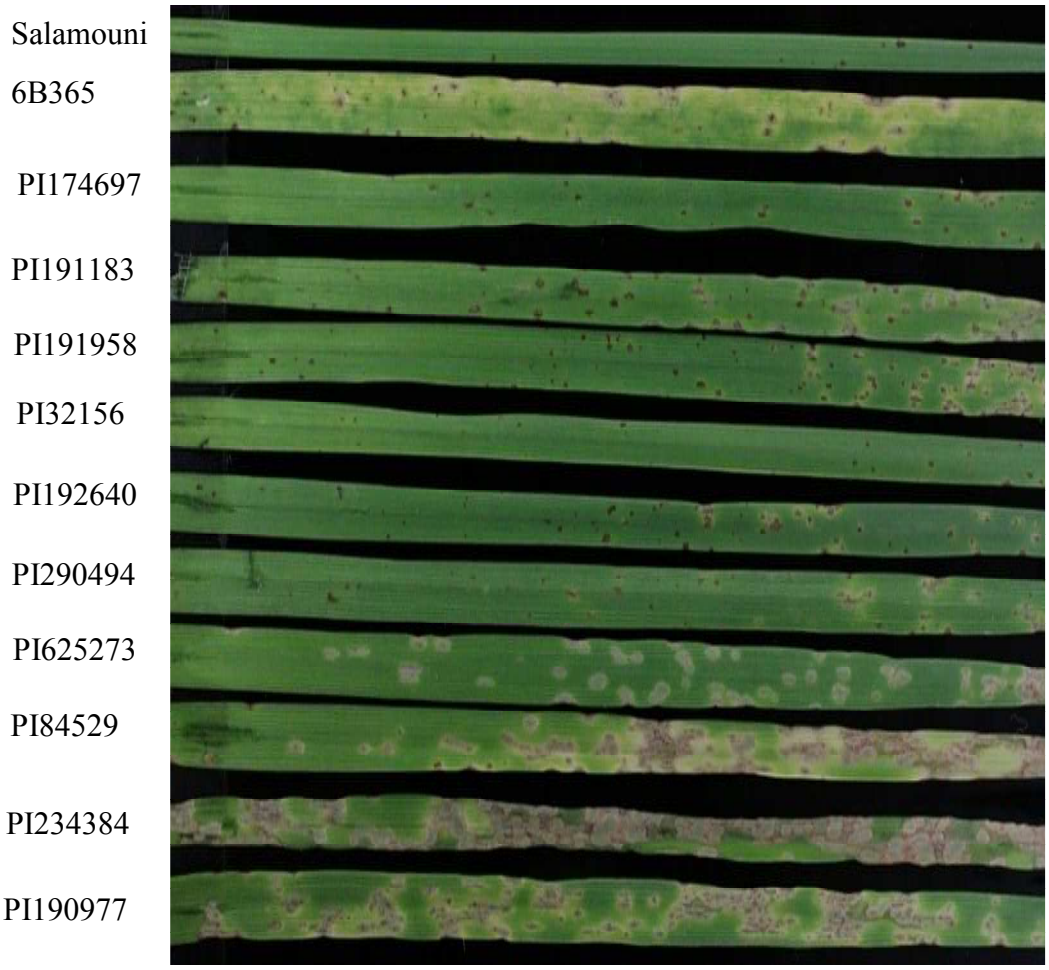

Figure 2. Examples of durum lines for the reaction to individual isolate inoculations. The *Pyrenophora tritici-repentis* fungal isolate Pti2 representing race 1 was shown. Salamouni and 6B365 are used as differential lines with Salamouni being the universal resistant line and 6B365 as a Ptr ToxC sensitive line.

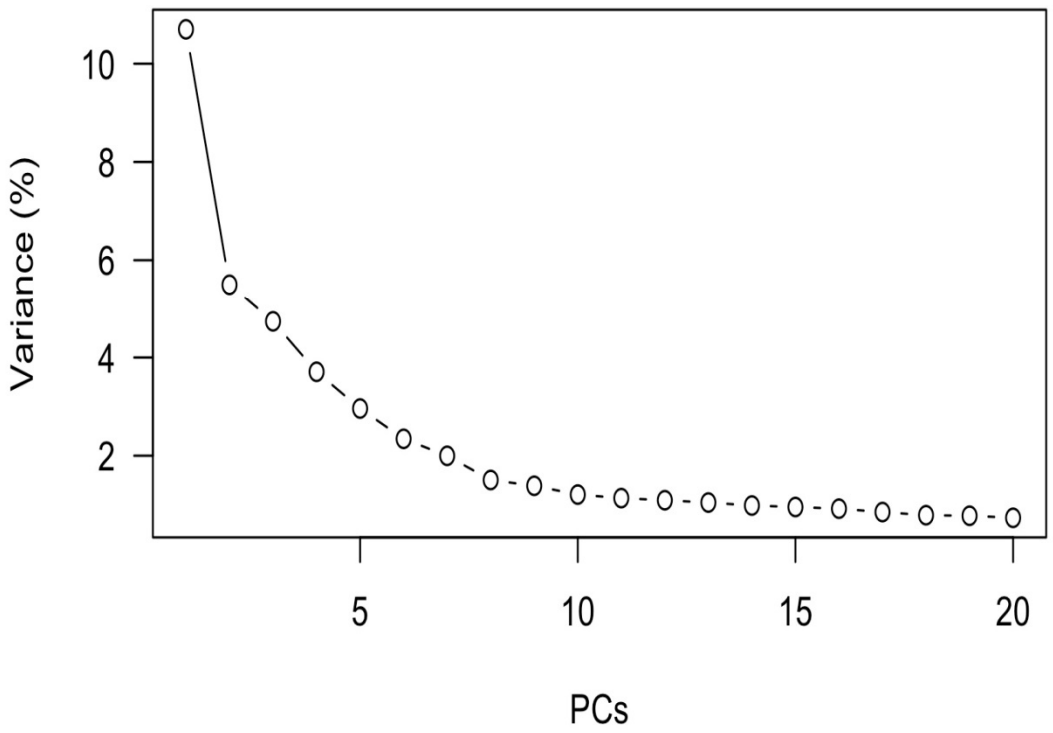

Figure 3. The scree plot between number of principal components and percentage of variance. The x-axis is number of principal components and the y-axis is percentage of variance. The first 20 PCs obtained in principal component analysis was shown. The elbow in the scree plot was used to determine a sufficient number of dimensions for the final analysis.

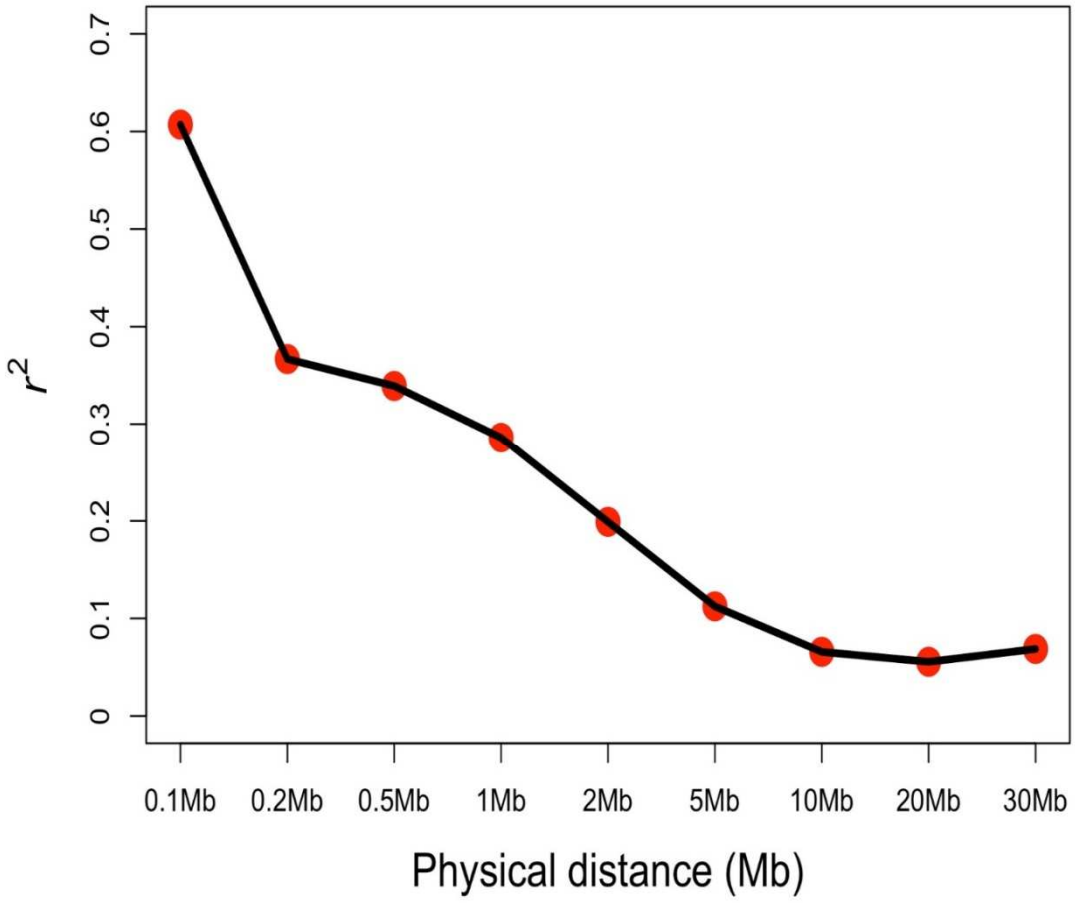

Figure 4. Scatter plot showing the average linkage disequilibrium (LD) r^2 values across the genome. The physical distances between pairs of SNP markers in mega base (Mb) is plotted against the LD estimate (r^2) for pairs of single-nucleotide polymorphisms.

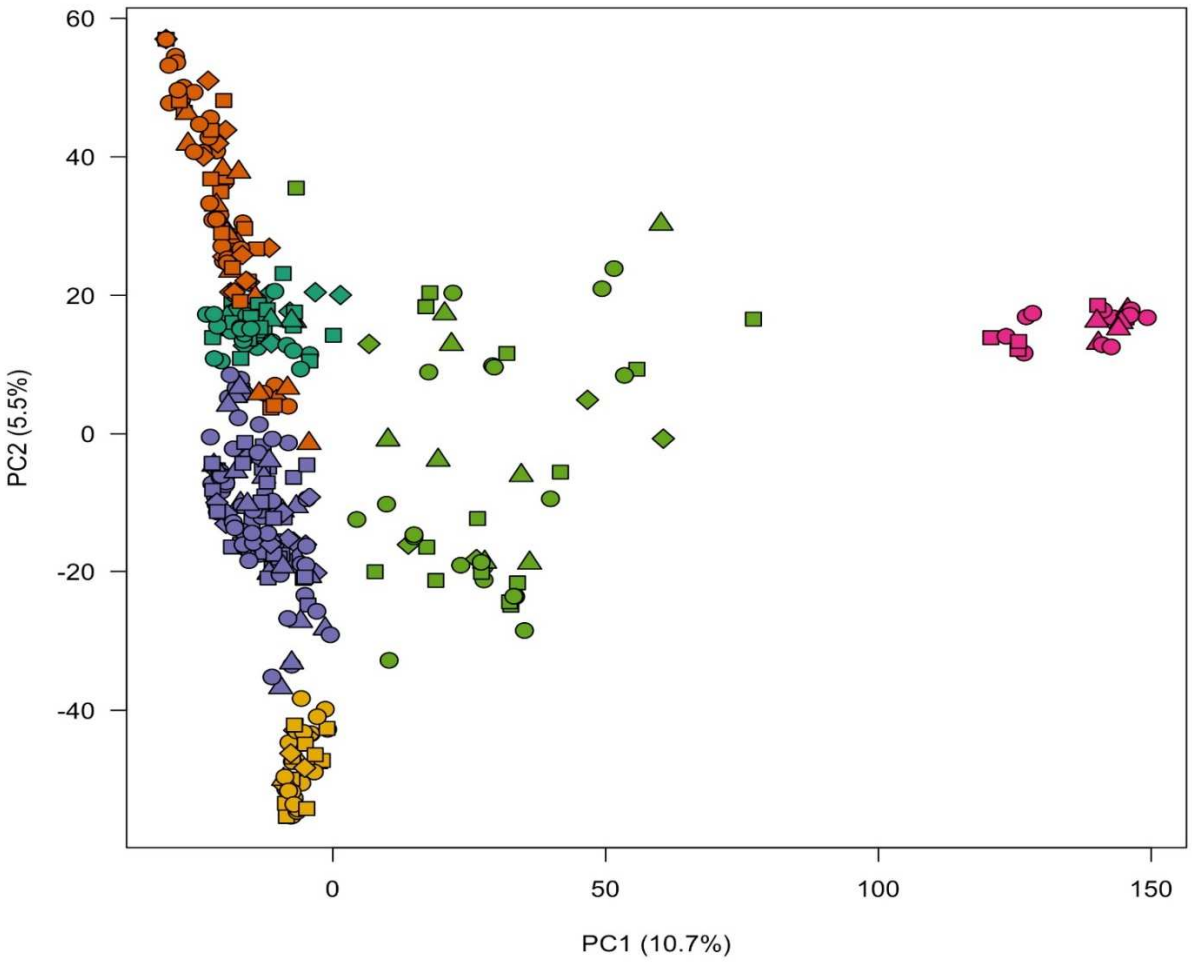

Figure 5. Population structure in T-CAP durum lines based on principal component analysis. The analysis was performed by using 13,263 polymorphic single-nucleotide polymorphism markers obtained on 371 durum wheat accessions. PC1 and PC2 explain 10.7, and 5.5 % variation, respectively. Different colors and shapes represent different clusters representing 9 subgroups and different improvement status.

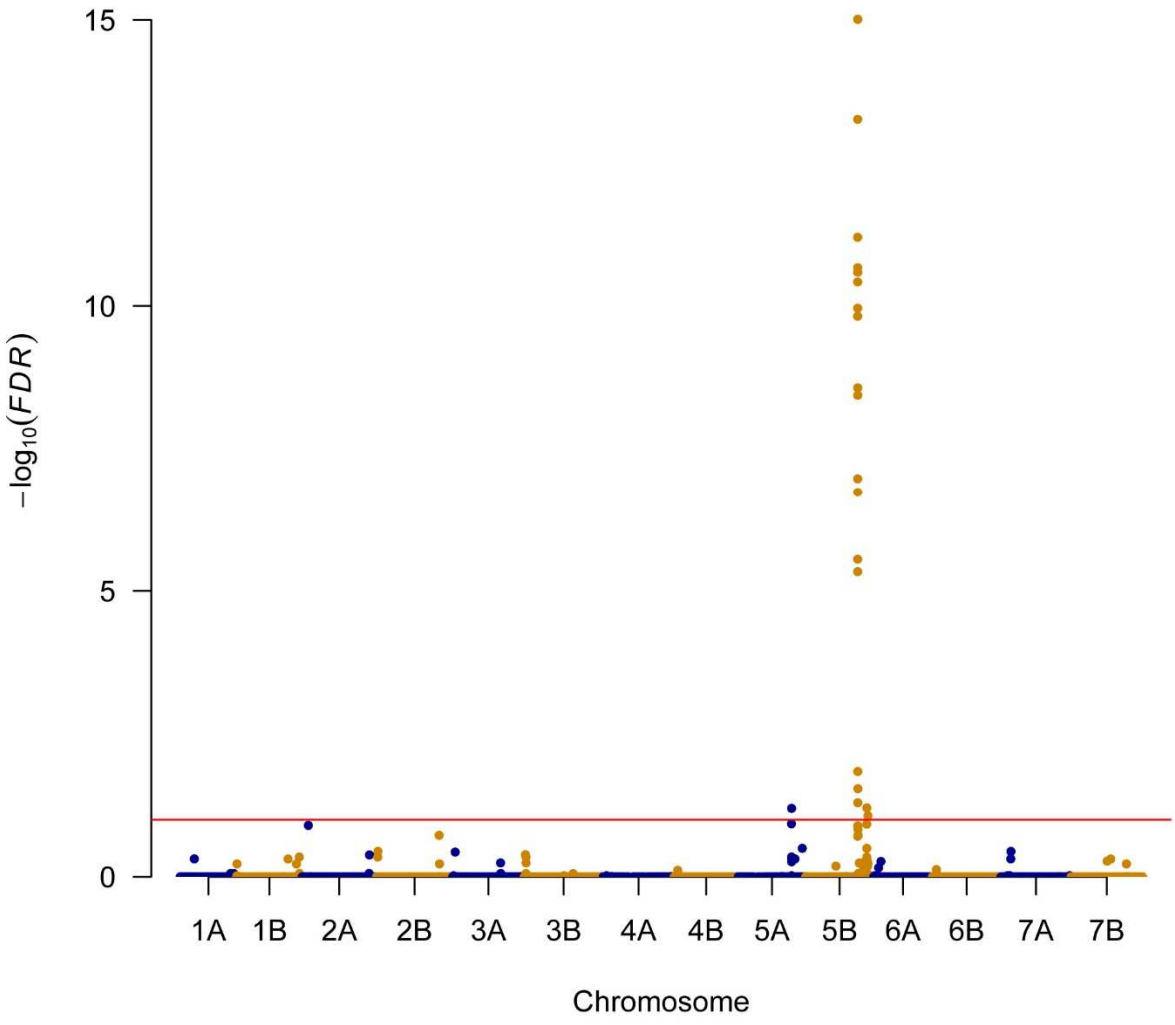

Figure 6. Manhattan plots showing adjusted *P*-values of single-nucleotide polymorphism (SNP) markers associated with reaction to Ptr ToxA infiltration at seedling plant stage. The red horizontal line indicates significant threshold at false discovery rate (FDR) = 0.1.

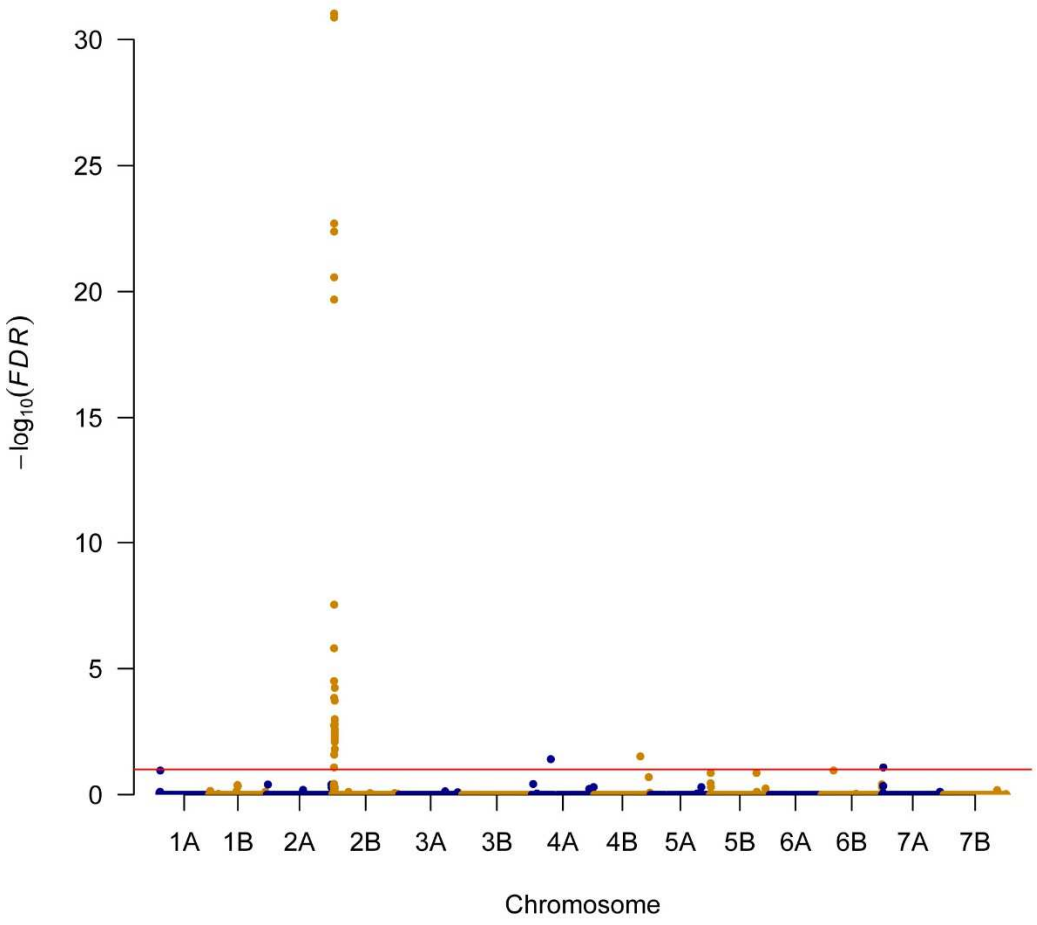

Figure 7. Manhattan plots showing adjusted *P*-values of single-nucleotide polymorphism (SNP) markers associated with reaction to Ptr ToxB infiltration at seedling plant stage. The red horizontal line indicates significant threshold at false discovery rate (FDR) = 0.1.

