GENETIC MAPPING OF QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO WHEAT

TAN SPOT USING TWO BI-PARENTAL POPULATIONS

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ABSTRACT

Tan spot, caused by *Pyrenophora tritici-repentis* (*Ptr*), is an economically important disease on both common wheat (*Triticum aestivum* L.) and durum (*T. turgidum* L. ssp. *durum*). Genetics of resistance to tan spot is complicated and needs to be further investigated for breeding cultivars with more complete resistance. The objective of this study was to map and characterize genetic resistance in two wheat bi-parental popuations. In Louise × Penawawa population, four quantative trait loci (QTL) were identified and the major race-nonspecific QTL, designated as *QTs.zhl-3BL*, was shown to have epistatis and additive effect on Ptr ToxA-*Tsn1*, Ptr ToxC-*Tsc1* interactions, respectively. Nine QTL were identified in the Lebsock × PI 94749 population with three likely being novel. This work improves our understanding of genetic resistance to tan spot and provides important tools for breeding resistant cultivars.

Finally, and the most importantly I would like to thank my parents Mr. Lalith Shantha Kariyawasam and Mrs. Chandani De Silva and my brother Mr. Dilshan Kariyawasam for their continuous support, encouragement and love. I dedicate this thesis to my loving wife Madushika Samarasekara to expresses my deepest gratitude for love, support, dedication and understanding she provided throughout this study.

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GENERAL INTRODUCTION

Wheat is one of the most important staple food crops for many countries throughout the history of mankind. Tan spot, caused by the fungus *Pyrenophora tritici-repentis*, is a common foliar disease on both common wheat (*Triticum aestivum* L.) and durum (*Triticum turgidum* L. ssp. *durum*) worldwide. Over the last century, the disease has evolved from a minor problem into a major disease in many major wheat-growing regions, including the northern Great Plains of the United States (Faris et al. 2013). Under favorable conditions, tan spot can cause yield losses up to 50% and adversely affect grain quality on susceptible cultivars (Rees et al. 1982; Schilder and Bergstrom 1994; Fernandez et al. 1997). Although crop rotation and fungicide application can be used to manage tan spot disease, utilization of genetic resistance is the most preferred way of combating this disease.

In the last 50 years, great progress has been made in our understanding of host resistance to tan spot and the biology of host-pathogen interactions. The fungal pathogen was shown to produce host selective toxins which now referred as necrotrophic effector (NE). The wheat-*Ptr* pathosystem has been known to follow an inverse gene-for-gene model where NEs produced by the pathogen are recognized specifically by the product of the corresponding dominant susceptibility genes that result in a compatible interaction and lead to the development of disease symptoms (Wolpert et al. 2002). Therefore, genetic resistance in this system is due to the absence of dominant susceptibility genes. Up to now, three such interactions have been identified, Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1* (Ciuffetti et al 2010; Faris et al. 2013 for review). Because these NE-host gene interactions play an important role in disease, isolates of the fungal pathogen have been classified into eight races solely based on the presence or absence of these NEs (Lamari and Bernier 1989a; reviewed in Strelkov and Lamari 2003;

reviewed in Lamari and Strelkov 2010). However, more studies have strongly indicated that the disease system is not just based on these three NEs-host sensitivity interactions. Friesen et al. (2003) found that a *Tsn1* mutant still developed strong disease after being inoculated with a race 2 isolate which is known to produce only Ptr ToxA. A number of studies have identified major resistance genes and mapped them into chromosome positions other than those of three sensitivity genes. Ali et al. (2010) identified some isolates that did not produce Ptr ToxA, but caused strong disease on 'Glenlea' which is the Ptr ToxA differential line. QTL mapping for resistance to tan spot also revealed other genomic regions controlling tan spot resistance. In particular, some of these regions confer resistance to all races, referred to as race-nonspecific resistance (Faris and Friesen 2005; Chu et al. 2008). All these indicate that genetic resistance/susceptibility to tan spot and host-pathogen interaction in this pathosystem is complicated and needs to be characterized in a wide range of wheat backgrounds. Race-nonspecific resistance is particularly interesting to breeding programs because it confers resistance to all races. Therefore, one of my research objectives was to identify race-nonspecific resistance QTL and characterize their relationships to disease susceptibility induced by the known NE-host sensitivity gene interactions. Furthermore, most genetic studies were done on the hexaploid wheat backgrounds. The second objective was to map genomic regions governing resistance to tan spot in tetraploid wheat using a bi-parental population.

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<center>**LITERATURE REVIEW**</center>

<center>**Wheat classification and evolution**</center>

Classification

Current classification system places all wheat species in the genus *Triticum* of family Poaceae. The Poaceae is a large family including many tribes and *Triticum* species are grouped in the tribe of Triticeae (Soreng et al. 2015). Approximately 500 species of annual and perennial species are classified in Triticeae. In addition to wheat, barley, rye and triticale are also in this group; therefore, Triticeae is one of the most important groups for human kind (Lu and Ellstrand 2014).