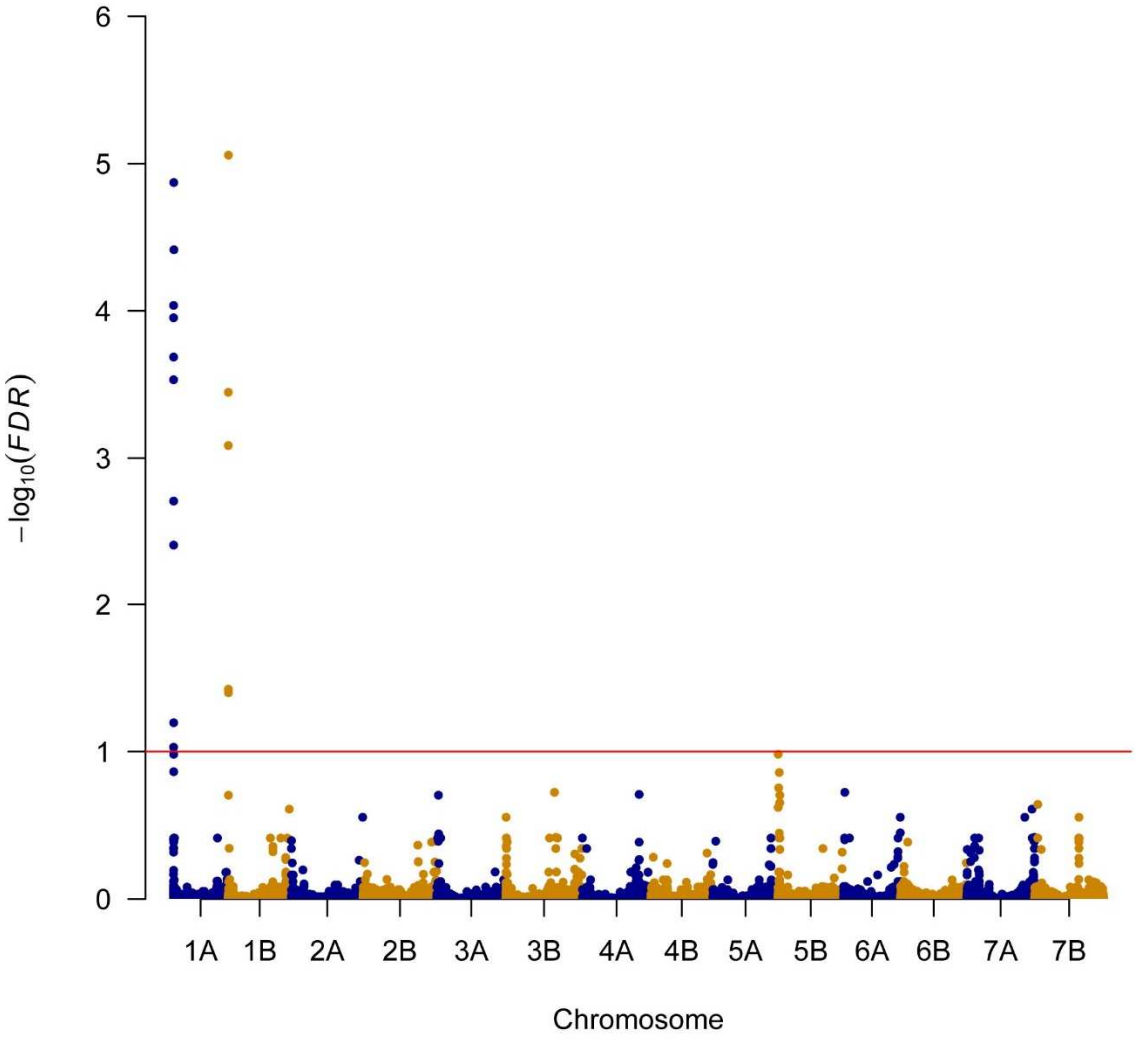

Figure 8. Manhattan plots showing adjusted *P*-values for single-nucleotide polymorphism (SNP) markers associated with reaction to tan spot caused by Pti2 (race 1) at seedling plant stage. The red horizontal line indicates significant threshold at false discovery rate (FDR) = 0.1.

Table 4. The significant markers associated with resistance to tan spot caused by *Pyrenophora tritici-repentis* race 1 (Pti2)

SNP	Chr[a]	Chromosome Position (bp)	Pti2	
			Marker R^{2} *	-log $_{10}$ (FDR)[b]
S1B_6049220	1B	6049220	0.11	5.06
S1B_6049233	1B	6049233	0.11	5.06
S1B_4859254	1B	4859254	0.08	3.45
S1B_4859266	1B	4859266	0.08	3.09
S1B_5697587	1B	5697587	0.06	1.43
S1B_5697588	1B	5697588	0.06	1.43
S1B_5697589	1B	5697589	0.06	1.43
S1B_6049215	1B	6049215	0.05	1.40
S1A_5025450	1A	5025450	0.11	4.87
S1A_6424367	1A	6424367	0.10	4.42
S1A_2988049	1A	2988049	0.09	4.04
S1A_2988007	1A	2988007	0.09	3.95
S1A_3120830	1A	3120830	0.09	3.69
S1A_2988008	1A	2988008	0.08	3.53
S1A_3493169	1A	3493169	0.07	2.71
S1A_3695742	1A	3695742	0.07	2.71
S1A_3695790	1A	3695790	0.07	2.71
S1A_3695792	1A	3695792	0.07	2.71
S1A_4260108	1A	4260108	0.07	2.71
S1A_4260122	1A	4260122	0.07	2.71
S1A_4260123	1A	4260123	0.07	2.71
S1A_4260140	1A	4260140	0.07	2.71
S1A_4260164	1A	4260164	0.07	2.71
S1A_3493172	1A	3493172	0.06	2.40
S1A_4045658	1A	4045658	0.05	1.20
S1A_3017672	1A	3017672	0.04	1.03
S1A_3017688	1A	3017688	0.04	1.03

[a]Chr = the Chromosomal locations of the SNP marker

*R^{2}indicates the proportion of phenotypic variation explained by the individual marker

[b]-log $_{10}$ (FDR) false discovery rate

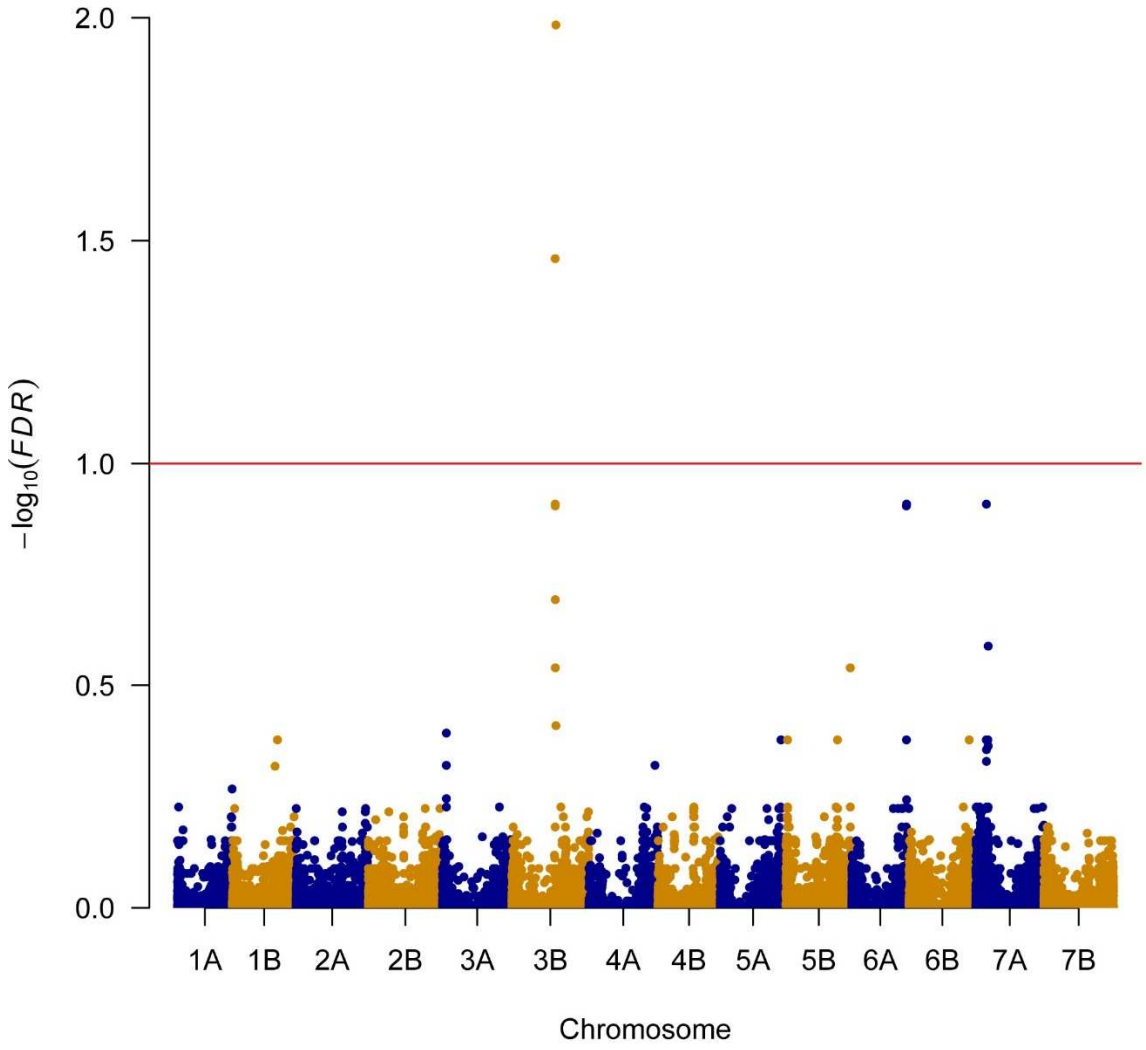

Figure 9. Manhattan plots showing adjusted *P*-values for single-nucleotide polymorphism (SNP) markers associated with response to tan spot caused by 86-124 (race 2) at seedling plant stage. The red horizontal line indicates significant threshold at false discovery rate (FDR) = 0.1.

Table 5. The significant markers associated with resistance to tan spot caused by *Pyrenophora tritici-repentis* race 2 (86-124)

SNP	Chr[a]	Chromosome Position (bp)	86-124	
			Marker R^{2*}	-log $_{10}$ (FDR)[b]
S3B_474316625	3B	474316625	0.07	1.98
S3B_466618145	3B	466618145	0.06	1.46

[a]Chr = the Chromosomal locations of the SNP marker
[*]R^2 indicates the proportion of phenotypic variation explained by the individual marker
[b]-log $_{10}$ (FDR) false discovery rate

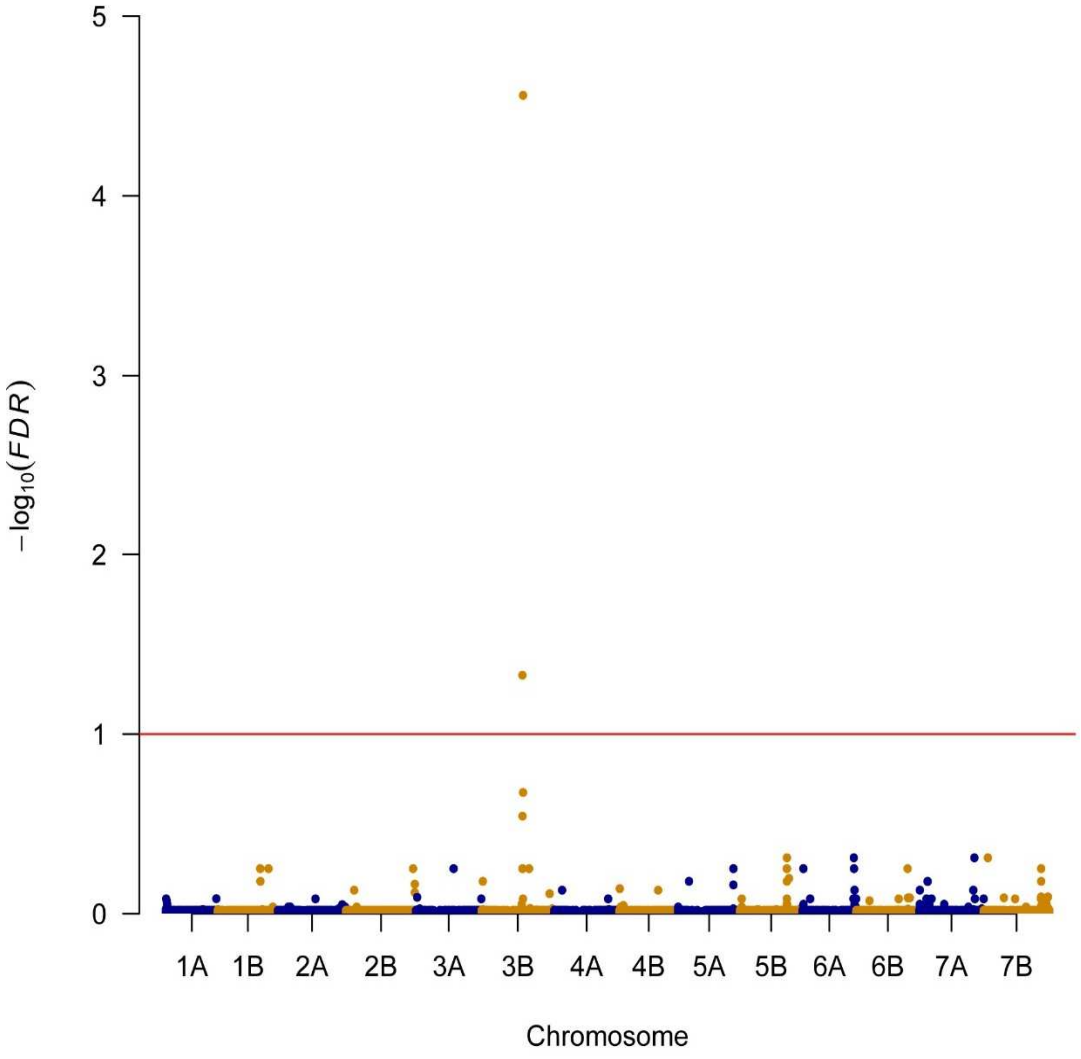

Figure 10. Manhattan plots showing adjusted *P*-values for single-nucleotide polymorphism (SNP) markers associated with response to tan spot caused by 331-9 (race 3) at seedling plant stage. The red horizontal line indicates significant threshold at false discovery rate (FDR) = 0.1.

Table 6. The significant markers associated with resistance to tan spot caused by *Pyrenophora tritici repentis* race 3 (331-9)

SNP	Chr[a]	Chromosome Position (bp)	331-9	
			Marker R^{2*}	-log $_{10}$ (FDR)[b]
S3B_474316625	3B	474316625	0.11	4.56
S3B_466618145	3B	466618145	0.06	1.33

[a]Chr = the Chromosomal locations of the SNP marker
[*]R^2 indicates the proportion of phenotypic variation explained by the individual marker
[b]-log $_{10}$ (FDR) false discovery rate

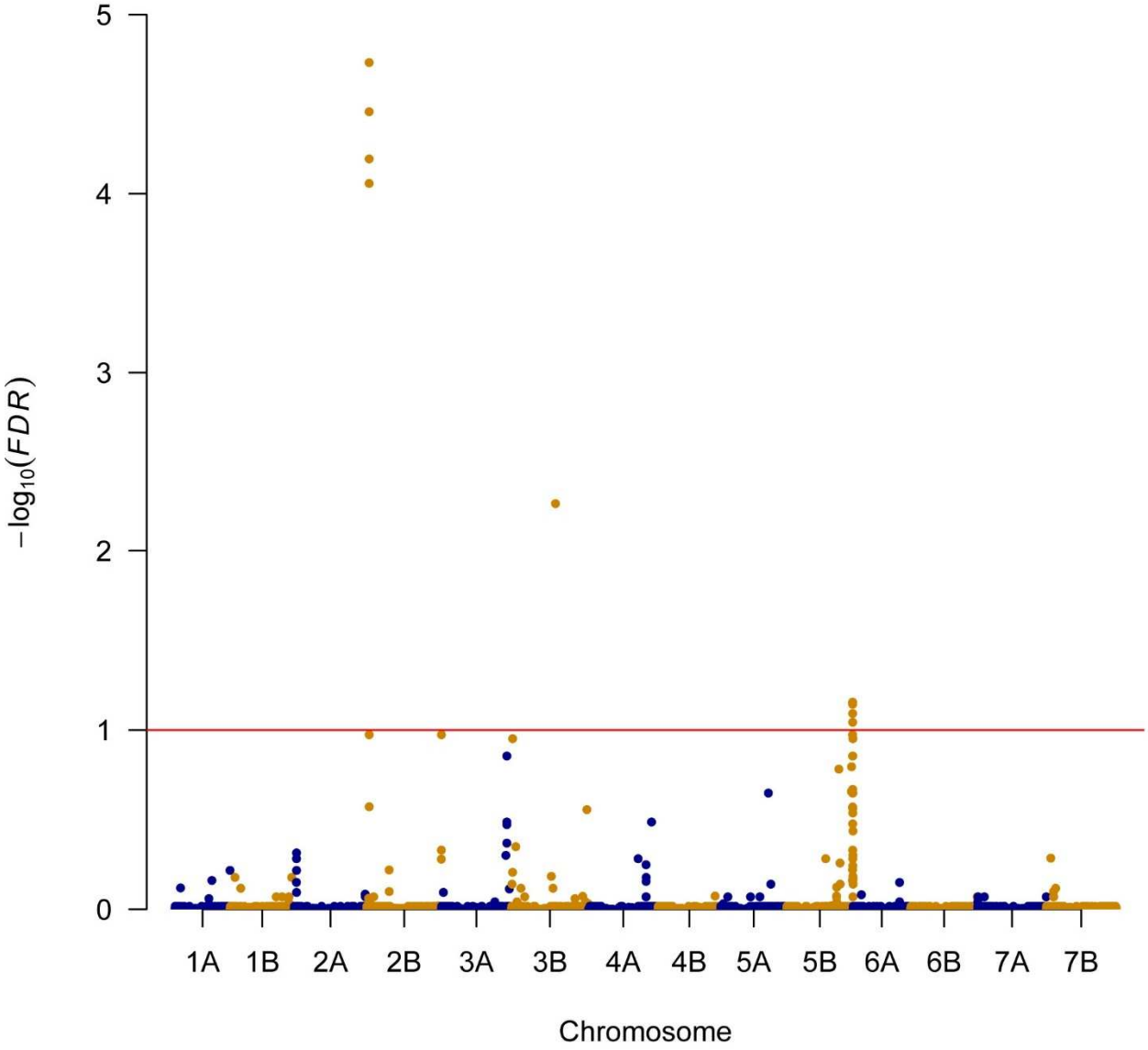

Figure 11. Manhattan plots showing adjusted *P*-values for single-nucleotide polymorphism (SNP) markers associated with response to tan spot caused by DW5 (race 5) at seedling plant stage. The red horizontal line indicates significant threshold at false discovery rate (FDR) = 0.1.