Evolution

Currently, the group of *Triticum* and *Aegilops* consists of 13 diploid and 18 allopolyploid species (12 at tetraploid and 6 at the hexaploid level) (Feldman et al. 2012). It was estimated that a common diploid ancestor ($2n = 2x = 14$) gave rise to the earliest progenitors of *Triticum* and *Aegilops* around 3 million years ago (Faris 2014). The evolution of current durum (*T. turgidum* L. ssp. *durum*, $2n = 4x = 28$, AABB genome) and bread wheat (*T. aestivum* L., $2n = 6x = 42$, AABBDD genome) was driven by two important events of wide crosses followed by amphiploidization, both of which was believed to take place in Fertile Crescent of Middle East (Luo et al. 2007). The first wide cross and amphiploidization events took place about half million years ago between wild diploid wheat *T. uratu* Tumanian ex Gandylian ($2n = 2x = 14$, AA genome) and *Ae. speltoides* ssp. *lingustica* ($2n = 2x = 14$, SS genome) that donated A and B genome, respectively, to form tetraploid wheat *T. turgidum* ssp. *dicoccoides* (Korn.) Thell ($2n = 4x = 28$, AABB genomes) (Dvorak et al. 1993). Known as wild emmer wheat, *T. turgidium* ssp. *dicoccoides* gave rise to modern cultivated forms of tetraploid wheats, such as emmer wheat (*T. turgidum* ssp. *dicoccum*) and durum (*T. turgidum*. ssp. *durum*) through human domestication

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process (Charmet 2011). It is around 8000 years ago that the second cross and amphiploidization event occurred between a *T. turgdium* (AABB) sub species and the diploid goatgrass *Ae. tauschii* Coss. (2n = 2x = 14, DD genome). This event likely led to the formation of the hexaploid wheat *T. aestivum* ssp. *spelta* (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 include brittle rachis, tenacious glume and free-threshing (Faris 2014).

Wheat production in the world, US and ND

World Production

Wheat is one of the staple food crop in the world and bread wheat alone accounts for 20% of the daily caloric intake for human (Faris et al. 2014). In 2014, wheat was cultivated over 223 million hectares and the total production was over 725 million tons (Economic Research Service, USDA, updated on 8/12/2015). As a major wheat producer in the world, the United States produced 55 million tons of wheat in 2014, ranking the 5th in total wheat production, only behind European Union, China, India and Russia (https://www.worldwheatproduction.com/). In 2014, wheat was grown over 56.8 million acre land in the US across 42 states and ranked as the third behind corn and soybean in term of planted acreage (Economic Research Service, USDA, updated on 8/12/2015).

US 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. These types of wheat are grown in different regions of the US and have distinctive morphological characteristics and quality traits as well as different usages (North Dakota Wheat Commission, updated on 2015). In general, HRW wheat accounted for 40% of the total wheat

production in US, followed by HRS, SRW, HW and durum wheat at 20%, 15-20%, 10-15% and 3-5%, respectively (Economic Research Service, USDA, updated on 8/12/2015).

Wheat is one of the most commonly grown commodities in the state of North Dakota covering 25% of the total land area. North Dakota produces mainly HRS and durum wheat and is the No.1 producer in US for these two types of wheat. In term of total wheat production, ND has been ranked as No.1 in the US in 2014, surpassing Kansas which is the major producer of HRW wheat in US. In 2013, North Dakota produced 273 million bushels of wheat with 78% being HRS wheat, 16% being durum wheat and 6% being winter wheat (North Dakota Wheat Commission, 2013). It was estimated that wheat industry usually generates 5 to 7 billion dollars of economic gain annually in ND (North Dakota Wheat Commission, 2013).

Tan spot

History

The tan spot fungus was initially identified as a saprophyte on grass in 1850s and was not considered as a pathogen of wheat and grasses for a long time. Diedicke did the first isolation of the fungus from *Agropyron repens*, a grass species and named it as *Pleospora trichostoma* which was renamed by him as *Pleospora tritici-repentis* (Hosford 1982; De Wolf et al. 1998). Drechsler (1923) identified and renamed the fungus as *Pyrenophora tritici-repentis* which has been used since then. Nisikoda (1928) isolated a similar fungus on wheat in Japan and described it as *Helminthosporium tritici-vulgaris* for the conidia state. Ito (1930) established the group of *Drechslera* and renamed the conidial state of the fungus to *D. tritici-vulgaris*. Shoemaker (1962) found that *D. tritici-vulgaris* is the same as *D. tritici-repentis*. *Pyrenophora trichostoma* has also been used to describe the fungus, but it was considered as synonym to *P. tritici-vulgaris* and *P. tritici-repentis* (Hosford 1971). It has been widely accepted that the sexual stage of the tan spot

fungus is *Pyrenophora tritici-repentis* (Died.) Drechs. and the asexual stage is *Drechslera tritici-repentis* (Died.) Shoem (De Wolf et al. 1998).

As mentioned above, the fungus was originally isolated from grasses and was not considered as a wheat pathogen. From the early 1930s, the fungus was readily identified from wheat and known to cause disease on wheat (Conners 1937; Mitra 1934). The disease was originally called yellow spot or yellow leaf blotch mainly due to the formation of the chlorotic symptoms (Conners 1940; Hosford 1971; Friesen et al. 2006). After 1940s, the reports of tan spot outbreaks was more common in many places of the world and the disease was observed to associate with the development of light brown and tan-colored necrotic lesions in addition to chlorosis symptom on leaves (Barrus 1942; Johnson 1942; Hosford 1971; Friesen et al. 2006). The disease might gain the name of "tan spot" at that time because of this severe form of symptoms. A recent study has strongly suggested that the fungal pathogen acquired the *ToxA* gene from another wheat fungal pathogen *Parastagonospora nodorum* which makes the fungus to cause the necrosis symptoms and more virulent (Friesen et al. 2006). By 1980s, the severe epidemics of tan spot have been reported in many countries around the world and the disease had evolved into a major disease in many wheat growing areas (Hosford 1982). The increase in disease incidence and severity appears to have coincided with the introduction of no-tillage farming practices. This farming practices has the intention to retain soil moisture, organic compounds and other beneficial microbes, but unintentionally increase the inoculum level of stubble-born disease, such as tan spot.

Distribution

The disease was first reported on wheat in Japan in 1928 (De Wolf et al. 1998) and the earliest reports of the disease were also found in Canada, India and USA during the 1930s to

1940s (Conners 1937; Mitra 1934; Sprague 1950). Later, tan spot has been reported as an important disease in many countries during 1970s-80s, including Australia (Valder and Shaw 1952), Belgium (Maraite and Weyns 1982), Brazil (Mehta and Almeida 1977), Canada (Tekauz 1976; Tekauz et al. 1983), India (Misra and Singh 1972), Mexico (Gilchrist et al. 1984), the U.S.A. (Hosford 1971). By 2003, tan spot had become a worldwide disease distributing in almost all major wheat producing countries (Strelkov and Lamari 2003). Currently, tan spot was considered as one of economically important disease in several countries and regions, for example, the Northern Great Plain of the US, the western Canada regions, and Australia. In North Dakota, it is a very common disease and has been ranked as No.1 disease in wheat disease surveys of last several years (NDSU, extension service https://www.ag.ndsu.edu/ndipm).

Economic importance

Tan spot is able to cause significant yield losses which has been documented in many studies ranging from 5 to 50% (Rees and Platz 1982; Ackermann 1987; De Wolf et al. 1998). Studies have shown that four major factors determines the yield lost due to tan spot, including amount of primary inoculum (Rees and Platz 1982), post-inoculation wet period (Hosford and Busch 1974), host genotype (Raymond et al. 1985) and growth stage of the wheat plant at infection (Shabeer and Bockus 1988). Rees and Platz (1983) showed that disease occurring at the seedling stage caused lesser yield loss than infection taking place after the jointing stage. Later, Shabeer and Bockus (1988) revealed that initial infection on mature plants at booting and flowering stage caused more yield losses compared to the infection at other growth stages. Tan spot causes yield losses by reducing kernel weight, number of grain per head, number of tillers grain size, leaf photosynthesis area, as well as delaying crop development (Rees and Platz 1983; Shabeer and Bockus 1988). Under favorable conditions, tan spot can cause yield losses as much

as 50% (Andrew and Klomparens 1952; Rees and Platz 1982). In addition, the disease can cause

reduction in grain quality. Red smudge or pink smudge occurs when *P. tritici-repentis* infects the

wheat seed during the filling period. Pink smudge causes the discoloration of grain leading to the

downgrading of wheat grain (Schilder and Bergstorm 1994). Red smudge is usually observed in

durum wheat (Fernandez et al. 1994). Therefore, in many wheat growing areas around world,

wheat production suffers great economic losses from tan spot epidemics. In Australia, tan spot

has surpassed other foliar diseases and become the No.1 wheat foliar disease, causing an annual

loss estimated at $212 million dollars even with the use of chemical control (Murray and

Brennan 2009).   Tan spot causes yield losses around 5-15% in North Dakota, which can

translate into multiple millions of US dollar losses (Marcia McMullen, personal

communications).

Disease cycle

Tan spot is a polycyclic disease. *P. tritici-repentis* overwinters as black pinhead sized

pseudothecia and mycelium on wheat stubbles from previous growing season and they release

ascospores and conidia in spring and early summer, which act as primary inoculum. Both sexual

spores and asexual spores are dispersed by wind and infect wheat leaves in juvenile stage under

wet conditions in a wide range of temperatures (Hosford et al. 1987; reviewed in De Wolf et al.

1998). Ascospores or conidia geminated and produce appressoria by which the fungus penetrates

into epidermal cells and the whole process can take place within 12 hours under favorable

conditions (Loughman and Deverall 1986). The fungus grows intercellular hyphae and

differentiate into vesicles within epidermal cells followed by the advancement of the intercellular

hyphae from epidermal cells into mesophyll tissue (Loughman and Deverall 1986). During this

period, the fungus likely releases necrotrophic effectors (NE), also known as host selective

toxins (HSTs) that cause cellular disruption in susceptible/sensitive wheat genotypes (Lamari and Bernier 1989). The actions of NEs result in the production of typical tan spot symptoms, including necrosis and chlorosis. The symptoms on susceptible genotypes is characterized by tan-colored, elliptical necrotic lesions with small, dark brown infection site in the center and often surrounded by chlorotic halos (Weise 1987). On the infected leaves, conidia can be produced in a diurnal manner where conidiophores are produced in the dark and conidia are produced in the light (Khan 1971). The formed conidia disperse to infect upper leaves or adjacent plants with the aid of rain splashing, relative humidity and wind (Platt and Morrall 1980; McMullen and Adhikari 2009). The asexual production can be repeated multiple times during the growing season, which leads to tan spot epidemics at the local scale (Wegulo 2011). At the end of the growing season, a large number of the overwintering structure pseudothecia formed on the wheat stubbles that are left in the field.

Disease management

Culture practices

Rees and Platz (1992) stated that most tan spot epidemics occurred in the past coincides with the lack of tilling. Since 1970s, most wheat-growing areas have widely practiced no or little tilling to avoid soil erosion. This practice increased the level of initial inoculum from the previous growing season, which is believed to be a major reason for the frequent and wider epidemics of tan spot in the past 50 years. Therefore, any cultural practices to minimize the primary inoculum are effective to reduce disease incidence and severity (Bockus and Claassen 1992). Crop rotation and residue management are strongly recommended to manage the tan spot disease in agricultural practices. Both spring and durum wheat can be rotated with the broadleaf crops, such as field pea, clover, and canola. Reduction of tan spot was observed when wheat was

grown after crop sequences of soybean, corn-barley, alfalfa-alfalfa (Sutton and Vyn 1990) and

sorghum (Bockus and Claassen 1992). However, it is not recommended to rotate wheat with corn

because this could pose threats of Fusarium head blight (Wegulo 2011). Residue management

can be done to cover some straws under the ground by chisel plowing; however, this could leave

enough wheat stubbles to carry potential inoculum to the coming season (McMullen and

Adhikari 2009).