Table 7. The significant markers associated with resistance to tan spot caused by *Pyrenophora tritici repentis* race 5 (DW5)

SNP	Chr[a]	Chromosome Position (bp)	DW5	
			Marker R^{2*}	-log $_{10}$ (FDR)[b]
S2B_24099403	2B	24099403	0.11	4.73
S2B_24099412	2B	24099412	0.11	4.73
S2B_24099418	2B	24099418	0.11	4.73
S2B_24100467	2B	24100467	0.11	4.73
S2B_24356781	2B	24356781	0.11	4.73
S2B_23971365	2B	23971365	0.10	4.46
S2B_24354200	2B	24354200	0.09	4.20
S2B_24210901	2B	24210901	0.09	4.06
S3B_474316625	3B	474316625	0.07	2.27
S5B_707540820	5B	707540820	0.05	1.16
S5B_707540861	5B	707540861	0.05	1.16
S5B_707886907	5B	707886907	0.05	1.14
S5B_708182335	5B	708182335	0.05	1.09
S5B_708091471	5B	708091471	0.05	1.04
S5B_708091512	5B	708091512	0.05	1.04

[a]Chr = the Chromosomal locations of the SNP marker
[*]R^2 indicates the proportion of phenotypic variation explained by the individual marker
[b]-log $_{10}$ (FDR) false discovery rate

Discussion

Tan spot is an important foliar disease on both common wheat and durum. In the last two decades, much progress has been made in understanding of genetic and molecular basis of the disease system. It is known that the pathosystem is a complex, which not only involves the race specific susceptibility determined by the fungal produced NE-host sensitivity gene interaction, but also other race-specific or nonspecific QTL conferring resistance/susceptibility. However, so far, much understanding has been largely based on the studies using common wheat. Although few studies were done on durum wheat, results have suggested some difference regarding the role of NE-host sensitivity gene interaction and presence of new interactions that cause necrosis

from durum specific isolates (Gamba and Lamari 1998; Faris et al. 2013; Virdi et al. 2016). In this study, we conducted disease evaluations and NE sensitivity assay on a world durum collection, known as T-CAP durum panel, and performed a GWAS in this panel to reveal genetics of tan spot resistance in durum backgrounds. From the disease evaluations, a total of nineteen durum lines were found to be highly resistant to multiple *Ptr* races. These lines likely contain race-nonspecific resistance (see below), which will be valuable for improving tan spot resistance in durum cultivars. The GWAS also identifies various genome regions significantly associated with sensitivity to NEs and resistance or susceptibility to tan spot caused by different races. The identified genomic regions provide important information for further understanding of the disease system in durum. This is also first GWAS performed in durum for tan spot resistance.

As expected, genomic regions associated with sensitivity to Ptr ToxA and Ptr ToxB were identified to the chromosome arms 5BL and 2BS with high confidence levels and the highest FDRs was 15 for sensitivity to Ptr ToxA and 31 for sensitivity to Ptr ToxB. The 5BL QTL and the 2BS QTL identified by these markers should correlate to the *Tsn1* and *Tsc2* loci, respectively. Several highly significant SNP markers for sensitivity to Ptr ToxB were also associated with susceptibility to DW5 which produces Ptr ToxB; In contrast, all significant markers for sensitivity to Ptr ToxA were not identified to be associated with susceptibility to Pti2 and 86-124, both of which produce Ptr ToxA. Statistical analyses also indicated moderately strong correlation between sensitivity to Ptr ToxB with disease caused by DW5 (R^2=0.27, p<0.0001), but a very weak correlation (R^2=0.03, p=0.0002 & R^2=0.08, p<0.0001) between sensitivity to Ptr ToxA and disease caused by Pti2 and 86-124. These suggest Ptr ToxB-*Tsc2* interaction plays an important role in disease development in durum background while Ptr ToxA-*Tsn1* plays no significant role in disease in this durum panel. The important role of Ptr

ToxB-*Tsc2* interaction in disease when presented has been demonstrated in both hexaploid wheat (Friesen and Faris 2004; Abeysekara et al. 2009) and tetraploid wheat (Virdi et al. 2016). In contrast, the importance of Ptr ToxA-*Tsn1* interaction in disease was dependent on the genetic backgrounds in hexaploid wheat (review by Faris et al. 2013) and has never been shown in durum wheat background (Virdi et al. 2016). Therefore, our results further confirmed unimportance of Ptr ToxA-*Tsn1* interaction in durum. QPCR conducted by Virdi et al (2016) showed both Pti2 and 86-124 had a very low expression of the *ToxA* gene after being inoculated onto durum wheat lines, which explain to some degree why Ptr ToxA-*Tsn1* interaction plays no significant role in durum. However, the *ToxA* gene expression of these isolates should be studied in more diverse line to see if the low expression of the *ToxA* gene is a universal phenomenon when the fungal pathogen interacts with durum wheat.

For Pti2, there were two genomic regions identified, one on 1AS and the other on 1BS. It is very likely that 1AS QTL corresponds to the *Tsc1* locus, which confers sensitivity to chlorosis inducing NE Ptr ToxC. This further suggests that Ptr ToxC-*Tsc1* interaction is important in durum backgrounds, which has never been demonstrated before. However, no known QTL have been identified on 1BS to be specifically associated with tan spot susceptibility or resistance to race 1 isolates. We also used GWAS to map the chlorosis data from Pti2 inoculations (scoring the presence and absence of chlorosis) and obtained a similar set of SNP markers on 1A and 1B (data not shown). This suggests either that Pti2 may produce another chlorosis-inducing NEs that acts on 1BS locus or that another gene on 1BS is presented in the Ptr ToxC-*Tsc1* reaction pathway. It is also possible that these significant 1BS markers are located on 1AS, but somehow were mistakenly assembled on the similar positions of 1BS. Further work is needed to figure this out.

It is very intriguing that no marker on 1AS was identified for the 331-9 isolate (race 3). This isolate was used in bi-parental QTL mapping of tan spot done by Kariyawasam et al. (2016) and shown to produce Ptr ToxC that interacts with *Tsc1*. There are two possible reasons for not detecting the *Tsc1* locus as a QTL for 331-9. First, 331-9 was isolated from durum wheat in Canada (Friesen et al. 2005) and it is possible that this isolate produce new necrosis-inducing NEs masking the Ptr ToxC-*Tsc1* reaction. The epistasis of Ptr ToxA-*Tsn1* interaction on some unknown chlorosis-inducing NEs have been reported (Manning and Ciuffetti 2015). Second, 331-9 might not produce much Ptr ToxC when being inoculated onto this set of durum wheat lines. Therefore, Ptr ToxC-*Tsc1* reaction (chlorosis) was not detectable in the association mapping.

There were two closed SNP markers identified on 3B chromosome for all the races except the race 1 isolate Pti2. This genomic region likely corresponds a race-nonspecific QTL identified in previous studies. Race-nonspecific resistance was first reported by Faris and Friesen (2005) in the common wheat variety 'BR34', which are conferred by two QTL, with one on 1BS (*QTs.fcu-1BS* and the other on 3BL (*QTs.fcu-3BL*). Kariyawasam et al. (2016) lately identified a major race-nonspecific QTL at the similar position of *QTs.fcu-3BL* in a hexaploid wheat population derived from the cross between Louise and Penawawa and also showed that this race-nonspecific QTL is dominant on Ptr ToxA-*Tsn1* interaction. The identification of race-nonspecific resistance QTL is also supported by the fact that nineteen durum lines in this panel were highly resistant to all the races. However, this genomic region was not identified for Pti2. We checked the allele frequency for the two SNP markers on 3B and found they are extreme low at 15 out of 345. When the larger set of marker data (68,382) was used, we identified three new SNP marker on the similar location of 3B significantly associated with resistance to Pti2 (data

not shown). Therefore, this locus represents race-nonspecific resistance QTL. Here, we first demonstrate that race-nonspecific resistance QTL is also present in durum wheat. These nineteen durum lines likely carry this race-nonspecific resistance QTL and will be very useful materials to improve tan spot resistance in durum cultivars.

There was another minor QTL on the chromosome 5BL (distal end) associated with the disease cause by the DW5 isolate (Fig. 11). This DW5 specific 5BL QTL should be different from the *Tsn1* locus because they are about 250 mega-bases away and also DW5 does not produce Ptr ToxA to interact with *Tsn1*. Kariyawasam (2015) also detected a significant QTL (*QTs.zhl-5B*) at the similar position on 5BL in a tetraploid doubled haploid population for DW5. This is a novel QTL that is specific to DW5.

In summary, we identified several durum lines at different improvement status and showed Ptr ToxB-*Tsc2* interaction and Ptr ToxC-*Tsc1* interaction, but not Ptr ToxA-*Tsn1* interactions play an important role in tan spot disease in durum. Additionally, race-nonspecific resistance QTL was also shown to be important for disease in durum. This work emphasizes the complexity of the wheat-*Ptr* pathosystem. Because all the SNP markers have been physically mapped to the wheat reference genome, the most significant markers linked to *Tsc1* and *Tsc2* as well as the 3B race-nonspecific QTL not only will be useful in breeding programs after converted to PCR-based markers, but also will provide important anchors for physically isolating these genes or QTL.

Literature Cited

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

Ali, S., Gurung, S., and Adhikari, T. B. 2010. Identification and characterization of novel isolates of *Pyrenophora tritici-repentis* from Arkansas. Plant Disease 94:229-235.

Aoun, M., Breiland, M., Turner, M. K., Loladze, A., Chao, S., Xu, S. S., Ammar, K., Anderson, J. A., Kolmer., and Acevado, M. 2016. Genome-Wide Association Mapping of Leaf Rust Response in a Durum Wheat Worldwide Germplasm Collection. The Plant Genome 9: 1-24.

Cheong, J., Wallwork, H., and Williams, K. J. 2004. Identification of a major QTL for yellow leaf spot resistance in the wheat varieties Brookton and Cranbrook. Crop and Pasture Science 55:315-319.

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

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

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

Effertz, R. J., Anderson, J. A., and Francl, L. J. 2001. Restriction fragment length polymorphism mapping of resistance to two races of *Pyrenophora tritici-repentis* in adult and seedling wheat. Phytopathology 91:572-578.

Elshire, R.J., J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto, E.S. Buckler, and S.E. Mitchell. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. (L Orban, Ed.). PLoS One 6: e19379.

Faris, J. D., Anderson, J. A., Francl, L. J., and Jordahl, J. G. 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 Friesen, T. L. 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., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J. P., Meinhardt, S. W., Rasmussen, J. B., Oliver, R. P., and Friesen, T. L. 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.

Faris, J. D., Abeysekara, N. S., McClean, P. E., Xu, S. S., and Friesen, T. L. 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., Liu, Z., and Xu, S. S. 2013. Genetics of tan spot resistance in wheat. Theoretical and Applied Genetics 126:2197-2217.

Fernandez, M. R., Clarke, J. M., and DePauw, R. M. 1994. Response of durum wheat kernels and leaves at different growth stages to *Pyrenophora tritici-repentis*. Plant Disease 78:597-600.

Fernadez, M. R., Clarke, J. M., DePauw, R. M., and Lefkovitch, L. P. 1997. Emergence and growth of durum wheat derived from red smudge-infected seed. Crop Science 37:510-514.

Fernandez, M. R., DePauw, R. M., Clarke, J. M., and Fox, S. L. 1998. Discoloration of wheat kernels by *Pyrenophora tritici-repentis*. Canadian Journal of Plant Pathology 20:380-383.

Fernandez, M. R., Clarke, J. M., and DePauw, R. M. 2002. The effect of plant height on tan spot on durum wheat in Southern Saskatchewan. Crop Science 42:159-164.

Flor, H. H. 1956. The complimentary genetics systems in flax and flax rust. Advance Genetics 8:29–54.

Friesen, T. L., and Faris, J. D. 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.

Glaubitz, J.C., T.M. Casstevens, F. Lu, J. Harriman, R.J. Elshire, Q. Sun, and E.S. Buckler. 2014a. TASSEL-GBS: A High Capacity Genotyping by Sequencing Analysis Pipeline. PLoS One 9: e90346.

Hosford Jr, R. M. 1971. A Form of *Pyrenophora trichostoma* Pathogenic to Wheat and Other Grasses. Phytopathology 61:28-32.

Joehanes, R., and Nelson, J. C. 2008. QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24:2788-2789.

Jordan, K.W., S. Wang, Y. Lun, L.-J. Gardiner, R. MacLachlan, P. Hucl, K. Wiebe, D. Wong, K.L. Forrest, A.G. Sharpe, C.H. Sidebottom, N. Hall, C. Toomajian, T. Close, J. Dubcovsky, A. Akhunova, L. Talbert, U.K. Bansal, H.S. Bariana, M.J. Hayden, C. Pozniak, J.A. Jeddeloh, A. Hall, and E. Akhunov. 2015. A haplotype map of allohexaploid wheat reveals distinct patterns of selection on homoeologous genomes. Genome Biology. 16: 48.

Kariyawasam, G. K., Carter, A. H., Rasmussen, J. B., Faris, J., Xu, S. S., Mergoum, M., and Liu, Z. 2016. Genetic relationships between race- nonspecific and race-specific interactions in the wheat- *Pyrenophora tritici repentis* pathosystem. Theoretical and Applied Genetics 129:897-908.

Kariyawasam, G. K. 2015. Genetic mapping of quantitative trait loci for resistance to wheat tan spot using two bi-parental populations, MS thesis, North Dakota State University, Fargo.

Kollers, S., Rodemann, B., Ling, J., Korzun, V., Ebmeyer, E., Argillier, O., Hinze, M., Plieske, J., Kulosa, D., Ganal, M. W., and Röder, M. S. 2014. Genome-wide association mapping of tan spot resistance (*Pyrenophora tritici-repentis*) in European winter wheat. Molecular Breeding 34:363- 371.

Lamari, L., and Bernier, C. C. 1989. 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 Strelkov, S. E. 2010. Minireview/Minisynthèse. The wheat/*Pyrenophora tritici-repentis* interaction: progress towards an understanding of tan spot disease. Canadian Journal of Plant Pathology 32: 4-10.

Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. Genomics 0: 1-3.

Liu, Z., El-Basyoni, I., Kariyawasam, G., Zhang, G., Fritz, A., Hansen, J. M., Marias, F., Friskop, A., Chao, S., Akhunov, E., and Baenziger, P. S. 2015. Evaluation and association mapping of resistance to tan spot and Stagonospora nodorum blotch in adapted winter wheat germplasm. Plant Disease 99:1333-1341.

Manning, V., and Ciuffetti, L. M. 2015. Necrotrophic effector epistasis in the *Pyrenophora tritici-repentis*-wheat interaction. PLoS One 10: e0123548.

Matsuoka, Y. 2011. Evolution of Polyploid Triticum Wgeats under Cultivation: The Role of Domestication, Natural Hybridization and Allopolyploid Speciation in their Diversification. Plant & Cell Physiology 52: 750–764.

Patel, J. S., Mamidi, S., Bonman, J. M., and Adhikari, T. B. 2013. Identification of QTL in spring wheat associated with resistance to a novel isolate of *Pyrenophora tritici-repentis*. Crop Science 53:842-852.

Poland, J.A., P.J. Brown, M.E. Sorrells, and J.-L. Jannink. 2012. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7: e32253.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–59.

Rees, R. G., Platz, G. J., and Mayer, R. J. 1982. Yield losses in wheat from yellow spot: comparison of estimates derived from single tillers and plots. Australian Journal of Agricultural Research 33:899–908.

Rees, R. G., and Platz, G. J. 1992. Tan spot and its control: some Australian experiences.

Riede, C. R., Francl, L. J., Anderson, J. A., Jordahl, J. G., and Meinhardt, S. W. 1996. Additional Sources of Resistance to Tan Spot of Wheat. Crop Science 36: 771-777.

SAS Institute 2011. SAS/IML 9.3 User's Guide.SAS Institute, Cary, North Carolina.

Singh, P. K., Gonzalez-Hernandez, J. L., Mergoum, M., Ali, S., Adhikari, T. B., Kianian, S. F., Elias, E. M. and Hughes, G. R. 2006. 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., Mergoum, M., Gonzalez-Hernandez, J. L., Ali, S., Adhikari, T. B., Kianian, S. F. Elias, E. M. and Hughes, G. R. 2008. Genetics and molecular mapping of resistance to necrosis inducing race 5 of *Pyrenophora tritici-repentis* in tetraploid wheat. Molecular Breeding 21:293-304.

Somers, D. J., Isaac, P., and Edwards, K. 2004. A high-density microsatellite consensus map for bread wheat (*Triticum aestivum L.*). Theoretical and Applied Genetics 109:1105-1114.

Stekhoven, D.J., and P. Bühlmann. 2012. MissForest--non-parametric missing value imputation for mixed-type data. Bioinformatics 28: 112–8.

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

Tadesse, W., Hsam, S. L. K., and Zeller, F. J. 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., Schmolke, M., Hsam, S. L. K., Mohler, V., Wenzel, G., and Zeller, F. J. 2010. Chromosomal location and molecular mapping of a tan spot resistance gene in the winter wheat cultivar Red Chief. Journal of Applied Genetics 51:235-242.

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

Virdi, S. K., Liu, Z., Overlander, M. E., Zhang, Z., Xu, S. S., Friesen, T. L., and Faris, J. D. 2016. New insights into the roles of host gene-necrotrophic effector interactions in governing susceptibility of durum wheat to tan spot and septoria nodorum blotch. G3 6:4139-4150.

Wolpert, T. J., Dunkle, L. D., and Ciuffetti, L. M. 2002. Host-selective toxins and avirulence determinants: what's in a name? Annual Review Phytopathology 40:251-285.

Zhang, Q., Axtman, J. E., Faris, J. D., Chao, S., Zhang, Z., Friesen, T. L., Zhong, S., Cai, X., Elias, E. M., and Xu, S. S. 2014. Identification and molecular mapping of quantitative trait loci for Fusarium head blight resistance in emmer and durum wheat using a single nucleotide polymorphism-based linkage map. Molecular Breeding 34:1677–1687.

PAPER 2: IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO TAN SPOT IN A DURUM RECOMBINANT INBRED LINE POPULATION DERIVED FROM BEN × PI 41025

Abstract

Tan spot, caused by the fungi *Pyrenophora tritici-repentis* (*Ptr*), is a major foliar disease on both bread wheat (*Triticum aestivum*) and durum (*T. turgidum* ssp. *durum)*. The pathosystem is complex, not only involving race-specific susceptibility determined by the necrotrophic effector (NE) and host sensitivity (S) gene interactions, but also including genetic factors conferring race-nonspecific resistance. To gain a better understanding of genetic basis of the disease system in durum, we conducted the genetic mapping of quantitative trait loci (QTL) for tan spot resistance in a durum recombinant inbred line population derived from Ben × PI 41025. Ben, an NDSU cultivar, is sensitive to Ptr ToxA and susceptible to tan spot while PI 41025, a cultivated emmer wheat, is insensitive and resistant. A major QTL on wheat chromosome 5A was identified conferring resistance to all races tested suggesting race-nonspecific resistance. Although the population segregated for reaction to Ptr ToxA, the *Tsn1* locus on 5BL did not significantly associate with susceptibility to the disease caused by races 1 and 2. This indicates that Ptr ToxA-*Tsn1* interaction plays no role in disease in this population. Two minor QTL were identified on the chromosomes 3A and 5B for specific races. This work indicates both race-nonspecific and race specific resistance presented in durum and the closely linked markers identified will be useful in transferring the resistance QTL into durum cultivars.

Introduction

The Tan spot, also known as yellow spot, caused by the fungus *Pyrenophora tritici-repentis* (*Ptr*), is an important disease on both bread wheat (*Triticum aestivum* L.) and durum (*T. turgidum* L. ssp. *durum*) worldwide. The fungus mainly overwinters on wheat stubbles and the crop residues left from the previous year serve as a source of primary inoculum (Singh et al. 2012). Throughout the last century, the disease has evolved into a main threat to global wheat production and it is believed that the increase of tan spot epidemics is largely due to the widespread adoption of no or minimum tillage farm practices (Hosford 1982; Murray and Brennan 2009; Faris et al. 2013). Under favorable conditions for the disease, severe infestation can occur, which can cause yield losses up to 50%, and possibly the reduction in grain quality, particularly in durum (Rees et al. 1982; Schilder and Bergstrom 1994). Although there are several ways to manage tan spot, such as crop rotation and fungicide applications, use of resistant varieties is the easiest, most cost-effective and environmentally friendly approach.

Genetics of wheat-*Ptr* interaction is known to follow an inverse gene-for-gene model (Ciuffetti et al. 2010; Faris et al. 2013; Liu et al. 2017). In this model, the pathogen produces necrotrophic effectors, previously known as host-selective toxins that interact with the corresponding host sensitivity (S) genes to induce disease (Wolpert et al. 2002). Furthermore, different NE-host sensitivity gene interactions can additively contribute to the disease development ((Friesen and Faris 2010; Liu et al. 2017). Up to now, three NE-host S gene interactions have been identified in the wheat-*Ptr* pathosystem, naming Ptr ToxA - *Tsn1*, Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1* (Ciuffetti et al. 2010; Faris et al. 2013 for review). The Ptr ToxA-*Tsn1* interaction induce necrosis, whereas the other two results in the development of chlorosis. The three host sensitivity genes: *Tsn1*, *Tsc1* and *Tsc2* have been mapped to the wheat

chromosome arms 5BL (Faris et al. 1996), 1AS (Effertz et al. 2001), and 2BS (Friesen and Faris 2004; Abeysekara et al. 2009), respectively. The *Tsn1* gene has been cloned, and resemble a plant resistance gene containing protein kinase, nucleotide binding, and leucine-rich repeat motifs (Faris et al. 2010). Because *Ptr* races differ in the production of NEs, host reaction is usually dependent on the race used, thus shown to be race-specific.