Fungicide application

Application of fungicides is another important way of managing tan spot disease.

Fungicides are available for the application in early season or late season. Early season

application is not recommended if the environmental factors are not favorable to the disease.

Protectant fungicides containing mancozeb, copper, or both of these ingredients have been used

to control this disease but the applications must be done before infection (McMullen and

Adhikari 2009). Protectant fungicides generally degrades after 7 to 10 days due to rain and

sunlight, thus at least two applications are usually required for the whole growth season.

Systemic fungicides which contain chemicals belonging to the classes of triazoles, strobilurins

and mixture of them, can also be applied in early and late season (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 (McMullen and Adhikari 2009). Furthermore, 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.

(http://www.ag.ndsu.nodak.edu/cropdisease). Farmers can decide if the fungicide application is need or not based on the prediction.

However, cultural practices and fungicide applications are not always practical and also increase production cost. Therefore, the most inexpensive and environmentally sound method of controlling tan spot is the use of genetically resistant cultivars.

Pathogen virulence and race classification

Genetic variability in pathogen virulence

Genetic variation in pathogen virulence has long been noticed in *P. tritici-repentis*. In the beginning, several quantitative parameters have been used to assess virulence of the fungus, including percent infection, lesion size (Misra and Singh 1972), percent leaf area infected (Luz and Hosford 1980), and necrotic leaf area (Schilder and Bergstorm 1990). Lamari and Bernier (1989a) proposed a method to evaluate virulence of the *P. tritici-repentis* based on lesion type, which has been widely used in genetic analysis of host resistance and pathogen virulence since then.

Tan spot symptoms mainly differentiated into two types: necrosis and chlorosis. In the landmark work done by Lamari and Bernier (1989a), a total of 92 *Ptr* isolates were classified into four pathotypes based on lesion type (necrosis and/or chlorosis) they produced on different wheat genotypes, 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. Two wheat cultivars 'Glenlea' and '6B365' was proposed in that study as differential lines to classify isolates into four pathotypes with pathotypes 1 and 2 producing necrosis on Glenlea and pathotypes 1 and 3 producing chlorosis on 6B365 (Lamari and Bernier 1989).

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Later, Lamari et al. (1995) identified a new pathotype from 39 Algerian isolates. These isolates induced chlorosis on susceptible wheat lines as pathotype 3 isolates, but yet could not induce chlorosis on differential line 6B365. Wheat lines, such as 'Katepwa' on which these Algerian isolates produced chlorosis were resistant to isolates in 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 and 'Salamouni' was included as universal resistant line.

Race classification

Using the established differential set, a total eight races have been described. 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 reaction on two differential lines with race 1 combining races 2 and 3 virulence, race 6 combining race 3 and 5 virulence and race 7 combining races 2 and 5 virulence. 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, were 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). However, other virulence races (6, 7 and 8) were identified in a limited geographic regions but have not been identified in North America (Lamari and Strelkov 2010). Now, it is known that the fungus produces necrotrophic effector to cause disease on wheat line 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, Ali et al. (2010) reported a set of isolates from Arkansas that did not conform to the current classification system indicating a new race.

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Host-pathogen interaction

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Inverse gene-for-gene model

Inverse gene-for-gene model was proposed based on the extensive studies on the disease systems involving the pathogen-produced necrotrophic effectors (Wolpert et al. 2002; Friesen et al. 2007; Ciuffeetti et al. 2010). Many NEs have been identified as low molecular weight secondary metabolites, for example victorin (Wolpert et al. 2002 for review). However, studies in wheat tan spot and Septoria nodorum blotch (SNB caused by *Parastagonospora nodorum*) revealed the involvement of proteinaceous NEs in disease, each of which is directly encoded by a fungal gene (Ciuffeetti et al. 1997; Liu et al. 2004, 2012). These proteinaceous NEs induce necrosis/chlorosis on plant by interacting with their corresponding host sensitivity genes in a gene-for-gene manner. The interaction between fungal-produced NE and host sensitivity gene induces programmed cell death in the host, similar to that from the interaction of *Avr* gene product and plant *R* gene. However, the result of NE-host sensitivity gene is susceptible reaction and the absence of either one of them leads to incompatible reaction (resistance). This is in contrast to the Flor's gene-for-gene model (1971), therefore, it has been referred to inverse gene-for-gene model. The NEs produced by necrotrophic fungal pathogens induce cell death in plant tissue and it was believed that programmed cell death of plant cells is detrimental to biotrophic pathogens, but favors necrotrophic pathogen which require dead tissue for nutrients (Wolpert et

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al. 2002; Liu et al. 2012). The wheat-*P. tritici-repentis* system has been known to follow inverse gene-for-gene model because three NE- host sensitivity gene interactions have been identified, including Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1* (Ciuffetti et al. 2010; Faris et al. 2013 for review).

Ptr ToxA- *Tsn1* interaction

Ptr ToxA was the first NE that was isolated and characterized from *Ptr* and it was also the first proteinaceous NE identified. Ptr ToxA is encoded by a single-copy gene *ToxA* and this gene is sufficient to render avirulent isolate pathogenic indicating Ptr ToxA is a pathogenicity factor (Ciuffetti et al. 1997). *ToxA* was later identified in *Parastagonospora nodorum* the causal agent of Septeria nodorum blotch. Strong evidence indicated that the *ToxA* was horizontally transferred from *P. nodorum* to *P. tritici-repentis* (Friesen et al. 2006). This gene transfer events might also be the reason for the emergence of *Ptr* as an economically important wheat pathogen (Friesen et al. 2006). Ptr ToxA is a small secreted protein consisting of 179 amino acids (aa) (Ciuffetti et al. 1997). The first 23 aa is signal peptide for secretion and the 38 aa right after signal peptide is pro-sequence (Tuori et al. 2000). Upon secretion, both signal peptide and pro-domain are cleaved leading to a mature protein with a molecular weight at 13.2 kDa (Tuori et al. 2000). The mature Ptr ToxA is a single domain protein with a β-barrel and a loop containing a RGD motif (Sarma et al. 2005). RGD motif is believed to regulate the uptake of Ptr ToxA into mesophyll cells of susceptible genotypes because mutations within this domain prevented the internalization and toxic activity of Ptr ToxA (Meinhardt et al. 2003; Manning and Ciuffetti 2005). Microscopic evidence showed that Ptr ToxA enter the plant cells of susceptible genotype and then is transported to chloroplast where it disrupts the function of photosynthesis (Manning and Ciuffetti, 2005; Manning et al. 2009). Ptr ToxA was further shown to directly interact with

plastcyanin and ToxABP1 in cholorplast (Manning et al. 2007; Tai et al. 2007); very recently, Ptr ToxA was also shown to directly interact with PR-1 protein (Lu et al. 2013)

Host gene *Tsn1* confers sensitivity to Ptr ToxA which was mapped to wheat chromosome 5BL (Faris et al. 1996). *Tsn1* was recently cloned using map-based strategy (Faris et al. 2010) and was shown to contain a N-terminal S/TPK domain and C-terminal NBS-LRR domains. Therefore, it possesses a structure similar to a classic resistance gene providing support for the notion that necrotrophic pathogen hijack plant resistance signal pathway. However, *Tsn1* was shown not to interact with Ptr ToxA or the other ToxA interacting factors (plastcyanin and ToxABP1) (Faris et al. 2010). Therefore, it was believed that the interaction between Ptr ToxA and Tsn1 protein is indirect (Faris et al. 2010). Several studies have shown that Ptr ToxA-*Tsn1* compatible interaction induce hallmarks of plant resistant reactions, including electrolyte leakage, accumulation of H_2O_2, defense gene up-regulations (Adhikari et al. 2008; Pandelova et al. 2009; Manning et al. 2009).

Ptr ToxB-*Tsc2* interaction

Ptr ToxB is a protein with a molecular weight of 6.5 kDa (Strelkov et al. 1999, 2002). Unlike Ptr ToxA, Ptr ToxB induces chlorosis on sensitive wheat genotypes. *ToxB* encodes Ptr ToxB and was first cloned from a race 5 isolate (Martinez et al. 2001), but its homolgous gene, non-functinal *toxb* was also found in other races that do not produce detectable Ptr ToxB (Strelkov et al. 2002, 2006; Martinez et al. 2004). The *ToxB* gene exists with multiple copies in race 5 isolates and the copy number is correlated with virulence of the isolate toward the Ptr ToxB-sensitive lines (Martinez et al. 2004; Strelkov et al. 2006; Ciuffetti et al. 2010). Ptr ToxB has been shown to degrade chlorophyll and also induce defense responses (Ciuffetti et al. 2010).

A recent study suggests that Ptr ToxB may remain in apoplast area and it may interact with another protein triggering signaling cascade that ultimately results in chlorosis (Figueroa et al. 2015). In sensitive genotypes, Ptr ToxB induce up-regulation of many genes encoding WRKY transcription factors, RLKs, pathogenicity related proteins, components of phenyl propanoid pathway and jasmonic acid pathway (Pandelova et al. 2012). Furthermore, it was also shown that ROS accumulation and decrease in chlorophyll a and b overlaps with the symptom development caused by Ptr ToxB (Pandelova et al. 2012; Ciuffetti et al. 2010).

Sensitivity to Ptr ToxB is governed by the host susceptibility gene *Tsc2*. *Tsc2* was first mapped on to chromosome arm 2BS by Friesen and Faris (2004) using ITMI (International Triticeae Mapping Initiative) population. Saturation mapping was done by Abeysekara et al. (2010) using a RIL population derived from Salamouni × Katepwa which delineated *Tsc2* to a 3.3 cM region. It was also confirmed that resistance to race 5 is recessive and *Tsc2* is a dominant susceptible gene using a F_2 population derived from a cross between Salamouni × Katepwa.

Ptr ToxC-*Tsc1* interaction

Ptr ToxC is a chlorosis-inducing NE (Lamari and Bernier 1991; Gamba and Lamari 1998; Gamba et al. 1998). 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 the purification of Ptr ToxC, the exact chemical structure of Ptr ToxC has not been determined and the gene(s) responsible for its biosynthesis have not been cloned. Little has been done on the mode of action of Ptr ToxC. The host gene conditioning reaction to Ptr ToxC is *Tsc1* and it has been mapped to wheat chromosome arm 1AS (Faris et al. 1997; Effertz et al. 2001, 2002). More work is needed on Ptr ToxC because chlorosis caused by this NE is importantly associated with

the development of tan spot disease in many wheat genetic backgrounds (Faris et al. 1997;

Effertz et al. 2001, 2002).

Genetic resistance to tan spot

Early genetic studies

Because genetic resistance is considered as the best option of controlling this disease,

many studies have been conducted to screen and characterize genetics of resistance to tan spot

since the 1980s.