Quantitative trait locus analysis often revealed that the host sensitivity loci confer a significant portion of disease variations indicating NE-host sensitivity gene interaction play an important role in disease development (review by Faris et al. 2013). The importance of Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1* interactions in disease has been demonstrated in all cases when they are presented for (Friesen and Faris 2004; Abeysekara et al. 2010; Singh et al. 2010; Virdi et al. 2016). However, the effect of Ptr ToxA-*Tsn1* interaction on disease can be variable from highly significant, moderately significant, to nonsignificant at all dependent on the genetic background and polyploidy levels (Faris et al. 2013 for review; Virdi et al. 2016).

In addition to the three susceptibility genes, several major resistance genes and a number of QTL for tan spot have been identified and mapped in different sources of resistant materials (review by Faris et al. 2013). The recessive nature of some qualitative resistance genes and their specificity to certain races strongly suggested the presence of additional NE-host S interactions in the wheat-*Ptr* pathosystem. However, a few resistance QTL were identified for different races and shown to race-nonspecific, some of which have a dominant effect on Ptr ToxA-*Tsn1* interactions (Faris and Friesen 2004; Chu et al. 2008, 2010; Kariyawasam et al. 2016). These highlights the complex of host-pathogen interaction for this disease system.

Among the studies mentioned above, only few were done in durum to characterize and map genetic resistance and quantify the effect of NE-host S gene interaction in disease. Two

linked recessive qualitative resistance genes (*tsr2* and *tsr5*) were mapped on the chromosome 3BL from a resistant *T. turgidum* ssp. *turgidum* accession for resistance to race 3 and 5 respectively (Singh et al. 2006, 2008). Chu et al. (2010) identified five resistance QTLs from *T. turgidum* ssp. *carthlicum* accession PI 94749 with two of them on 5A and one each on 3A, 3B and 7A. This study also revealed Ptr ToxA-*Tsn1* interaction plays no role in disease for races 1 and 2. Very recently, Virdi et al. (2016) showed Ptr ToxB-*Tsc2* interaction, but not Ptr ToxA-*Tsn1* interaction is important for tan spot in the tetraploid population derived from Altar 84 × Langdon. Here, we conducted QTL mapping of resistance to tan spot and determine the effect of NE-host S gene interaction on disease in a bi-parental population derived from a durum cultivar and a cultivated emmer wheat accession.

Materials and Methods

Plant Materials and Experimental Design

The mapping population consisted of 200 $F_{2:7}$ recombinant inbred lines (RILs) derived from the cross between Ben and PI 41025, which has been referred to BP025 (Faris et al. 2014). The population was kindly provided by Drs. Steven S. Xu and Justin Faris, USDA-ARS, Red River Valley Agricultural Research Center in Fargo, ND. Ben is a North Dakota hard amber durum variety released by North Dakota State University (Elias et al. 1998). PI 41025 is a cultivated emmer wheat accession, which originate from Samara, Russia. These two parental lines and all RILs were evaluated under the controlled conditions for reactions to tan spot caused by races 1, 2, 3 and 5 and new race as well as Ptr ToxA. Four common wheat lines ‘Salamouni’, ‘Glenlea’, ‘6B365’ and ‘6B662’ were included because they are commonly used as tan spot differential lines.

All experiments were done under controlled conditions in greenhouse and growth chambers. The parental lines, differential lines and RILs were planted individually in super cell containers (Stuewe and Sons, Inc., Corvallis,OR), which were filled with Sunshine SB100 soil (Sun Grro Horticulture, Bellevue, WA). All the containers were placed in RL98 racks. After planting, a pinch of the fertilizer Osmocote Plus 15-19-12 (Scotts Sierra Horticultural Product Company, Maysville, OH) were applied to each container. Three seeds were planted in a container for each line as experimental unit. The highly susceptible genotype "Jerry', a North Dakota hard red winter wheat cultivar, was planted along the border containers of each rack to reduce the edge effect (Liu et al. 2015). Plants were grown in a greenhouse room with temperature between 20-25°C. When the seedling plants reached two-three leaf stage, they were used for fungal inoculations and NE infiltration. The inoculation for each isolate was repeated three times, which were considered as three replications. For each time, lines were arranged using randomized complete block design (RCBD).

Fungal Inoculations and Disease Evaluation

Five *Ptr* isolates were used to evaluate the BP025 population, including Pti2, 86-124, 331-9, DW5 and ARCrossB10. Regarding the known NE production, Pti2 produces Ptr ToxA and Ptr ToxC, 86-124 produces only Ptr ToxA, 331-9 and produces only Ptr ToxC, and DW5 produces only Ptr ToxB, which have been classified as races 1, 2, 3 and 5, respectively. AR CrossB10 produces Ptr ToxC, no Ptr ToxA, but caused disease on Ptr ToxA differential line, Glenlea. Therefore, this isolate was designated as a new race.

Fungal spore suspension was prepared by following the standard procedure described by Lamari and Bernier (1989). Before inoculation, the concentration of the spore suspension was adjusted approximately to 2000-3000 spores per ml and Tween 20, a surfactant reagent, was

added to the spore suspension at a rate of two drops per 100 ml. Three leaf stage seedling plants (approximately two weeks after planting) were inoculated in a closed cold room by using an air-pressured spray gun. After the spraying, the plants were placed in a mist chamber with 100% relative humidity, continuous lighting and the temperature of 21˚C for 24 hours. After that, the plants were moved into a growth chamber with conditions of 12-hour photoperiod and the temperature of 21˚C. At the 7th day after inoculation, disease responses were rated using a 1-5 rating scale (Lamari and Bernier 1989) with 1 being highly resistant and 5 being highly susceptible. If the plant had an equal amount of two lesion types, an intermediate score between the two was given.

Necrotrophic Effector Infiltration

The parental lines were first tested for sensitivity to Ptr ToxA and Ptr ToxB, and then the whole population was infiltrated if there is a difference in reaction between the parental lines. Ptr ToxA and Ptr ToxB were produced from genetically engineered *Pichia pastoris* strains expressing the corresponding NEs. The yeast strains, yeast culturing and culture filtrate harvest followed the descriptions by Liu et al. (2009). Roughly, 25 µl of this harvested culture filtrates were injected in to fully expand secondary leaf using a 1 ml syringe with the needle removed. The area of infiltration was marked using a permanent marker. Then these infiltrated plants were put in to a growth chamber with temperature of 21˚C for 3 days or 5 days and the responses were rated using 0-3 rating scale which was implemented by Friesen and Faris (2012) in which 0 being insensitive and 1, 2, 3 being sensitive.

Statistical Analysis and QTL Mapping

Statistical analysis was carried out using SAS 9.4 software with equivalent command codes (SAS Institute 2012). The disease data of each isolate was initially tested for normal

distribution using Shapiro-Wilk in the PROC UNIVARIATE procedure (SAS Institute 2012). Homogeneity of variance among replicates of each isolate was then tested using Bartlett's χ^2 test if the data had a normal distribution (Snedecor and Cochran 1989), or Levene's test if it did not (Levene 1960) at $P < 0.05$ significant level. Disease data from homogenized experiments for each isolate was combined and the means was computed, which were used for the subsequent analysis and QTL mapping.

This BP025 population along with the parental lines have been genotyped by using Illumina iSelect 9K array (Cavanagh et al. 2013) and SSR method, which had led to the generation of 2593 DNA markers covering all 14 chromosomes. Four sequence-tagged site (STS) markers developed by Abeysekara et al. (2010) for the *Tsc2* locus on chromosome 2B were also mapped in the BP025 population (Faris et al. 2014). This population and the generated map has been used by Faris et al. (2014) to map wheat domestication-related traits. To identify QTL associated with tan spot resistance, the disease means of lines from three homogenous replicates were used with the genetic maps of BP025 population generated by Faris et al. (2014). QGene 4.3.10v software was employed to conduct QTL analysis in BP025 population (Joehanes et al. 2008). Single marker analysis was first performed to identify the chromosomes containing possible QTL. After the chromosomes were identified, the linkage mapping for these chromosomes were redone by using Mapdisto version 1.7.7 (Lorieux 2012) to reduce marker density to average 5-10 cM per marker for using in single trait multiple interval mapping. The information about all the QTL was presented using results from single trait multiple interval mapping function. A permutation test consisting of 1000 permutations yielded an LOD threshold of 4.0 for an experiment-wise significance level of 0.05.

Results

Reaction of Parental Lines and the BP025 Population to NEs and Fungal Isolates

Ben was sensitive to Ptr ToxA while PI 41025 was insensitive (Fig. 12) and both Ben and PI 41025 were insensitive to Ptr ToxB (Fig. 12). The two parental lines also differed in their reactions to conidial inoculations with all the isolates (Fig. 1). PI 41025 mainly developed small pin-point or very small size dark lesions on the leaves for all the races and the disease means ranged from 2.0 for 86-124 (race 2) to 2.40 for Pti2 (race 1) (Table 8). In contrast, Ben mainly displayed large necrotic lesions to all the isolates tested indicating a susceptible or moderate susceptible reaction to tan spot caused by these races. The average disease score for Ben ranged from 2.67 for 331-9 (race 3) to 4.00 for 86-124 (Table 8). However, there was no obvious chlorosis symptom developed on either parent after inoculated with all the isolates tested (Fig. 12).

The population segregated for reaction to Ptr ToxA as 107 sensitive and 85 insensitive lines, which statistically fits the 1:1 ratio ($\chi 2$=2.521, *P*=0.1124). Because both Ben and PI 41025 were insensitive to Ptr ToxB, BP025 population was not tested for reaction to Ptr ToxB. The BP025 population segregated for reaction to tan spot caused by all the races from highly resistant to highly susceptible (Table 8). Compared to the disease reaction, the population showed a clear transgressive segregation for most races. The average disease scores of the population were 3.07, 3.01, 3.02, 2.91, and 3.16 for Pti2, 86-124, 331-9, DW5 and AR CrossB10, respectively. Normality tests (Shapiro- Wilk) rejected the hypothesis that the disease reactions of the BP025 population to all the isolates had a normal distribution (*P*= 0.0018 for Pti2, 0.0004 for 86-124, 0.0005 for 331-9, 0.0001 for DW5 and 0.0001 for AR CrossB10) indicating presence of major

QTL governing the reactions. The histograms of disease reactions for each isolate were shown in Fig. 13.

Table 8. Lesion type means of the parental lines and the BP025 population to conidial inoculations of different *Pyrenophora tritici-repentis* races.

Isolates [a]	Ben	PI 41025	Population mean	Population range
Pti2 (race 1) (Ptr ToxA+, Ptr ToxC+)	3.25	2.38	3.07	1.0-4.83
86-124 (race 2) (Ptr ToxA+, Ptr ToxC-)	4.00	2.00	3.01	1.0-4.83
331-9 (race 3) (Ptr ToxA-, Ptr ToxC+)	2.67	2.17	3.02	1.17-4.50
DW5 (race 5) (Ptr ToxA-, Ptr ToxC-)	3.33	2.10	2.91	1.0-4.33
AR CRossB10 (Ptr ToxA-, Ptr ToxC+)	2.67	2.17	3.16	1.0-4.50

[a]Five isolates representing different *Pyrenophora tritici-repentis* races were used to evaluate the BP025 population and parental lines for reaction to tan spot. The NEs they produce are indicated in parenthesis where '+' = production of the NE and '–' = no production of the NE.
Disease was scored using a 1-5 scale with 1 being highly resistant and 5 being highly susceptible.

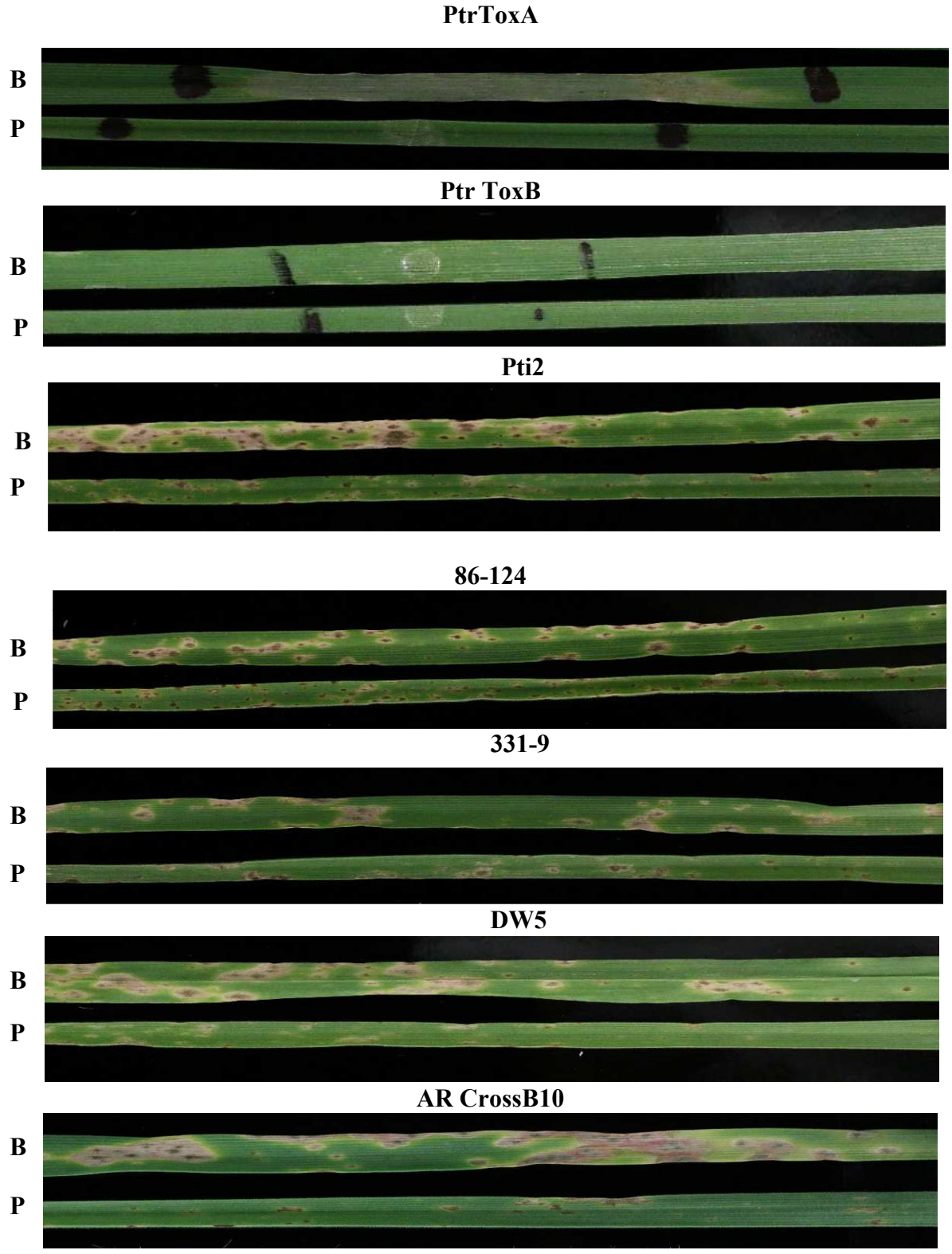

Figure 12. Reaction of Ben and PI41025 to *Pyrenophora tritici-repentis* necrotrophic effector infiltration and fungal inoculation. Ptr ToxA and Ptr ToxB were tested for NE infiltration and five isolates representing different races Pti2 (race 1), 86-124 (race 2), 331-9 (race 3), DW5 (race 5) and AR CrossB10 were used in fungal inoculations. B: Ben, P: PI 41025.

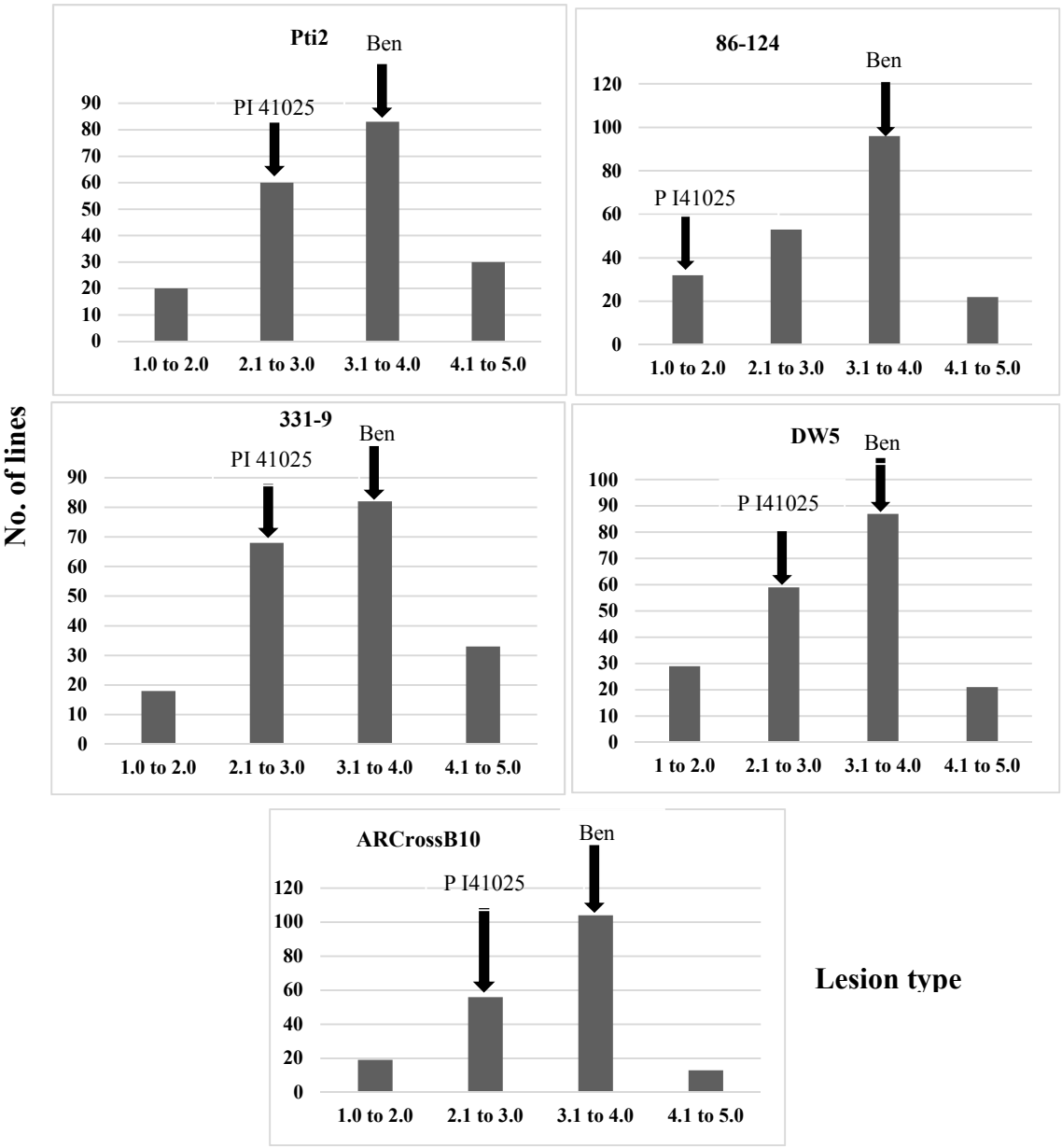

Figure 13. Histograms of disease reaction of the Ben × PI 41025 population to individual *Pyrenophora tritici-repentis* isolates. Four isolates representing different races are Pti2 (race 1), 86-124 (race 2), 331-9 (race 3), DW5 (race 5) and AR CrossB10 (unclassified race). The disease was scored using a 1-5 lesion type-based scale with 1 being highly resistant and 5 being highly susceptible. The x-axis denotes the disease scale and y-axis denotes the number of recombinant inbred lines.

Correlation of Sensitivity to Ptr ToxA and Disease Reaction to Races 1 and 2

Sensitivity to Ptr ToxA was mapped to the same place as Xfcp623, which is a marker developed from the *Tsn1* gene itself (Faris et al. 2010). Therefore, this confirms the *Tsn1* gene confers sensitivity to Ptr ToxA. Correlation analysis using Ptr Tox A infiltration data and disease data caused by Pti2 and 86-124 isolates indicated there is no significant correlation between sensitivity to Ptr ToxA and disease susceptibility caused by Pti2 and 86-124 inoculations (R^2=0.02, p=0.0655 for Pti2 and R^2=0.01, p=0.1653 for 86-124 respectively). This suggests that Ptr ToxA-*Tsn1* interaction is not important for disease development in the BP025 population.

QTL Identification

Because disease reactions of the BP025 population significantly deviated from a normal distribution, Levene's test was used to determine the homogeneity of variances of disease ratings among the three experiments for each isolate. The results indicated the data from different experiments were homogeneous (P = 0.06-0.75) for all the isolates (appendix A). Therefore, the disease data was combined to compute average disease scores for each RILs, which was then used in the subsequent QTL analyses.

In total, three QTLs associated with tan spot resistance in the BP025 population were identified, and they were located to the chromosome 5A, 5B, and 3A. The resistance alleles for all the QTL were contributed by the resistant parent PI 41025. The 5A QTL was significantly associated with resistance to all five isolates, whereas the 3A QTL and the 5B QTL are specific to only one isolate with the former for 86-124 isolate and the latter for DW5 (Table 9).

The 5A QTL, which conferred resistance to all isolates, had the largest effect on the disease. It had LOD values ranging from 7.10 (331-9) to 4.23 (AR CrossB10) and explained disease variations (R^2) from 15% (331-9) to 9% (AR CrossB10) (Table 9). The genomic region

harboring this QTL was flanked by the SNP markers *IWA7579* and *IWA4449*. Two SNP markers *IWA3085* and *IWA3362* were underlying the peak of the QTL (Fig. 14)

The 5B QTL, which associated with disease caused by DW5, was located to the position between markers *IWA3226* and *IWA6718* on the 5B chromosome (Table 9). This position is about 12 cM distal to the *Tsn1* gene (Fig 15). It had a LOD value of 4.77 and a R^2 value of 10% (Table 9).

The 3A QTL had a similar size of effect as the 5B QTL, but only associated with disease caused by 86-124. It was flanked by *IWA536* and *IWA5316*, which is at the 75-77 cM region on the chromosome 3A. The LOD and R^2 for this QTL were 4.77 and 10%, respectively (Fig 16).

Table 9. Single trait multiple interval mapping analysis of QTLs associated with resistance to tan spot caused by *Pyrenophora tritici-repentis* races 1, 2, 3, 5 and ARCrossB10 in the Ben × PI 41025 recombinant inbred line population.

Chromosomal location	**Interval (cM)**	**Flanking markers**	**R^{2*}**					**LOD[a]**					**Source[b]**
			Pti2	86-124	331-9	DW5	AR Cross B10	Pti2	86-124	331-9	DW5	AR Cross B10	
5A	121 - 125	*IWA7579-IWA4449*	0.14	0.13	0.15	0.10	0.09	6.53	6.09	7.10	4.35	4.23	P
3A	75 -78	*IWA536-IWA5316*	NS	0.10	NS	NS	NS	NS	4.78	NS	NS	NS	P
5B	115-117	*IWA3226-IWA6718*	NS	NS	NS	0.10	NS	NS	NS	NS	4.77	NS	P

*R^2 = the coefficient of determination. The R^2 values × 100 represents the amount of phenotypic variation explained. NS indicates the QTL was not significant.

[a]LOD was determined by the execution of 1000 permutations on marker and phenotypic data sets, which yielded a value of 4.0 as the cutoff for the detection of significant QTLs.

[b]The source of each QTL indicates the resistance allele was contributed by one of the parental lines with B being Ben and P being PI 41025.

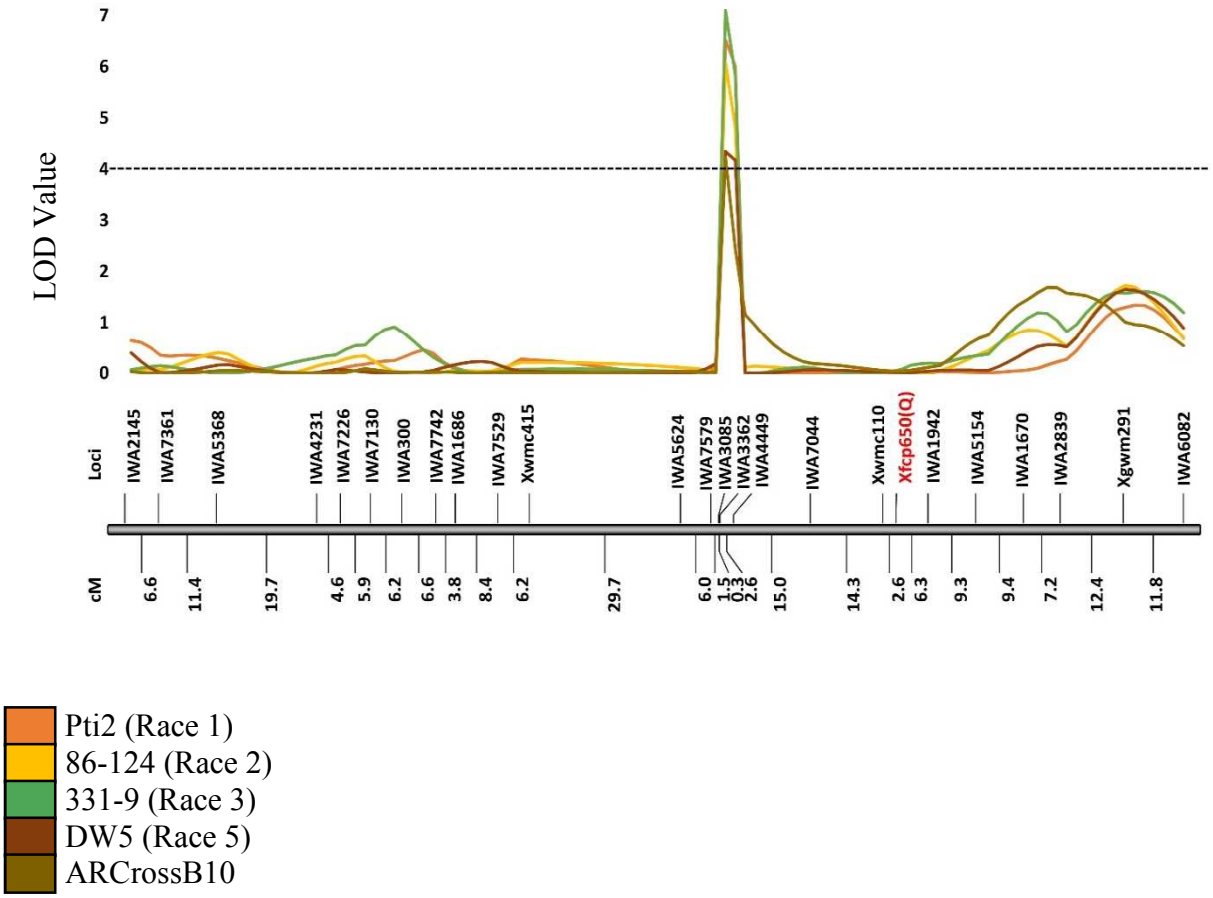

Figure 14. Single trait multiple interval map of chromosome 5A containing QTLs significantly associated with resistance to tan spot. QTL mapping was conducted on the BP025 population for four *Pyrenophora tritici-repentis* isolates representing different races including Pti2 (race 1, orange), 86-124 (race 2, yellow), 331-9 (race 3, green), DW5 (race 5, brown) and ARCrossB10 (light brown) The positions of marker loci are shown below the linkage groups and genetic scales in centiMorgan (cM) are shown along each chromosome. A dash line represents the logarithm of the odds (LOD) significance threshold of 4.0.

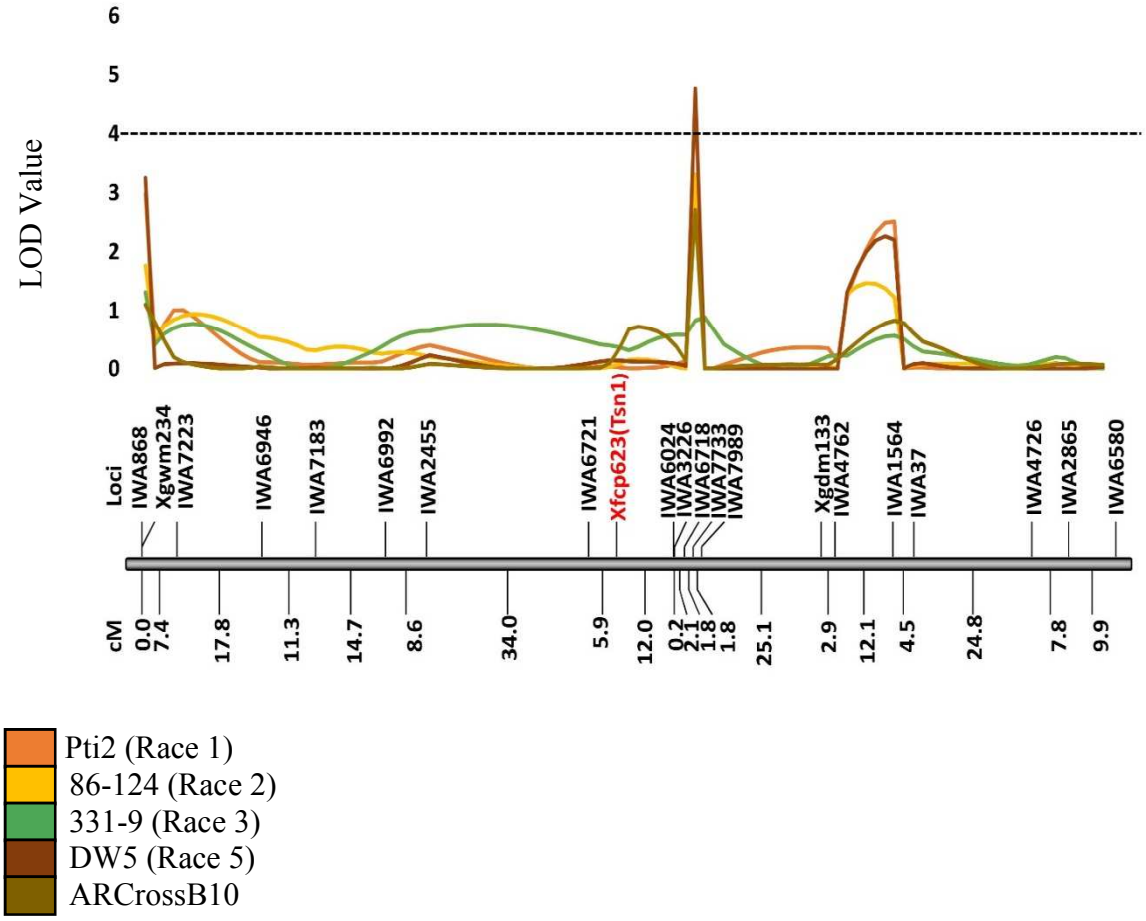

Figure 15. Single trait multiple interval map of chromosome 5B containing QTLs significantly associated with resistance to tan spot. QTL mapping was conducted on the BP025 population for four *Pyrenophora tritici-repentis* isolates representing different races including Pti2 (race 1, orange), 86-124 (race 2, yellow), 331-9 (race 3, green), DW5 (race 5, brown) and ARCrossB10 (light brown). The positions of marker loci are shown below the linkage groups and genetic scales in centi Morgan (cM) are shown along each chromosome. A dash line represents the logarithm of the odds (LOD) significance threshold of 4.0.

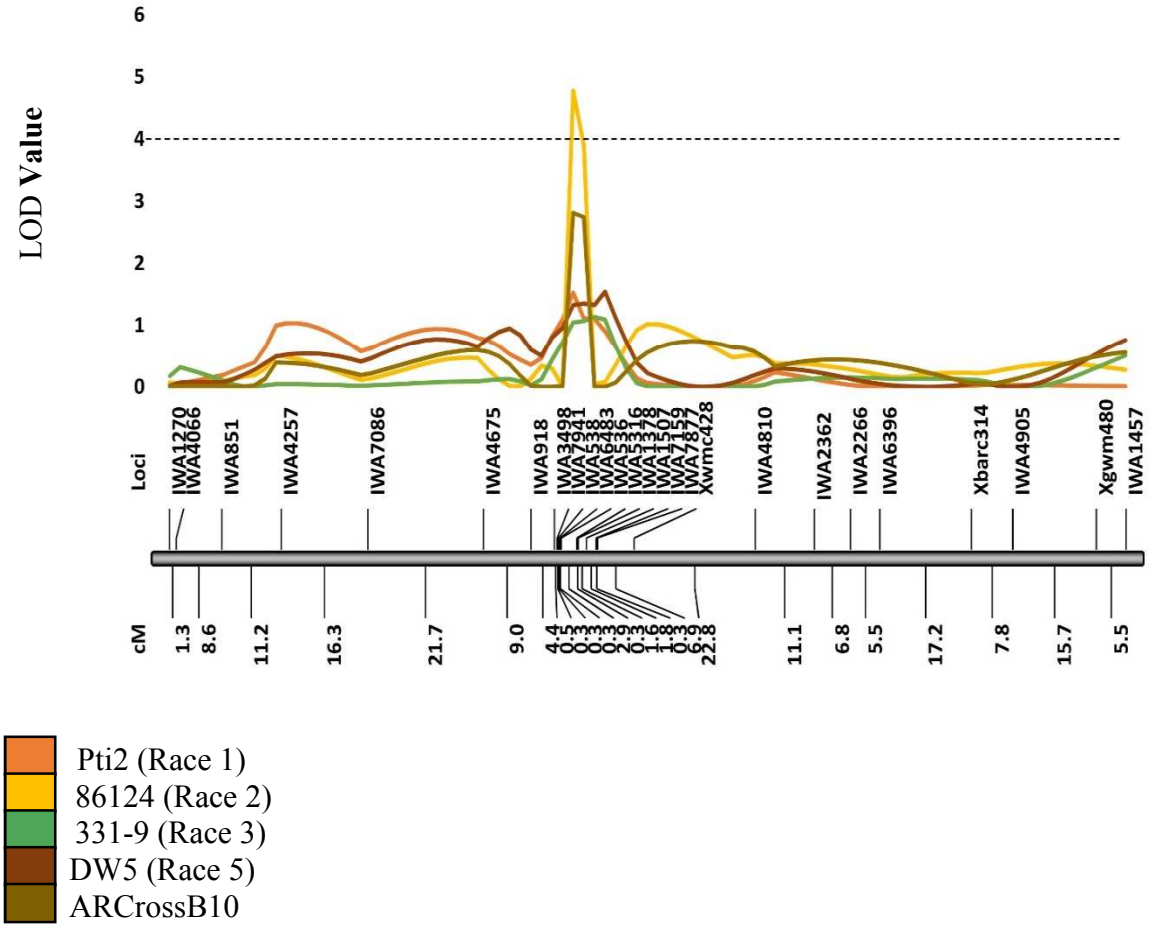

Figure 16. Single trait multiple interval map of chromosome 3A containing QTLs significantly associated with resistance to tan spot. QTL mapping was conducted on the BP025 population for four *Pyrenophora tritici-repentis* isolates representing different races including Pti2 (race 1, orange), 86-124 (race 2, yellow), 331-9 (race 3, green), DW5 (race 5, brown) and ARCrossB10 (light brown). The positions of marker loci are shown below the linkage groups and genetic scales in centi Morgan (cM) are shown along each chromosome. A dash line represents the logarithm of the odds (LOD) significance threshold of 4.0.

Discussion

P. tritici-repentis is a devastating wheat pathogen and also is diverse in virulence with more than eight races having been described (Lamari and Strelkov 2003; Faris et al. 2013; Ali et al. 2010). Host resistance in wheat germplasm should be evaluated and characterized for reaction to all the virulent races if it is possible. Characterization of genetic resistance to tan spot has been mainly on hexaploid wheat, not much on tetraploid durum wheat germplasm. Therefore, this study was conducted to characterize and map tan spot resistance in a tetraploid population derived from the resistant emmer wheat PI 41025 and the susceptible durum cultivar Ben. In this study, we showed again that Ptr ToxA-*Tsn1* interaction is not important for tan spot development and identified three resistance QTL from PI 41025. Our work provides further insight into durum-*Ptr* pathosystem and tools for improving tan spot resistance in durum cultivars.

The BP025 population segregated for sensitivity to Ptr ToxA and sensitivity to Ptr ToxA was mapped to the *Tsn1* locus. The fact that sensitivity to Ptr ToxA did not correlate with disease caused by Pti2 and 86-124 and no QTL was identified on the *Tsn1* locus strongly suggests the unimportance of Ptr ToxA-*Tsn1* interaction in this tetraploid population. This agrees with the previous studies (Chu et al. 2008a, 2010; Virdi et al. 2016) as well as our study in paper 1. Different from the situation on tetraploid wheat, the importance of Ptr ToxA-*Tsn1* interaction in hexaploid populations highly variable from very important to unimportant depending on the genotypes used (Faris et al.2013; Virdi et al. 2016). Virdi et al. (2016) also revealed that ToxA gene in the fungus had no detectable expression during the infection in the durum cultivar Langdon. It is very possible that the *ToxA* gene in Pti2 and 86-124 is not expressed when the fungus was inoculated onto the BP025 population. Both Ben and PI 41025 were insensitive to Ptr ToxB suggesting that the BP025 population does not segregating for the *Tsc2* gene. This is also

supported by the QTL mapping in which no QTL was identified on the chromosome arm 2BS where *Tsc2* is located. The Ptr ToxC-*Tsc1* was likely not presented in this population because we did not observe obvious chlorosis development in the parental lines and RILs and no QTL was identified on 1AS at the *Tsc1* locus.

One of QTL identified in BP025 population confers resistance to all the races tested indicating it is race-nonspecific. Race-nonspecific resistance QTL was first reported by Faris and Friesen (2005) on the chromosomes 1B and 3B in the Brazilian common wheat cultivar 'BR34'. Kariyawasam et al. (2016) mapped a major QTL on 3BL conferring race-nonspecific resistance in the common wheat cultivar 'Penawawa'. Small race-nonspecific resistance QTL were also identified in other chromosome arms, including 2AS, 5BL, 5DL and 7BS (Chu et al. 2008b; Faris et al. 2012). In the last chapter on durum association mapping, we detected the race-nonspecific resistance QTL on 3BL in durum. Here, we found another race-nonspecific resistance QTL on the chromosome 5A which is from the emmer wheat PI 41025. The QTL on the chromosome 5A associated with tan spot resistance has also been reported in both hexaploid wheat and durum populations (Chu et al. 2008, 2010; Kariyawasam et al. 2016). However, these 5A QTL were reported to be specific to one or two races not all the races tested. Further studies are needed to determine the relationship among these 5A QTL.

The 5B QTL we identified in BP025 population was specific to DW5 and it was located to the distal side of the *Tsn1* locus. In the last chapter for durum association mapping, we also identified a genomic region at the similar location on 5BL associated with DW5. Kariyawasam et al. (unpublished data) also identified a QTL on the distal end of *Tsn1* associated with resistance to DW5 in a tetraploid double haploid. Therefore, this QTL seems to be specific to race 5 isolate in durum. DW5 was isolated from durum in North Dakota (Ali et al. 2003) and it

was suspected that DW5 may produce novel NE causing necrosis on durum. This QTL may represent the locus conferring sensitivity to unidentified necrosis-inducing NE produced by DW5.

We also identified a QTL on 3AS in BP025 population that is specific to 86-124. Singh et al. (2008) identified a QTL at the distal end of 3AS for resistance to tan spot caused by a race 1 isolate in a hexaploid wheat population derived from resistant spring wheat cultivar 'WH542' and a moderately susceptible cultivar 'HD29'. The resistance gene *tsr4* in winter wheat cultivar 'Red Chief' was also mapped to the chromosome 3A close to SSR markers *Xgwm2* and *Xgwm5* (Tadesse et al. 2010). Due to the lack of common marker, it is difficult to determine if the *tsr4* gene and other 3AS QTL is the same as the 3A QTL obtained in this study.