Sources of resistance have been identified from many countries including the United

States, Brazil, Mexico, Chile, China, Germany, France, Ecuador, and Japan (Lamari et al. 1992;

Luz 1995; Rees and Platz 1992). Lamari et al. (1992) screened more than 1200 wheat accessions

at different ploidy levels and identified 329 resistant hexaploids and 288 resistant tetraploids to

tan spot. Riede et al. (1996) did a pedigree analysis of selected resistant lines and suggested that

sources of resistance are mainly derived from four lines including: Frontana, Bluebird, Kavkaz, a

wheat-*Agropyron distichum* derivative that originated in Brazil, Mexico, Russia, respectively,

and grass species *A. distichum*.

Nagle et al. (1982) was the first to investigate the inheritance of genetic resistance to tan

spot. Using segregating populations (F_2 and BC_1F_1) derived from crosses between ND495

(susceptible parent) and 'Eklund' (resistant parent), the researcher found that resistance to tan

spot is complicatedly inherited and likely controlled by more than two genes. In addition,

diallelic analysis among ten hexaploid wheat accessions indicated that additive effects play a

significant role in inheritance of resistance. Lee and Gough (1984) found that the segregation

ratio of resistant and susceptible line was close to 1:3 in a F_3 families derived from susceptible

parent 'TAM W-101' and resistant parent 'Carifen 12' suggesting the recessive nature of tan spot

resistance. Using a segregating tetraploid population derived from durum wheat accession 'PI 184526' (resistant) and 'Calvin' (moderately susceptible), Elias et al. (1989) revealed a relatively high heritability (0.73) for tan spot resistance and also a significant additive effect for resistance genes. Sykes and Bernier (1991) conducted a comprehensive genetic study in hexaploid, tetraploid and diploid wheat using a few resistant and susceptible lines at each polyploid level and it was shown that resistance is controlled by a single recessive gene in tetraploid and diploid wheat, but two recessive genes in hexaploid wheat.

In the end of 1980s, it has been recognized that pathogen cause two distinct symptoms: necrosis and extensive chlorosis on different wheat genotypes (Lamari and Bernier 1989a, b; see above). Therefore, Lamari and Bernier (1991) investigated the inheritance of resistance to necrosis and extensive chlorosis individually. By using the populations segregating for necrosis only, chlorosis only or both, it was revealed that resistance to necrosis and extensive chlorosis caused by tan spot is govern by two genetically distinct genes, resistance to necrosis development is recessive, but resistance to extensive chrolosis is dominant. This research was very significant at that time because we know now two different NE-host sensitivity interactions control the development of necrosis and chrolosis (see above).

Genetic mapping of resistance genes

Since 1990s, the availability of wheat cytogenetic stock and molecular markers has allowed researchers to map tan spot resistance gene to individual chromosome and to a specific genomic region.

Following the identification of Ptr ToxA, a few studies were conducted showing that insensitivity to Ptr ToxA is highly correlated with resistance to necrosis-inducing isolate (Tomas and Bockus 1987; Lamari and Bernier 1989c; Faris et al. 1996). Faris et al. (1996) revealed that

sensitivity to Ptr ToxA, thus susceptibility to the fungus (necrosis-inducing isolate) is dominant. The gene (*Tsn1*) conferring sensitivity to Ptr ToxA was mapped to the chromosome 5BL using RFLP marker (Faris et al. 1996). In a very similar way, sensitivity to Ptr ToxB and Ptr ToxC were found to correlate with susceptibility to race 5 and race 3 isolates, respectively (Friesen and Faris 2004; Faris et al. 1997; Effertz et al. 2002), and the wheat genes conferring sensitivity to these two NEs were mapped to chromosome arms 2BS and 1AS, respectively (Friesen and Faris 2004; Effertz et al. 2002). Therefore, in this pathosystem, resistance to tan spot is mainly due to the lack of dominant susceptibility genes (*Tsn1, Tsc1* and *Tsc2*) that has been described as inverse gene-for-gene model (see above).

In addition to *tsn1, tsc2* and *tsc1*, several qualitative genes conditioning resistance to specific *P. tritici-repentis* races/isolates have also been identified. Singh et al. (2006) identified *tsr2* on the chromosome arm 3BL which confers recessive resistance to necrosis caused by the race 3 isolate 331-9 in the tetraploid wheat. Tadesse et al. (2006a) identified another recessive resistance gene (*tsr3*) on the chromosome 3D with the race 1 isolate ASC1b using populations derived from resistant hexaploid synthetics XX41, XX45, XX110 and susceptible cultivar Chinese Spring. 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'. Singh et al. (2008b) mapped *tsr5* which is also on the chromosome 3B conferring recessive resistance to DW13 (race 5) in durum wheat. This gene is 8.3 cM apart from the *tsr2* gene that was previously mapped by Singh et al. (2006). The recessive nature of these resistance genes suggests again the lack of dominant susceptibility genes which interact with additional NEs that have not been discovered (Faris et al. 2013).

QTL mapping of tan spot resistance

Quantitative traits are usually controlled by multiple genes located in different regions of a species genome. These regions are called quantitative trait loci (QTL) and can be identified using QTL mapping which involves the development of segregating host population, construction of genetic 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 were developed and applied to tan spot QTL mapping (Table 1.1).

Most populations used were derived from two hexaploid spring wheat genotypes with one being tan spot resistant and Ptr ToxA insensitive and the other being tan spot susceptible and Ptr ToxA sensitive. Therefore, these populations segregated for reaction to both fungal isolate and Ptr ToxA, which allows the investigation of the role of Ptr ToxA in the disease. In some cases, *tsn1* underlined a major resistance QTL for the disease caused by race 1 and race 2 isolates of different origins indicating the importance of Ptr ToxA in the disease (Cheong et al. 2004; Singh et al. 2008; Faris et al. 2012). However, in other cases there was no QTL identified at the *tsn1* locus suggesting Ptr ToxA-*Tsn1* interaction is not important for disease development (Faris and Friesen 2005; Chu et al. 2008, 2010). A few studies used the populations derived from two genotypes that are both insensitive to Ptr ToxA and revealed QTLs at the *Tsc1* and *Tsc2* loci indicating that they segregated for reaction to Ptr ToxC and Ptr ToxB and these two NEs play

important roles in the disease in those populations (Faris et al. 1997; Friesen and Faris 2004; Sun et al. 2010).