In conclusion, we identified three QTLs in the BP025 population associated with tan spot resistance. One of them is race-nonspecific QTL derived from the emmer wheat PI 41025, which can be transferred to durum cultivar to improve tan spot resistance. The identified markers underlying this QTL can be useful in transferring the QTL. We also showed that the *Tsn1* locus is not associated with disease for races 1 and 2 in this population. This provide further evidence that Ptr ToxA-*Tsn1* interaction is not important for disease development in durum background.

Literature Cited

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

Abeysekara, N. S., Friesen, T. L., Liu, Z. H., McClean, P. E., and Faris, J. D. 2010. Marker development and saturation mapping of the tan spot Ptr ToxB sensitivity locus *Tsc2* in hexaploid wheat. Plant Genome 3:179-189.

Ali, S., Gurung, S., and Adhikari, T. B. 2010. Identification and characterization of novel isolates of *Pyrenophora tritici-repentis* from Arkansas. Plant Disease 94:229-235.

Aoun, M., Breiland, M., Turner, M. K., Loladze, A., Chao, S., Xu, S. S., Ammar, K., Anderson, J. A., Kolmer., and Acevado, M. 2016. Genome-wide association mapping of leaf rust response in a durum wheat worldwide germplasm collection. The Plant Genome 9: 1-24.

Beecher, B. S., Carter, A. H., and See, D. R. 2012. Genetic mapping of new seed-expressed polyphenol oxidase genes in wheat (*Triticum aestivum* L.). Theoretical and Applied Genetics 124:1463-1473.

Carter, A. H., Chen, X. M., Garland-Campbell, K., and Kidwell, K. K. 2009. Identifying QTL for high-temperature adult-plant resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in the spring wheat (*Triticum aestivum* L.) cultivar 'Louise'. Theoretical and Applied Genetics 119:1119-1128.

Cheong, J., Wallwork, H., and Williams, K. J. 2004. Identification of a major QTL for yellow leaf spot resistance in the wheat varieties Brookton and Cranbrook. Crop and Pasture Science 55:315-319.

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

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

Ciuffetti, L. M., Francl, L. J., Balance, G. M., Bockus, W. W., Lamari, L., Meinhardt, S. W., and Rasmussen, J. B. 1998. Standardization of toxin nomenclature in the *Pyrenophora tritici-repentis*/wheat interaction. Canadian Journal of Plant Pathology 20:421-424.

Ciuffetti, L. M., Manning, V. A., Martinez, J. P., Pandelova, I., and Andrie, R. M. 2003 Proteinaceous toxins of *Pyrenophora tritici-repentis* and investigation of the site-of-action of Ptr ToxA. In: Rasmussen, J. B., Friesen, T. L., Ali, S. (eds). Proceedings of the fourth international wheat tan spot and spot blotch workshop. North Dakota Agricultural Experiment Station, Fargo (p. 96-102).

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

Effertz, R. J., Anderson, J. A., and Francl, L. J. 2001. Restriction fragment length polymorphism mapping of resistance to two races of *Pyrenophora tritici-repentis* in adult and seedling wheat. Phytopathology 91:572-578.

Effertz, R. J., Meinhardt, S. W., Anderson, J. A., Jordahl, J. G., and Francl, L. J. 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.

Faris, J. D., Anderson, J. A., Francl, L. J., and Jordahl, J. G. 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., Anderson, J. A., Francl, L. J., and Jordahl, J. G. 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., Anderson, J. A., Francl, L. J., and Jordahl, J. G. 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 Friesen, T. L. 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., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J. P., Meinhardt, S. W., Rasmussen, J. B., Oliver, R. P., and Friesen, T. L. 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.

Faris, J. D., Abeysekara, N. S., McClean, P. E., Xu, S. S., and Friesen, T. L. 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., Liu, Z., and Xu, S. S. 2013. Genetics of tan spot resistance in wheat. Theoretical and Applied Genetics 126:2197-2217.

Faris, J. D., Zhang, Q. J., Chao, S., Zhang, Z., and Xu, S. S. 2014. Analysis of agronomic and domestication traits in a durum x cultivated emmer wheat population using a high-density single nucleotide polymorphism-based linkage map. Theoretical and Applied Genetics 127:2333-2348.

Flor, H. H. 1956. The complimentary genetics systems in flax and flax rust. Advance Genetics 8:29–54.

Friesen, T. L., Ali, S., Kianian, S., Francl, L. J., and Rasmussen, J. B. 2003. Role of host sensitivity to Ptr ToxA in development of tan spot of wheat. Phytopathology 93:397-401.

Friesen, T. L., and Faris, J. D. 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., Xu, S. S., and Harris, M. O. 2008. Stem rust, tan spot, Stagonospora nodorum blotch, and hessian fly resistance in Langdon durum-synthetic hexaploid wheat lines. Crop Science 48:1062-1070.

Friesen, T. L., and Faris, J. D. 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.

Hosford, R. M. Jr. 1982. Tan spot-developing knowledge 1902-1981, virulent races and differentials, methodology, rating systems, other leaf diseases, literature. In: Hosford, RM Jr (ed) Tan spot of wheat and related diseases workshop. North Dakota Agricultural Experiment Station, Fargo (p. 1-24).

Joehanes, R., and Nelson, J. C. 2008. QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24:2788-2789.

Kariyawasam, G. K., Carter, A. H., Rasmussen, J. B., Faris, J., Xu, S. S., Mergoum, M., and Liu, Z. 2016. Genetic relationships between race- nonspecific and race-specific interactions in the wheat- *Pyrenophora tritici-repentis* pathosystem. Theoretical and Applied Genetics 129:897-908.

Kollers, S., Rodemann, B., Ling, J., Korzun, V., Ebmeyer, E., Argillier, O., Hinze, M., Plieske, J., Kulosa, D., Ganal, M. W., and Röder, M. S. 2014. Genome-wide association mapping of tan spot resistance (*Pyrenophora tritici-repentis*) in European winter wheat. Molecular Breeding 34:363- 371.

Lamari, L., and Bernier, C. C. 1989b. 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 Strelkov, S. E. 2010. The wheat/*Pyrenophora tritici-repentis* interaction: progress towards an understanding of tan spot disease. Canadian Journal of Plant Pathology 32:4-10.

Levene, H. 1960 Robust tests for equality of variances. In: Olkin I, Ghurye SH, Hoeffding W, Madow WG, Maan HB (eds) Contributions to probability and statistics: Essays in honor of Harold Hotelling. Stanford University Press, Stanford.

Liu, Z. H., El-Basyoni, I., Kariyawasam, G., Zhang, G., Fritz, A., Hansen, J. M., Marais, F., Friskop, A. J., Chao, S., Akhunov, E., and Baenziger, P. S. 2015. Evaluation and association mapping of resistance to tan spot and Stagonospora nodorum blotch in adapted winter wheat germplasm. Plant Disease 99:1333-1341.

Liu, Z. H., Faris, J. D., Oliver, R. P., Tan, K., Solomon, P. S., Mcdonald, M. C., Mcdonald, B. A., Nunez, A., Lu, S., Rasmussen, J. B., and Friesen, T. L. 2009. SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the *Snn3* gene. Public Library of Science Pathology 5:1-15.

Lorieux, M. 2012. MapDisto: fast and efficient computation of genetic linkage maps. Molecular Breeding 30:1231-1235.

Manning, V., and Ciuffetti, L. M. 2015. Necrotrophic effector epistasis in the *Pyrenophora tritici-repentis*-wheat interaction. Public Library of Science One 10: e0123548 doi: 10.1371/journal.pone.0123548.

Matsuoka, Y. 2011. Evolution of Polyploid Triticum Wgeats under Cultivation: The Role

of Domestication, Natural Hybridization and Allopolyploid Speciation in their Diversification. Plant & Cell Physiology 52: 750–764.

Meinhardt, S. W., Ali, S., Ling, H., and Francl, L. J. 2003. A new race of *Pyrenophora tritici-repentis* that produces a putative host-selective toxin. In: Rasmussen, J. B., Friesen, T. L., Ali, S. (eds) Proceedings of the fourth international wheat tan spot and spot blotch workshop. North Dakota Agricultural Experiment Station, Fargo, (p. 117-119).

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

Noriel, A. J., Sun, X. C., Bockus, W., and Bai, G. 2011. Resistance to tan spot and insensitivity to Ptr ToxA in wheat. Crop Science 51:1059-1067.

Patel, J. S., Mamidi, S., Bonman, J. M., and Adhikari, T. B. 2013. Identification of QTL in spring wheat associated with resistance to a novel isolate of *Pyrenophora tritici-repentis*. Crop Science 53:842-852.

Rees, R. G., Platz, G. J., and Mayer, R. J. 1982. Yield losses in wheat from yellow spot: comparison of estimates derived from single tillers and plots. Australian Journal of Agricultural Research 33:899–908.

Riede, C. R., Francl, L. J., Anderson, J. A., Jordahl, J. G., and Meinhardt, S. W. 1996. Additional Sources of Resistance to Tan Spot of Wheat. Crop Science 36: 771-777.

SAS Institute. 2011. SAS/IML 9.3 User's Guide. SAS Institute, Cary, NC.

Schilder, A. M. C., and Bergstrom, G. C. 1994. Infection of wheat seed by *Pyrenophora tritici-repentis*. Canadian Journal of Plant Pathology 72:510-519.

Singh, P. K., Gonzalez-Hernandez, J. L., Mergoum, M., Ali, S., Adhikari, T. B., Kianian, S. F., Elias, E. M. and Hughes, G. R. 2006. 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., Mergoum, M., Gonzalez-Hernandez, J. L., Ali, S., Adhikari, T. B., Kianian, S. F. Elias, E. M. and Hughes, G. R. 2008. Genetics and molecular mapping of resistance to necrosis inducing race 5 of *Pyrenophora tritici-repentis* in tetraploid wheat. Molecular Breeding 21:293-304.

Snedecor, G. W., and Cochran, W. G. 1989. Statistical Methods, Eighth Edition, Iowa State University Press.

Sun, X. C., Bockus, W., and Bai, G. 2010. Quantitative trait loci for resistance to *Pyrenophora tritici-repentis* race 1 in a Chinese wheat. Phytopathology 100:468-473.

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

Tadesse, W., Hsam, S. L. K., and Zeller, F. J. 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.

Virdi, S. K., Liu, Z., Overlander, M. E., Zhang, Z., Xu, S. S., Friesen, T. L., and Faris, J. D. 2016. New insights into the roles of host gene-necrotrophic effector interactions in governing susceptibility of durum wheat to tan spot and septoria nodorum blotch. G3 6:4139-4150.

Wolpert, T. J., Dunkle, L. D., and Ciuffetti, L. M. 2002. Host-selective toxins and avirulence determinants: what's in a name? Annual Review of Phytopathology 40:251-285.

Zeng Z-B. 1994. Precision mapping of quantitative trait loci. Genetics 136: 1457-1468.

Zhang, Q., Axtman, J. E., Faris, J. D., Chao, S., Zhang, Z., Friesen, T. L., Zhong, S., Cai, X., Elias, E. M., and Xu, S. S. 2014. Identification and molecular mapping of quantitative trait loci for Fusarium head blight resistance in emmer and durum wheat using a single nucleotide polymorphism-based linkage map. Molecular Breeding 34:1677–1687.

APPENDIX A: PHENOTYPIC DATA FOR THE TAN SPOT CAUSED BY PTI2 (RACE 1), 86-124 (RACE 2), 331-9 (RACE 3), DW5 (RACE 5) AND NE, PTR TOXA AND PTR TOXB ON TCAP DURUM PANEL

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
1	CItr5083	.	.	.	.	.	.
2	CItr5122	4.17	2.63	3.33	3.25	.	0.00
3	CItr6874	3.75	2.83	2.83	3.17	3.00	0.00
4	CItr6876	4.25	3.38	2.83	3.25	0.00	1.00
5	CItr7669	3.50	3.67	3.33	2.83	0.00	0.00
6	CItr8164	4.83	4.63	4.00	4.33	3.00	1.00
7	CItr8882	2.17	2.75	2.83	2.00	0.00	0.00
8	CItr11476	4.50	3.38	4.33	2.67	0.00	0.00
9	CItr11496	3.50	3.17	3.83	3.33	0.00	0.00
10	CItr12032	2.83	2.67	2.83	3.33	.	0.00
11	CItr12818	4.33	4.50	4.33	3.67	1.00	0.00
12	CItr13246	3.33	3.25	2.83	2.25	0.50	0.00
13	CItr13338	2.67	3.13	2.33	2.50	2.00	1.00
14	CItr14080	3.67	4.00	4.50	4.33	0.00	1.00
15	CItr14091	3.75	2.88	2.50	2.67	3.00	1.00
16	CItr14093	3.50	3.38	3.50	3.50	0.00	0.00
17	CItr14094	2.67	3.38	4.00	3.67	0.00	1.00
18	CItr14099	4.83	4.38	3.50	3.33	0.50	0.00
19	CItr14268	2.50	2.38	1.75	2.50	.	1.00
20	CItr14374	4.67	4.38	4.33	4.00	0.00	1.00
21	CItr14434	4.00	3.25	3.67	4.00	0.00	1.00
22	CItr14438	4.75	4.38	3.67	2.50	0.00	0.00
23	CItr14559	3.17	3.50	3.83	3.00	0.00	0.00
24	CItr14618	4.33	4.00	3.50	2.83	.	0.00
25	CItr14623	3.75	4.00	3.50	3.00	3.00	0.00
26	CItr14699	4.50	3.67	3.67	3.33	1.25	0.00
27	CItr14798	4.00	3.38	4.50	2.67	3.00	0.00
28	CItr14809	3.83	4.00	4.67	4.67	0.00	1.00
29	CItr14810	4.50	3.63	4.00	4.17	0.00	1.00
30	CItr14813	4.50	3.38	4.00	3.33	3.00	.
31	CItr14814	4.83	4.63	4.25	4.00	3.00	0.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
32	CItr14816	3.00	3.25	2.50	2.83	0.00	.
33	CItr14954	3.33	3.13	3.83	3.00	0.00	0.00
34	CItr14965	4.00	2.88	4.00	2.33	0.00	0.00
35	CItr14978	3.83	3.50	4.00	3.33	.	0.00
36	CItr15153	4.17	3.50	4.50	4.25	.	1.00
37	CItr15159	4.17	3.33	4.33	3.00	.	0.00
38	CItr15422	3.83	3.63	2.83	2.33	3.00	0.00
39	CItr15500	3.67	3.75	4.00	4.33	0.00	1.00
40	CItr15513	4.50	3.50	4.17	4.17	0.00	1.00
41	CItr15769	3.33	2.25	2.83	2.17	3.00	0.00
42	CItr15814	3.00	3.38	3.17	2.00	0.00	0.00
43	CItr15911	3.83	3.50	4.33	3.50	0.00	0.00
44	CItr17337	1.67	2.50	2.50	1.25	1.00	0.00
45	CItr17341	2.67	3.00	3.50	3.00	1.00	1.00
46	CItr17757	3.67	3.50	3.50	3.00	1.75	0.00
47	PI6020	3.00	3.13	3.67	3.33	0.00	0.00
48	PI7464	4.17	3.25	4.17	4.00	0.00	1.00
49	PI32156	1.67	1.50	1.33	1.00	0.00	0.00
50	PI38624	4.00	3.25	3.83	4.17	0.00	1.00
51	PI40938	4.83	3.33	4.00	4.00	0.00	0.00
52	PI40939	3.25	2.63	2.25	2.75	3.00	0.00
53	PI41050	3.33	3.50	2.67	3.17	3.00	1.00
54	PI41343	5.00	3.50	3.00	2.25	.	.
55	PI41353	3.50	3.50	4.50	2.17	.	0.00
56	PI43247	2.17	1.50	1.75	1.50	0.00	0.00
57	PI43340	2.75	2.17	3.17	3.00	0.00	1.00
58	PI43341	3.00	2.33	3.00	2.67	0.00	1.00
59	PI43342	2.67	2.13	3.00	3.33	0.00	1.00
60	PI45441	3.00	2.63	3.00	3.50	0.00	1.00
61	PI45442	3.50	3.38	3.50	3.67	0.00	0.00
62	PI47888	4.50	3.83	4.50	3.50	.	0.00
63	PI47891	3.17	3.38	3.67	3.67	3.00	1.00
64	PI50929	4.50	3.88	4.00	2.75	3.00	0.00
65	PI51210	3.83	3.88	4.17	3.00	0.00	0.00
66	PI54432	4.17	4.25	4.00	4.00	0.00	.
67	PI55543	3.00	3.33	3.33	2.50	2.00	0.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
68	PI56245	4.25	2.88	3.67	3.33	0.00	0.00
69	PI57194	2.83	3.13	3.25	2.83	2.00	0.00
70	PI57555	2.50	3.25	3.17	2.00	0.00	0.00
71	PI57556	1.50	2.50	2.25	2.17	0.00	0.00
72	PI57558	2.50	2.88	3.83	3.00	0.00	1.00
73	PI57560	3.00	3.63	3.67	3.33	0.50	1.00
74	PI57562	2.50	3.50	3.50	2.17	0.00	0.00
75	PI57593	2.67	3.25	3.17	2.50	0.00	0.00
76	PI57595	2.50	3.00	2.83	2.83	0.00	0.00
77	PI57599	2.17	3.25	2.75	2.50	0.00	1.00
78	PI57602	4.17	3.75	4.75	3.25	0.00	0.00
79	PI68260	.	.	.	.	.	.
80	PI68266	.	.	.	.	.	.
81	PI68271	.	.	.	.	.	.
82	PI68275	4.00	4.17	4.33	4.00	0.00	1.00
83	PI68288	.	.	.	.	.	.
84	PI70658	3.83	3.00	3.83	3.33	0.00	1.00
85	PI70718	4.00	3.88	4.33	3.50	.	1.00
86	PI70720	3.00	3.67	2.83	3.17	.	.
87	PI70724	3.00	4.13	3.33	4.00	.	1.00
88	PI70728	3.33	3.67	3.33	3.50	0.00	1.00
89	PI70732	4.50	3.88	3.83	3.33	1.00	0.00
90	PI73366	3.50	3.75	4.25	4.00	0.00	1.00
91	PI74830	3.50	3.50	3.33	3.33	0.00	0.00
92	PI78810	.	.	.	.	.	.
93	PI78811	.	.	.	.	.	.
94	PI81792	4.25	3.67	3.50	3.75	0.00	.
95	PI84529	3.50	3.13	3.83	3.17	0.00	0.00
96	PI89642	3.50	3.83	4.50	3.83	0.00	0.00
97	PI91956	3.50	3.67	4.33	3.00	0.00	0.00
98	PI94701	3.83	3.88	3.33	3.00	0.00	1.00
99	PI94703	4.50	4.00	3.83	2.50	0.00	0.00
100	PI94705	3.83	4.38	3.33	3.17	0.00	0.00
101	PI94710	4.50	2.75	3.67	2.00	0.00	0.00
102	PI94713	3.00	3.50	3.67	4.00	2.00	1.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
103	PI94758	4.17	4.38	3.83	4.00	3.00	0.00
104	PI113395	4.00	3.88	4.17	3.17	3.00	0.00
105	PI113397	4.25	4.00	4.17	3.50	3.00	.
106	PI113398	5.00	3.67	4.25	4.00	0.00	0.00
107	PI113951	4.50	3.63	4.67	3.83	1.00	0.00
108	PI113964	4.25	3.88	4.50	3.67	1.00	0.00
109	PI115514	4.17	3.88	4.00	3.25	2.00	0.00
110	PI134929	3.67	3.00	3.67	2.67	0.00	0.00
111	PI138971	3.83	3.75	3.50	4.33	0.00	1.00
112	PI152567	4.33	3.88	3.17	3.83	3.00	0.00
113	PI153727	3.50	3.50	3.83	4.33	0.00	1.00
114	PI157953	4.00	3.63	3.25	3.50	0.00	0.00
115	PI157957	3.00	3.75	3.17	2.67	0.00	0.00
116	PI163274	3.00	2.33	2.67	2.00	0.00	1.00
117	PI165476	4.33	2.38	3.83	2.67	3.00	0.00
118	PI166327	4.33	3.38	3.50	3.17	1.00	0.00
119	PI167652	4.00	4.38	4.00	3.83	2.00	1.00
120	PI167718	3.67	1.63	2.00	1.17	0.00	0.00
121	PI167730	.	.	.	.	.	.
122	PI168913	3.08	2.17	3.25	3.00	0.00	0.00
123	PI168916	4.17	3.38	3.67	3.17	0.00	0.00
124	PI168922	2.75	2.33	3.50	2.17	0.00	0.00
125	PI172556	.	.	.	.	.	.
126	PI174625	4.25	3.50	4.00	3.83	0.00	0.00
127	PI174645	3.17	1.83	3.17	1.83	0.00	0.00
128	PI174646	4.33	2.25	3.33	2.17	0.75	0.00
129	PI174697	1.50	2.13	2.33	1.83	0.50	0.00
130	PI176228	4.50	4.50	3.50	2.33	3.00	0.00
131	PI176289	3.00	4.00	3.17	1.83	.	0.00
132	PI176291	4.00	3.75	4.00	3.00	3.00	0.00
133	PI177919	2.00	1.88	2.25	3.67	0.00	1.00
134	PI178143	2.67	3.00	1.83	4.00	3.00	1.00
135	PI178156	2.67	2.63	3.00	3.17	0.00	1.00
136	PI178758	4.50	4.38	3.50	3.67	0.50	1.00
137	PI182113	2.75	2.33	2.00	2.25	.	.

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
138	PI182412	3.17	3.17	2.67	3.33	0.00	0.00
139	PI182668	3.50	4.00	4.17	4.00	.	1.00
140	PI182669	4.33	4.67	4.00	2.67	3.00	0.00
141	PI182671	3.33	3.50	3.50	3.33	3.00	0.00
142	PI182674	.	.	.	.	.	.
143	PI182676	4.25	3.75	4.17	4.50	0.00	1.00
144	PI182708	4.00	3.83	3.00	3.67	.	1.00
145	PI182717	4.00	4.00	4.25	4.17	1.75	1.00
146	PI182738	4.00	3.63	4.00	4.75	0.00	1.00
147	PI183269	4.00	4.13	4.50	3.75	0.00	1.00
148	PI183909	4.17	4.00	4.00	4.00	1.00	1.00
149	PI184540	3.83	3.63	4.33	3.17	2.00	0.00
150	PI184641	3.50	2.75	3.67	2.50	0.00	0.00
151	PI185233	3.17	2.38	3.33	4.17	0.00	1.00
152	PI185300	4.17	3.25	3.50	1.67	0.00	0.00
153	PI185301	4.83	3.83	4.00	4.17	3.00	1.00
154	PI185722	2.50	1.63	3.00	3.83	0.00	1.00
155	PI185762	2.33	2.13	2.83	2.33	0.00	0.00
156	PI190937	3.17	4.00	4.33	3.67	0.00	1.00
157	PI190977	3.50	3.50	3.17	2.33	3.00	0.00
158	PI191011	4.67	2.63	3.50	2.00	0.00	0.00
159	PI191078	4.00	3.38	3.67	3.50	0.00	0.00
160	PI191183	2.67	2.75	3.00	3.33	3.00	0.00
161	PI191191	2.75	3.13	2.50	2.17	1.00	1.00
162	PI191356	4.00	4.25	3.83	3.50	0.00	0.00
163	PI191448	3.17	3.50	3.67	3.67	3.00	0.00
164	PI191488	3.00	2.63	3.33	3.50	0.00	1.00
165	PI191571	2.75	3.38	3.33	3.83	0.00	1.00
166	PI191615	3.33	3.13	3.33	3.33	3.00	1.00
167	PI191624	4.17	3.50	3.83	4.00	0.00	1.00
168	PI191645	2.33	1.88	2.17	1.83	0.00	0.00
169	PI191654	1.33	1.17	1.17	1.33	0.00	0.00
170	PI191816	.	.	.	.	.	.
171	PI191958	1.33	1.25	1.50	1.00	0.00	0.00
172	PI191963	3.83	3.50	3.83	3.83	.	0.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
173	PI191972	3.00	2.63	3.33	3.33	0.00	0.00
174	PI192044	2.83	2.50	3.17	3.33	.	1.00
175	PI192051	4.33	4.00	4.50	3.75	0.00	1.00
176	PI192109	4.17	3.75	4.17	3.33	0.00	0.00
177	PI192179	3.83	4.00	4.33	4.17	0.00	0.00
178	PI192334	1.50	1.63	1.83	4.00	0.50	1.00
179	PI192399	.	.	.	.	.	.
180	PI192454	4.17	2.00	4.00	2.33	.	0.00
181	PI192640	1.33	1.13	1.33	1.50	0.00	0.00
182	PI192657	3.33	2.50	3.83	1.50	0.00	0.00
183	PI192665	3.17	2.50	4.00	3.00	0.00	1.00
184	PI192711	3.25	3.25	4.33	3.33	0.00	1.00
185	PI192820	2.83	2.83	2.67	3.33	0.00	0.00
186	PI192836	3.17	2.88	3.83	2.83	0.00	0.00
187	PI192848	4.00	3.25	3.67	2.83	0.00	.
188	PI192852	1.67	1.50	2.00	2.67	0.00	0.00
189	PI193920	3.67	3.13	3.33	3.33	0.00	0.00
190	PI195693	3.50	3.63	3.67	3.25	1.00	0.00
191	PI195695	4.00	4.00	4.17	4.50	0.00	1.00
192	PI195905	4.00	4.00	3.50	3.50	0.00	0.00
193	PI196093	4.67	4.25	3.67	3.50	1.50	0.00
194	PI204033	3.67	2.25	3.00	1.50	0.00	1.00
195	PI208910	4.00	3.88	4.50	4.33	0.00	1.00
196	PI209274	3.67	2.63	3.67	2.83	0.00	0.00
197	PI209277	4.33	3.88	4.17	3.17	.	0.00
198	PI210381	3.33	3.50	3.00	4.00	0.00	1.00
199	PI210912	3.17	3.50	3.00	3.17	0.00	0.00
200	PI210944	4.50	3.00	4.33	2.17	0.00	0.00
201	PI210946	3.67	3.13	3.50	3.17	0.00	0.00
202	PI210947	3.17	3.00	2.83	3.67	0.00	1.00
203	PI210954	3.17	4.00	3.67	3.00	0.00	0.00
204	PI221409	2.67	3.25	3.67	3.00	0.00	0.00
205	PI221702	2.83	3.00	3.67	3.50	0.00	0.00
206	PI223152	4.50	3.88	4.17	3.67	0.00	1.00
207	PI223155	3.50	3.50	2.75	3.83	3.00	1.00
208	PI223165	2.00	2.38	2.33	3.83	0.00	1.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
209	PI223168	4.67	4.13	4.50	3.83	.	1.00
210	PI223169	4.00	4.13	3.67	3.33	0.00	0.00
211	PI223170	3.00	2.83	4.00	2.67	0.00	.
212	PI225324	4.00	3.88	4.33	3.67	3.00	1.00
213	PI225325	3.83	3.63	3.67	3.83	3.00	1.00
214	PI226576	4.17	4.00	3.83	3.33	3.00	0.00
215	PI230366	4.50	4.00	3.67	3.67	0.00	0.00
216	PI230367	3.25	3.75	4.00	4.67	0.00	1.00
217	PI231305	3.50	3.13	3.75	4.00	0.00	1.00
218	PI234381	4.50	3.88	4.75	3.50	0.25	0.00
219	PI234382	4.33	3.83	4.33	3.83	0.00	1.00
220	PI234384	4.83	3.75	4.67	4.17	0.50	1.00
221	PI234386	4.67	4.50	4.67	4.00	0.25	1.00
222	PI234387	4.67	3.88	4.17	4.00	0.00	1.00
223	PI234388	4.50	4.13	4.33	4.17	0.00	1.00
224	PI234860	4.17	4.25	3.33	2.83	0.00	0.00
225	PI237628	4.25	3.25	4.17	3.33	0.00	1.00
226	PI238388	4.33	3.50	4.17	3.50	0.00	0.00
227	PI243692	3.33	3.33	4.25	4.50	3.00	1.00
228	PI244061	3.83	3.38	2.17	2.00	.	0.00
229	PI249822	3.83	3.13	3.83	3.33	.	0.00
230	PI251918	4.33	3.67	3.50	3.33	0.00	0.00
231	PI253958	4.00	2.75	3.83	3.33	.	1.00
232	PI253960	3.50	3.63	3.50	4.00	0.00	1.00
233	PI253964	3.83	3.88	4.17	4.50	0.00	1.00
234	PI254011	3.00	3.00	4.00	2.83	0.00	0.00
235	PI254012	3.33	3.75	3.67	3.33	0.00	1.00
236	PI254013	2.17	3.00	3.33	3.00	.	1.00
237	PI254014	2.75	3.38	3.83	2.50	.	0.00
238	PI254015	3.00	2.75	2.25	2.83	0.00	1.00
239	PI260061	2.33	2.88	3.00	2.00	0.00	0.00
240	PI261823	2.33	3.00	2.83	2.83	0.00	0.00
241	PI262675	4.50	3.17	4.17	3.33	0.00	0.00
242	PI264434	4.00	3.75	3.67	3.33	0.00	0.00
243	PI264440	3.00	3.25	3.83	3.83	0.00	1.00
244	PI264930	3.50	3.88	4.00	2.17	2.50	0.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
245	PI264938	4.33	3.50	4.00	4.33	0.00	1.00
246	PI264947	3.50	3.50	3.00	3.33	0.00	0.00
247	PI264948	3.83	4.00	4.17	3.33	1.00	0.00
248	PI264992	2.83	3.00	3.67	2.17	0.00	0.00
249	PI266978	5.00	3.38	4.00	4.17	0.00	1.00
250	PI270001	4.17	3.75	3.17	3.67	0.00	0.00
251	PI272476	2.50	2.63	3.00	2.83	0.00	0.00
252	PI272545	5.00	4.00	4.33	3.00	3.00	0.00
253	PI272553	4.50	3.50	5.00	3.75	1.50	0.00
254	PI274670	2.00	1.75	2.17	1.83	0.00	0.00
255	PI274671	2.50	2.75	3.00	3.33	0.00	0.00
256	PI274672	2.33	2.50	3.17	1.67	0.00	0.00
257	PI274676	2.50	2.88	2.67	3.67	0.00	0.00
258	PI274678	1.50	1.17	1.50	1.17	0.00	0.00
259	PI274681	.	.	.	.	.	.
260	PI274682	3.50	1.50	2.50	1.50	0.00	0.00
261	PI278259	4.33	3.25	3.75	2.33	.	0.00
262	PI278265	.	.	.	.	.	.
263	PI278352	3.00	3.25	3.25	2.83	3.00	.
264	PI278376	3.17	3.33	3.33	2.50	0.00	1.00
265	PI278377	3.17	3.00	3.17	3.17	0.00	1.00
266	PI278378	3.75	3.88	4.33	5.00	0.00	1.00
267	PI278379	3.67	3.56	3.33	3.50	3.00	0.00
268	PI278380	4.33	4.00	4.17	3.25	.	0.00
269	PI278383	3.00	3.50	3.33	4.17	0.00	1.00
270	PI278443	3.33	3.38	3.33	2.75	0.00	1.00
271	PI278502	3.33	3.83	4.00	3.00	0.00	0.00
272	PI278503	3.00	2.63	2.83	2.33	0.00	0.00
273	PI282911	4.67	5.00	4.50	3.67	3.00	1.00
274	PI283151	.	.	.	.	.	.
275	PI283154	4.75	3.63	4.00	4.00	3.00	1.00
276	PI283155	4.17	3.88	4.00	3.83	3.00	1.00
277	PI283854	3.67	4.25	3.00	3.50	3.00	1.00
278	PI283855	3.25	3.75	3.50	3.00	.	0.00
279	PI283856	4.25	3.50	4.17	.	.	0.00
280	PI286063	3.00	3.25	2.67	3.50	0.00	0.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
281	PI286076	3.50	3.63	3.83	2.50	1.50	0.00
282	PI286539	4.00	3.33	3.50	3.33	0.00	1.00
283	PI289604	3.17	2.83	3.00	2.83	0.00	1.00
284	PI290473	2.50	2.75	3.00	2.50	0.00	0.00
285	PI290486	3.33	3.00	3.50	3.00	0.00	1.00
286	PI290490	2.67	3.13	3.00	4.00	.	0.00
287	PI290494	1.67	2.50	2.00	3.50	0.00	1.00
288	PI290531	.	.	.	.	.	.
289	PI292034	3.17	3.88	3.50	2.50	.	0.00
290	PI294587	4.17	3.38	3.67	2.83	0.00	0.00
291	PI294588	3.83	3.63	4.00	3.17	2.00	1.00
292	PI295967	4.00	3.25	4.17	3.00	.	0.00
293	PI297849	4.00	3.75	4.00	3.83	1.50	.
294	PI298547	4.00	3.50	4.17	3.17	3.00	0.00
295	PI304919	3.33	3.88	3.67	4.00	.	1.00
296	PI306530	3.33	3.13	3.25	3.00	0.00	0.00
297	PI306570	3.50	3.88	4.00	4.17	0.00	1.00
298	PI306578	3.83	3.50	3.83	3.50	.	0.00
299	PI306646	3.67	3.50	3.83	3.17	0.00	0.00
300	PI306657	2.83	2.67	2.67	3.67	0.00	1.00
301	PI306658	4.00	3.50	3.33	2.33	0.00	0.00
302	PI313096	3.67	2.50	3.17	1.67	.	0.00
303	PI316083	3.83	3.88	3.17	3.67	3.00	.
304	PI316092	2.50	2.50	3.50	4.17	0.00	1.00
305	PI316096	3.33	3.13	3.00	3.67	0.50	1.00
306	PI320097	3.83	4.33	3.83	3.00	2.00	0.00
307	PI320114	4.33	4.38	3.33	3.67	3.00	0.00
308	PI321699	3.17	3.75	3.33	3.00	3.00	0.00
309	PI324480	4.17	4.50	4.50	3.17	3.00	0.00
310	PI324482	3.00	3.13	4.25	2.67	3.00	0.00
311	PI324517	4.50	4.00	4.17	4.33	0.00	1.00
312	PI324518	3.33	3.50	3.17	4.00	0.00	1.00
313	PI324519	4.00	3.88	4.33	4.17	0.00	1.00
314	PI324850	4.17	4.00	4.00	3.00	.	0.00
315	PI324927	3.83	4.13	4.33	3.25	.	0.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
316	PI324928	4.00	4.13	3.83	4.00	3.00	0.00
317	PI325850	4.00	3.88	4.00	3.67	3.00	0.00
318	PI330529	3.50	3.00	3.50	1.83	.	0.00
319	PI330546	3.17	2.63	3.83	2.83	0.00	1.00
320	PI330547	.	.	.	.	.	.
321	PI337647	.	.	.	.	.	.
322	PI338529	4.33	3.38	4.50	3.33	0.00	0.00
323	PI341729	3.00	3.50	3.50	3.83	3.00	1.00
324	PI342646	4.33	3.83	4.17	4.33	0.00	1.00
325	PI342647	4.00	4.25	4.17	3.67	0.50	1.00
326	PI347152	3.67	3.67	3.67	3.50	.	1.00
327	PI347157	.	.	.	.	.	.
328	PI350145	3.00	2.00	2.33	1.50	0.00	0.00
329	PI367224	3.50	3.25	3.83	3.17	0.00	1.00
330	PI367227	2.83	3.38	3.33	3.67	0.00	0.00
331	PI367240	3.25	3.38	4.00	4.33	0.00	1.00
332	PI371820	2.33	3.17	3.67	3.00	0.00	1.00
333	PI371824	4.67	4.50	4.50	3.67	2.00	1.00
334	PI372445	4.17	4.38	4.33	3.50	0.00	0.00
335	PI372448	2.33	2.13	1.83	1.67	0.00	0.00
336	PI372451	3.33	3.63	3.83	3.17	3.00	0.00
337	PI372452	4.00	3.63	3.83	3.00	3.00	0.00
338	PI374482	2.17	2.75	3.33	2.67	.	0.00
339	PI381997	4.17	4.25	3.67	3.67	0.00	1.00
340	PI383416	2.17	2.63	3.17	1.83	0.00	0.00
341	PI383914	4.83	4.13	4.17	3.17	0.00	0.00
342	PI383915	3.50	2.88	3.75	3.17	0.00	0.00
343	PI383916	4.67	3.25	4.33	4.00	0.00	1.00
344	PI384037	3.17	3.38	3.50	4.33	0.00	0.00
345	PI384038	3.83	3.88	4.00	4.00	0.00	1.00
346	PI384044	2.83	2.63	2.83	2.67	0.00	0.00
347	PI384045	4.33	4.17	4.17	3.17	3.00	1.00
348	PI384244	4.83	4.50	4.33	4.00	3.00	0.00
349	PI384391	4.17	3.50	3.83	3.33	0.00	0.00
350	PI384392	3.83	3.25	4.17	2.50	0.00	0.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
351	PI384393	3.00	2.88	3.00	3.50	0.00	0.00
352	PI384394	3.17	2.63	2.83	3.00	0.00	0.00
353	PI384401	.	.	.	.	.	.
354	PI387263	4.50	3.67	3.50	2.33	3.00	0.00
355	PI387346	3.50	4.00	3.33	3.00	3.00	0.00
356	PI387635	3.83	3.33	3.67	3.33	3.00	0.00
357	PI388035	3.75	3.50	3.00	3.00	3.00	0.00
358	PI388133	3.17	3.50	3.50	3.17	2.00	0.00
359	PI390208	.	.	.	.	.	.
360	PI390348	.	.	.	.	.	.
361	PI405906	2.33	2.00	3.17	4.00	0.00	1.00
362	PI405907	4.33	2.63	3.67	2.00	0.00	0.00
363	PI412984	4.50	3.25	4.25	3.33	.	0.00
364	PI429317	4.17	3.69	4.00	3.33	0.00	0.00
365	PI429324	3.00	3.13	3.17	3.33	0.75	0.00
366	PI447421	3.33	2.63	3.00	3.33	0.00	0.00
367	PI462105	3.50	3.25	2.83	2.00	2.00	0.00
368	PI462110	3.83	4.00	3.83	3.83	.	0.00
369	PI469013	4.00	2.50	3.83	4.00	0.00	1.00
370	PI470826	3.50	2.75	3.50	3.33	3.00	0.00
371	PI470833	3.33	3.13	2.83	4.00	0.00	0.00
372	PI470834	2.67	2.63	3.33	3.50	0.00	0.00
373	PI470843	3.00	2.63	3.17	2.83	0.00	1.00
374	PI470868	3.00	2.50	3.00	2.83	1.00	1.00
375	PI470875	2.83	2.75	3.17	3.33	0.00	1.00
376	PI470893	2.50	2.38	2.83	3.00	0.00	0.00
377	PI470903	2.50	3.13	3.83	3.00	0.00	1.00
378	PI470904	3.33	3.50	4.17	3.00	3.00	0.00
379	PI477867	3.83	3.63	3.33	1.67	0.00	1.00
380	PI477881	4.33	4.00	4.33	3.33	0.00	0.00
381	PI477895	3.67	3.00	3.33	1.75	0.00	1.00
382	PI477911	4.00	4.00	4.17	4.33	.	1.00
383	PI478304	2.17	2.88	3.17	1.83	.	0.00
384	PI478427	3.17	2.13	2.67	2.83	0.00	0.00
385	PI479941	4.50	4.00	4.17	4.00	.	0.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
386	PI479983	3.75	3.63	3.83	3.83	3.00	0.00
387	PI480017	4.33	4.25	3.67	3.50	3.00	0.00
388	PI480139	3.50	3.13	3.33	3.33	3.00	0.00
389	PI481580	4.17	3.75	4.33	4.25	0.50	1.00
390	PI481584	3.33	3.63	4.00	4.67	0.00	1.00
391	PI481585	2.83	3.50	3.33	4.33	.	1.00
392	PI487290	4.00	2.63	3.67	3.50	.	1.00
393	PI497927	2.67	2.63	2.33	2.00	0.00	0.00
394	PI499974	4.67	4.13	4.00	3.50	1.00	1.00
395	PI510694	2.83	2.63	2.83	2.83	1.00	0.00
396	PI519170	3.00	3.13	3.50	3.17	0.00	0.00
397	PI519171	3.00	2.83	2.83	3.50	.	1.00
398	PI519174	3.50	3.25	3.17	3.50	0.00	1.00
399	PI519445	2.83	3.38	3.33	3.17	3.00	0.00
400	PI519453	3.50	2.83	3.83	3.25	0.00	0.00
401	PI519544	3.67	3.63	3.00	3.00	1.75	0.00
402	PI519556	2.33	2.13	3.00	2.50	0.00	0.00
403	PI519557	2.00	2.25	2.75	2.67	0.00	0.00
404	PI519559	2.50	2.75	2.83	2.50	3.00	0.00
405	PI519566	3.50	2.88	3.33	3.67	0.00	0.00
406	PI519567	1.75	1.83	2.83	1.25	0.00	.
407	PI519598	4.83	3.88	4.50	3.83	0.00	0.00
408	PI519619	2.67	3.38	2.33	2.33	0.00	0.00
409	PI519620	3.00	3.25	3.50	3.00	0.00	0.00
410	PI519639	3.33	3.50	3.33	3.00	0.00	0.00
411	PI519642	1.75	2.13	1.67	2.67	0.00	0.00
412	PI519732	4.00	3.38	3.67	3.83	3.00	0.00
413	PI519750	2.00	2.50	2.17	1.50	3.00	0.00
414	PI519753	2.75	2.38	3.67	3.83	0.00	1.00
415	PI519759	2.17	1.63	2.00	1.67	3.00	0.00
416	PI519777	1.83	2.63	3.00	2.83	0.50	0.00
417	PI519811	3.00	2.38	3.33	3.00	0.00	0.00
418	PI519832	3.33	3.17	3.17	2.00	0.00	0.00
419	PI519862	2.50	1.75	2.50	1.33	0.50	0.00
420	PI519887	3.25	3.13	3.67	3.33	2.00	0.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
421	PI520023	2.50	2.13	2.83	2.83	0.00	0.00
422	PI520027	2.83	2.50	3.50	3.00	0.50	1.00
423	PI520029	2.00	2.17	2.50	2.00	3.00	0.00
424	PI520044	2.00	1.75	2.33	1.83	0.25	0.00
425	PI520092	4.00	3.38	4.00	3.50	0.75	1.00
426	PI520165	3.17	3.25	3.17	3.50	1.00	0.00
427	PI520299	2.17	2.50	2.67	2.33	3.00	0.00
428	PI520362	2.17	2.63	3.67	3.17	0.00	1.00
429	PI520392	2.00	1.17	1.00	1.50	0.00	0.00
430	PI520406	2.83	3.00	3.17	2.17	0.00	0.00
431	PI520407	3.67	3.13	3.67	2.75	0.00	0.00
432	PI520413	4.00	3.17	4.50	2.50	3.00	0.00
433	PI520416	2.33	3.33	2.67	1.83	1.00	0.00
434	PI520518	2.33	2.38	2.33	2.33	3.00	0.00
435	PI525334	4.50	3.00	3.33	3.83	0.00	1.00
436	PI525341	4.33	3.38	3.83	4.17	0.00	1.00
437	PI525371	4.33	3.25	3.17	2.00	0.00	0.00
438	PI525395	4.00	4.38	4.17	3.17	0.00	0.00
439	PI525428	3.50	3.63	3.33	4.00	0.00	1.00
440	PI525438	3.00	2.50	3.33	3.00	0.00	0.00
441	PI532140	3.50	3.38	4.33	3.17	.	0.00
442	PI532239	4.33	4.13	3.50	3.50	0.00	0.00
443	PI532242	4.00	4.00	3.00	3.17	1.00	0.00
444	PI532288	3.50	3.38	3.17	2.67	3.00	0.00
445	PI532289	3.17	3.25	2.17	2.17	3.00	0.00
446	PI532292	3.00	2.63	2.33	2.00	1.00	0.00
447	PI534304	3.75	4.00	3.83	3.33	3.00	0.00
448	PI534343	3.50	4.25	4.00	4.50	3.00	1.00
449	PI534351	4.33	3.88	4.50	4.33	0.00	1.00
450	PI534367	4.17	4.25	3.83	4.17	0.00	1.00
451	PI534370	4.67	3.88	3.67	4.33	0.00	1.00
452	PI534471	2.50	3.00	3.33	3.00	3.00	1.00
453	PI534501	4.00	4.13	4.00	3.33	3.00	0.00
454	PI537310	2.33	1.75	2.50	1.50	3.00	0.00
455	PI565264	2.00	1.75	1.50	2.50	0.00	1.00
456	PI585023	3.83	3.63	3.33	3.17	3.00	.