In addition to three NE insensitivity loci, there were 21 QTLs identified on 11 wheat chromosomes accounting for disease variations ranging from 0.05 to 0.41 (Table 1). Some of them might be the same based on their similar genetic locations, for example, the 4AL QTL that were identified by Faris et al. (1997), Friesen and Faris (2004) and Chu et al. (2008).

Similar to the three NE insensitivity loci, the majority of QTL identified are effective against one specific race, which can be considered as race specific resistance. However, Faris and Friesen (2005) identified two QTL *QTs.fcu-1BS* and *QTs.fcu-3BL* conferring resistance to multiple races with an effect up to 41%, suggesting that, for the first time, the presence of race nonspecific resistance in wheat tan spot system.

Association mapping

Very recently, association mapping (AM) is another tool that has been used to identify QTL associated with tan spot disease. In AM, QTL mapping employs multiple genetically diverse lines from a natural population or a germplasm collection (Abdurakhmonov and Abdukarimov 2008), which is thought to have more genetic diversity than bi-parental QTL mapping. 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) screened 535 spring wheat lines for tan spot resistance using isolate AR Cross B10 and was able to identify QTL on chromosomes 1A, 1D, 2B, 2D, 6A, and 7A. In another study, 358 European winter

wheat lines and 14 spring wheat lines were studied for tan spot resistance and they were able to

identify *Tsn1*, *tsn2* or *tsn5*, *Tsc2* or *Tsc6* and resistant QTL on chromosome arms, 1DL, 2BL,

3BS, 3DL, and 3AL (Kollers et al. 2014). In a latest association mapping study 170 lines of

historical bread wheat germplasm developed at CIMMYT were tested for the resistance to tan

spot caused by race1 isolate Ptr-1 (Singh et al. 2015). From this study significant marker

associations were revealed on chromosome arms 1AS, 1BS, 2BL, 3BL, 4AL, 5BL, 6AL (two

QTL), 6BS and 7BL. They reported two QTL found on chromosome arm 6AL and the QTL

found on chromosome 7BL as novel regions related to tan spot resistance.

Table 1. Summary of QTLs identified in various bi-parental wheat populations

Population[*]	Ptr ToxA Reaction[Δ]	Population type[δ]	Polyploid level[‡]	Isolate[α]	QTL identified	Chromosome	Position[β] cM	$R^{2\gamma}$				Reference
								R1	R2	R3	R5	
W-7984 (SHW) × **Opata 85** (HRSW)	W-7984 - I Opata 85 - I	RIL	H	Pti2 (R1) D308 (R3) 86-124 (R2)	QTsc.ndsu-1A	1AS 4AL	tsc1	0.351 0.137	NS NS	0.35 0.137		Faris et al. 1997
Cranbook × Halberd **Brookton** × Krichauff		DH DH	H	Queensland, 2/99,2,20/99, 19/99,Kapunda		5BL	tsn1	0.60 0.36				Cheong et al. 2004
W-7984 (SHW) × **Opata 85** (HRSW)	W-7984 - I Opata 85 - I	RIL	H	DW5 (R5)	Xcdo447 tsc2 Xmwg2025 Xksu916(Oxo)	2AS 2BS 2BL 4AL	20.0 tsc2 120.0 80.0				0.117 0.692 0.119 0.200	Friesen and Faris 2004
BR34 (HRSW)× Grandin (HRSW)	BR34 – I Grandin - S	RIL	H	Pti2(R1), 86-124(R2), OH99 (R3), DW5 (R5)	QTs.fcu-1BS QTs.fcu-3BS QTs.fcu-3BL	1BS 3BS 3BL	10.0 55.0 128.0	0.27 NS 0.17	0.14 NS 0.24	0.29 0.12 0.13	0.13 NS 0.41	Faris and Friesen 2005
TA4152-60 (SHW) × ND495 (SW)	TA4152-60 I ND495-S	DH	H	Pti2(R1), 86-124(R2), OH99 (R3), DW5 (R5)	QTs.fcu-2AS QTs.fcu-4AL QTs.fcu-5AL QTs.fcu-5BL.1 QTs.fcu-5BL.2	2AS 4AL 5AL 5BL.1 5BL.2	84.0-85.5 151.8 138.4-140.1 57.6-59.7 105.2-107.1	0.14 NS 0.10 0.22 0.17	0.22 NS 0.09 0.22 0.14	0.14 0.10 NS 0.26 NS	0.19 NS 0.14 0.14 NS	Chu et al. 2008
WH542 (SW) × HD29 (SW)		RIL	H	R1 isolate from Kansas	QTs.ksu-3AS QTs.ksu-5BL	3AS 5BL	tsn1	0.23 0.27				Singh et al. 2008
Lebsock (Durum) × PI94749 (*T. turgidum* ssp. *carthlicum*)	Lebsock- S PI94749-I	DH	T	Pti2 (R1), 86-124 (R2)	QTs.fcu-3A QTs.fcu-3B QTs.fcu-5A.1 QTs.fcu-5A.2 QTs.fcu-7B	3AS 3BS 5AL.1 5AL.2 7BL	0 29.1 20.2 118.3 70.7	0.11 0.08 0.22 0.08 0.08	0.08 NS 0.15 0.13 0.06			Chu et al. 2010
Wangshuibai (WSB) (Landrace)× Ning7840 (SW)	WSB-I Ning7840- I	RIL	H	AZ-00 (R1)	QTs.ksu-1AS	1AS	2.0	0.39				Sun et al. 2010
Erina (SRWW) × Batavia		DH	H	Isolate from Australia		2BS		0.298-0.382 (Race unknown)				Li et al. 2011
Salamouni (Landrace) × Katepwa (SW)	Salamouni – I Katepwa-S	RIL	H	Pti2 (R1), AscI (R1), 86-124 (R2), AR LonB2	QTs.fcu-5B QTs.fcu-5D QTs.fcu-7B QTs.fcu-7D	5BL 5DL 7BS 7DS	tsn1 128.1 21.3 84.3	0.25 Only for Asc1 – 0.13 0.08 Only for AR Lon B2 – 0.07	0.32 0.05			Faris et al. 2012