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
457	PI623461	3.83	4.25	4.25	4.00	.	1.00
458	PI623709	3.50	4.25	4.00	3.33	0.50	1.00
459	PI623914	3.00	4.38	3.67	4.17	0.50	1.00
460	PI623926	3.00	3.50	3.00	3.83	0.00	1.00
461	PI623957	3.83	4.00	3.83	3.67	.	1.00
462	PI623997	3.17	4.00	3.50	2.83	0.00	0.00
463	PI624030	4.17	4.00	4.00	4.33	3.00	1.00
464	PI624052	3.75	4.25	3.83	3.67	.	1.00
465	PI624091	3.83	4.13	4.00	3.67	3.00	1.00
466	PI624127	3.67	3.63	4.00	4.17	1.00	1.00
467	PI624170	4.17	4.38	4.17	3.00	0.00	0.00
468	PI624210	4.00	3.50	4.00	3.83	3.00	1.00
469	PI624388	4.50	4.75	4.00	3.67	3.00	0.00
470	PI624394	3.50	3.63	3.83	3.33	3.00	0.00
471	PI624407	2.83	3.75	3.25	3.67	0.00	1.00
472	PI624428	3.83	4.38	4.00	3.83	3.00	1.00
473	PI624435	3.83	4.50	3.83	3.17	3.00	0.00
474	PI624456	4.33	4.13	4.50	3.25	3.00	0.00
475	PI624467	3.83	4.00	3.00	4.00	2.00	1.00
476	PI624501	2.50	2.75	2.83	3.83	0.00	1.00
477	PI624506	4.33	4.00	4.17	3.33	.	0.00
478	PI624516	3.67	3.50	3.83	2.50	0.00	0.00
479	PI624528	3.33	3.88	3.67	3.67	3.00	1.00
480	PI624529	4.00	4.25	4.17	3.50	3.00	1.00
481	PI624557	3.50	3.50	3.83	2.75	0.00	0.00
482	PI624568	3.67	4.25	4.17	3.33	3.00	1.00
483	PI624669	4.17	4.00	3.67	3.83	.	0.00
484	PI624694	3.17	3.50	3.83	3.75	0.00	1.00
485	PI624695	3.00	3.25	3.50	3.83	0.00	1.00
486	PI624731	2.67	2.50	2.33	2.33	0.00	0.00
487	PI624769	3.33	4.38	4.17	4.17	3.00	1.00
488	PI624801	4.50	3.63	4.00	4.00	3.00	1.00
489	PI624829	4.17	4.63	3.83	3.67	3.00	0.00
490	PI624850	4.17	2.50	3.25	3.83	0.00	1.00
491	PI624854	4.33	4.50	4.00	3.67	2.00	1.00

TCAP line	Line	Pti2	86-124	331-9	DW5	Ptr ToxA	Ptr ToxB
492	PI625273	3.67	3.63	3.50	3.33	3.00	0.00
493	PI625361	2.00	3.25	3.17	4.00	0.00	1.00
494	PI626482	.	.	.	.	.	.
495	PI626483	3.50	3.75	3.67	3.83	0.00	1.00
496	PI634315	2.33	2.13	3.50	1.83	0.00	0.00
497	PI636501	3.17	2.75	2.17	2.50	2.00	0.00

APPENDIX B: PHENOTYPIC DATA FOR THE TAN SPOT CAUSED BY PTI2 (RACE 1), 86-124 (RACE 2), 331-9 (RACE 3), DW5 (RACE 5), AR CROSSB10 AND NE, PTR TOXA ON BEN × PI41025 RECOMBINANT INBRED LINE POPULATION

BP025 line	Pti2	86-124	331-9	DW5	AR CrossB10	Ptr ToxA
1	2.83	2.50	4.00	3.50	3.33	3.00
2	1.17	1.00	1.67	1.00	1.33	3.00
3	2.83	3.17	3.00	2.67	3.17	0.00
4	3.33	3.33	3.00	4.00	4.00	3.00
5	2.00	1.50	2.83	1.17	1.50	3.00
6	2.33	1.67	2.83	2.50	2.67	0.00
7	4.00	3.83	3.83	3.67	2.83	0.00
8	1.83	2.50	2.33	1.83	3.33	3.00
9	2.50	2.67	3.83	3.00	3.00	3.00
10	3.50	3.83	4.00	3.83	3.83	3.00
11	1.33	1.00	2.00	1.33	1.83	3.00
12	4.50	4.00	4.50	4.00	3.33	0.00
13	3.33	3.17	3.00	4.33	3.00	0.00
14	3.00	4.17	3.67	2.67	3.33	0.00
15	3.50	2.67	1.67	3.17	2.50	3.00
16	2.33	1.33	1.17	1.75	1.67	0.00
17	2.33	1.50	3.00	2.00	1.67	0.00
18	3.33	3.00	2.67	3.00	2.67	0.00
19	3.83	4.67	4.50	4.00	4.00	3.00
20	2.50	2.67	2.50	3.67	2.33	0.00
21	3.67	3.67	3.83	3.17	3.50	3.00
22	3.17	3.67	1.83	2.67	2.67	0.00
23	4.00	4.50	4.33	3.83	3.83	3.00
24	3.67	3.50	3.67	3.83	3.67	3.00
25	2.50	2.83	3.17	3.50	3.33	0.00
26	4.17	3.00	3.83	3.33	3.33	3.00
27	4.17	3.67	4.33	3.50	3.67	0.00
28	4.00	3.67	3.83	4.00	3.50	3.00

BP025 line	Pti2	86-124	331-9	DW5	AR CrossB10	Ptr ToxA
29	2.33	3.17	2.83	2.00	3.67	0.00
30	2.33	2.33	1.67	2.00	3.83	0.00
31	4.17	3.50	3.83	3.33	3.33	3.00
32	3.67	3.50	2.67	2.50	3.67	3.00
33	3.33	3.33	3.50	2.67	3.33	0.00
34	1.67	2.67	1.83	1.00	2.33	3.00
35	3.00	3.00	2.33	2.50	2.50	3.00
36	4.17	4.67	3.67	4.00	4.33	0.00
37	3.50	3.67	3.00	3.67	3.67	3.00
38	4.33	4.33	3.50	3.83	4.00	0.00
39	3.00	3.00	.	3.00	4.17	0.00
40	1.83	1.33	1.33	2.33	2.00	3.00
41	4.33	3.67	3.50	3.50	3.50	3.00
42	3.33	2.00	2.00	3.27	2.50	3.00
43	.	.	.	.	.	.
44	1.00	1.00	1.17	1.17	1.00	3.00
45	3.00	3.83	3.50	3.50	3.33	0.00
46	2.67	1.00	3.67	1.67	2.83	3.00
47	3.67	3.83	3.50	4.33	4.00	0.00
48	3.17	4.50	4.00	4.17	4.17	3.00
49	2.83	2.00	2.17	2.00	2.17	0.00
50	3.17	3.50	3.00	2.83	3.17	3.00
51	1.83	2.17	1.67	2.17	2.83	3.00
52	4.00	3.50	4.17	3.50	4.33	0.00
53	2.83	3.00	3.00	2.50	3.17	3.00
54	3.00	3.67	3.17	4.00	2.67	0.00
55	3.50	4.50	3.75	3.67	.	.
56	2.17	1.67	1.50	1.83	1.67	0.00
57	3.33	3.83	2.83	3.83	3.17	0.00
58	2.00	3.00	2.00	1.33	2.67	3.00
59	4.83	4.83	4.17	3.83	3.33	0.00
60	2.83	3.33	2.33	3.83	2.50	3.00
61	3.83	3.50	4.17	3.17	3.83	3.00
62	2.67	2.83	1.67	2.67	2.83	3.00
63	2.83	3.00	2.33	1.83	3.00	0.00

BP025 line	Pti2	86-124	331-9	DW5	AR CrossB10	Ptr ToxA
64	3.17	3.50	2.83	2.17	2.33	3.00
65	3.17	2.83	2.50	4.17	3.67	3.00
66	3.67	4.00	4.33	3.67	3.83	0.00
67	2.50	3.33	2.33	3.00	3.33	0.00
68	3.33	4.67	3.83	3.33	3.67	3.00
69	4.33	4.33	4.17	4.00	4.00	0.00
70	3.50	2.50	3.00	2.67	2.33	0.00
71	3.83	3.67	4.00	3.17	3.00	0.00
72	3.83	3.67	3.67	3.17	3.33	3.00
73	2.67	2.67	2.67	2.33	2.83	3.00
74	2.67	2.00	2.83	3.17	3.50	0.00
75	1.50	1.33	2.67	1.00	2.67	0.00
76	3.33	3.50	3.83	2.67	2.67	3.00
77	2.67	3.00	2.17	2.67	3.33	3.00
78	2.83	3.50	3.83	3.33	4.33	3.00
79	4.00	3.50	3.17	4.00	3.67	3.00
80	4.00	4.17	2.17	2.83	3.83	3.00
81	2.50	2.67	2.17	2.50	2.33	0.00
82	3.33	2.50	3.17	3.33	3.17	3.00
83	4.17	3.67	3.83	4.17	4.00	3.00
84	.	.	.	1.00	.	.
85	3.33	2.50	2.67	3.00	3.00	3.00
86	3.17	4.17	3.67	3.50	3.83	3.00
87	2.33	1.83	2.33	2.50	3.17	3.00
88	2.00	1.83	2.33	2.50	2.17	3.00
89	4.00	3.00	2.67	4.00	3.83	3.00
90	4.17	4.00	4.00	3.33	3.83	3.00
91	1.75	2.00	2.00	1.83	2.17	3.00
92	2.17	3.67	3.50	3.50	3.67	0.00
93	3.33	3.67	3.83	3.00	3.83	0.00
94	3.33	3.83	3.83	3.33	4.00	0.00
95	4.33	3.83	4.33	3.67	3.50	3.00
96	2.83	3.83	3.33	2.83	4.33	3.00
97	3.33	3.67	3.33	4.17	3.67	3.00

BP025 line	Pti2	86-124	331-9	DW5	AR CrossB10	Ptr ToxA
98	1.83	2.67	3.00	2.17	3.67	3.00
99	2.00	2.67	.	2.50	3.67	3.00
100	3.50	3.17	3.50	2.83	3.67	0.00
101	3.67	4.00	3.33	3.33	4.17	3.00
102	2.67	3.00	3.33	2.33	2.67	3.00
103	2.83	3.33	4.17	3.17	4.00	0.00
104	4.17	3.67	3.83	3.83	3.83	3.00
105	3.50	3.33	2.83	3.17	4.50	3.00
106	2.83	2.33	2.67	1.67	2.83	3.00
107	3.17	2.67	4.00	2.50	3.33	3.00
108	2.83	3.00	2.17	2.00	3.50	3.00
109	4.17	4.17	3.50	3.17	3.67	0.00
110	3.83	3.83	3.33	3.67	3.50	0.00
111	2.33	1.17	2.17	2.50	2.33	3.00
112	2.67	3.00	3.00	3.83	3.83	3.00
113	3.33	3.50	3.17	3.00	3.50	3.00
114	3.00	3.00	2.33	2.67	3.67	0.00
115	3.67	3.00	2.50	4.00	3.83	0.00
116	2.17	2.17	2.33	2.17	2.50	0.00
117	3.33	2.67	3.33	3.33	3.50	3.00
118	3.67	4.33	3.67	3.67	4.33	0.00
119	2.75	2.83	2.17	2.50	2.67	0.00
120	3.83	3.33	2.83	3.67	3.33	3.00
121	4.17	3.00	4.00	3.83	3.50	0.00
122	3.67	2.83	3.67	3.50	2.50	0.00
123	2.67	1.50	3.00	2.00	2.00	3.00
124	3.50	2.17	2.83	3.17	3.83	3.00
125	2.00	1.67	2.00	2.67	2.17	3.00
126	1.75	1.67	3.83	1.00	1.67	3.00
127	3.33	3.67	3.67	3.50	3.00	0.00
128	.	.	.	1.00	.	.
129	2.67	2.83	2.83	3.17	3.00	0.00
130	2.67	2.00	3.00	3.17	2.50	0.00
131	1.33	2.33	1.67	2.17	3.17	3.00
132	3.50	4.33	4.17	3.83	3.83	0.00

BP025 line	Pti2	86-124	331-9	DW5	AR CrossB10	Ptr ToxA
133	4.17	3.50	4.00	3.67	4.17	3.00
134	2.67	2.83	2.83	3.17	3.33	3.00
135	3.83	2.83	2.17	2.67	2.83	0.00
136	2.50	2.33	3.00	1.33	2.50	3.00
137	3.33	3.67	4.17	3.67	3.33	3.00
138	3.17	2.50	2.67	2.17	1.50	0.00
139	4.00	3.33	3.50	3.33	3.67	0.00
140	3.67	2.67	3.83	3.67	2.83	0.00
141	2.83	3.00	3.25	3.00	3.50	3.00
142	3.00	3.00	3.50	3.67	4.00	0.00
143	1.25	1.33	1.33	1.75	2.00	3.00
144	.	2.50	.	2.50	.	.
145	1.67	1.17	2.00	1.83	1.67	0.00
146	3.83	4.17	3.33	3.33	3.33	3.00
147	3.50	3.67	3.67	1.67	2.67	0.00
148	3.33	3.83	3.83	2.50	3.17	0.00
149	3.17	3.67	2.67	3.00	3.17	3.00
150	2.67	2.67	2.67	2.17	3.00	3.00
151	3.67	3.67	3.50	2.83	3.83	0.00
152	1.83	1.33	1.17	1.50	1.83	0.00
153	4.00	3.00	3.33	3.67	3.83	3.00
154	3.50	3.50	2.83	3.50	3.33	0.00
155	3.00	2.50	3.33	3.50	3.83	3.00
156	4.17	3.67	3.83	3.17	4.00	0.00
157	2.00	2.00	1.67	1.00	2.17	3.00
158	.	.	.	2.50	.	.
159	.	.	.	.	.	.
160	3.83	3.67	3.67	4.00	3.83	0.00
161	.	2.00	.	.	1.50	.
162	2.00	3.17	3.83	2.50	4.17	0.00
163	3.33	3.17	3.83	3.33	3.67	3.00
164	3.50	3.00	3.67	2.33	3.00	3.00
165	2.67	3.00	2.50	3.00	2.33	0.00
166	3.83	3.33	3.33	3.67	3.50	0.00
167	3.83	3.67	3.00	3.83	4.17	0.00

BP025 line	Pti2	86-124	331-9	DW5	AR CrossB10	Ptr ToxA
168	3.33	3.17	2.00	2.83	3.17	3.00
169	1.50	2.50	2.00	3.00	2.33	3.00
170	4.17	4.50	4.00	3.33	4.33	0.00
171	2.17	1.67	2.67	1.83	2.33	3.00
172	3.33	3.50	3.50	3.33	4.00	3.00
173	3.33	2.00	2.83	3.17	3.33	0.00
174	1.67	1.33	2.33	2.00	2.33	0.00
175	1.67	1.50	1.83	1.67	1.50	3.00
176	2.83	3.67	3.17	2.67	3.50	0.00
177	2.67	3.00	3.00	3.33	2.67	3.00
178	2.83	2.50	2.33	2.67	3.00	0.00
179	4.00	3.33	2.33	3.33	3.33	3.00
180	3.83	2.67	2.17	2.83	3.33	0.00
181	2.50	3.33	2.33	3.00	3.17	0.00
182	2.33	2.33	2.67	2.50	2.50	0.00
183	3.50	3.33	2.83	3.17	2.83	3.00
184	2.50	2.50	2.67	1.83	1.67	3.00
185	3.00	2.83	2.83	4.17	3.17	0.00
186	3.75	3.00	3.17	3.50	3.50	3.00
187	3.33	3.33	3.83	3.50	2.83	3.00
188	2.50	3.00	3.00	2.83	3.33	0.00
189	3.00	2.67	2.67	2.67	2.17	0.00
190	1.67	1.17	1.67	1.33	2.00	3.00
191	3.50	3.83	2.50	3.50	3.83	3.00
192	4.17	3.17	3.50	4.00	3.83	0.00
193	1.75	3.00	2.50	1.50	3.50	3.00
194	2.75	3.67	3.50	2.83	4.50	3.00
195	3.33	3.25	3.83	4.00	3.33	3.00
196	3.17	3.17	2.67	3.00	3.67	3.00
197	2.83	2.83	2.67	2.50	4.00	3.00
198	2.67	2.33	1.83	1.67	1.50	3.00
199	4.00	4.00	4.00	4.17	3.67	0.00
200	3.00	2.33	2.33	2.17	3.67	0.00