*Wheat type: HRSW- Hard red spring wheat, SW- other type of spring wheat, SRWW- Soft red winter wheat, SHW- Synthetic hexaploid wheat. Resistant parental line was indicated in **bold.** .

$^{\Delta}$Ptr ToxA reaction of the parents: I- Insensitive, S- Sensitive.

$^{\delta}$Population type: RIL- Recombinant Inbred Lines, DH-Doubled Haploid

‡Polyploid level: H- Hexaploid, T- Tetraploid.

$^{\alpha,\,\gamma}$ Race of the isolates: R1 – race 1, R2 – race 2, R3-race 3, R5- race 5, AR- Arkansas isolates, newly identified race

$^{\beta}$Position- information on the genetic position or the range of QTL was taken from the published studies.

$^{\gamma}R^2$- Disease variation explained by the individual QTL. NS= nonsignificant for the specific race

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PAPER 1: GENETIC RELATIONSHIPS BETWEEN RACE-NONSPECIFIC AND RACE SPECIFIC INTERACTIONS IN THE WHEAT-*PYRENOPHORA TRITICI-REPENTIS* PATHOSYSTEM

Abstract

Tan spot, caused by the fungus *Pyrenophora tritici-repentis* (*Ptr*), is a destructive disease of wheat worldwide. The disease system is known to include inverse gene-for-gene, race specific interactions involving the recognition of fungal-produced necrotrophic effectors (NEs) by corresponding host sensitivity genes. However, quantitative trait loci (QTLs) conferring race-nonspecific resistance have also been identified. In this work, I identified a major race-nonspecific resistance QTL and characterized its genetic relationships with the NE-host gene interactions Ptr ToxA-*Tsn1* and Ptr ToxC-*Tsc1* in a wheat recombinant inbred population derived from the cross between 'Louise' and 'Penawawa'. Both parental lines were sensitive to Ptr ToxA, but Penawawa and Louise were highly resistant and susceptible, respectively, to conidial inoculations of all races. Resistance was predominantly governed by a major race-nonspecific QTL on chromosome arm 3BL for resistance to all races. Another significant QTL was detected at the distal end of chromosome arm 1AS for resistance to the Ptr ToxC-producing isolates, which corresponded to the known location of the *Tsc1* locus. The effects of the 3B and 1A QTLs were largely additive, and the 3B resistance QTL was epistatic to the Ptr ToxA-*Tsn1* interaction. Resistance to race 2 in F_1 plants was completely dominant, however race 3-inoculated F_1 plants were only moderately resistant because they developed chlorosis presumably due to the Ptr ToxC-*Tsc1* interaction. This work provides further understanding of genetic resistance in the wheat-tan spot system as well as important guidance for tan spot resistance breeding.

Introduction

Tan spot, also known as yellow leaf spot, is caused by the fungus *Pyrenophora tritici-repentis* (*Ptr*) and can occur on both common wheat (*Triticum aestivum* L.) and durum (*T. turgidum* L. ssp. *durum*). In the last century, the disease has evolved from a minor problem to a major threat to wheat production around the world (Hosford 1982; Murray and Brennan 2009; Faris et al. 2013). The wide adoption of minimum tillage practices is thought to be the main reason for the increase of tan spot because the fungus overwinters on wheat residue left from the previous year's crop providing a direct source of inoculum. If infestation is severe, tan spot can cause yield losses approaching 50% and negatively affect grain quality (Rees et al. 1982; Schilder and Bergstrom 1994). Although crop rotation and fungicide applications can be used to reduce losses due to tan spot, the development and deployment of resistant varieties is the most economical, environmentally friendly, and sustainable way to manage the disease. In order to breed tan spot resistant cultivars, a good understanding of genetic resistance/susceptibility and associated mechanisms in the wheat-*Ptr* system is needed.

Ptr is a necrotrophic pathogen, meaning that it requires dead or dying tissue to acquire nutrients and proliferate. Necrotrophic specialists such as *Ptr* are known to produce necrotrophic effectors (NE), previously known as host-selective toxins (HSTs). The NEs are recognized by corresponding sensitivity/susceptibility genes in the host in an inverse gene-for-gene manner (Wolpert et al. 2002; Friesen et al. 2008; Ciuffetti et al. 2010). In this model, recognition of an NE by the corresponding host sensitivity gene leads to a compatible interaction and ultimately necrotrophic effector-triggered susceptibility (NETS) (Liu et al. 2009). If the pathogen does not produce the NE, or if the host does not possess the corresponding sensitivity gene, an incompatible interaction occurs resulting in resistance. This scenario is in contrast to the classic

gene-for-gene model (Flor 1956) where resistance occurs upon the recognition of an avirulence gene product by the corresponding plant resistance gene product. The interaction leads to resistance and is known as effector-triggered immunity (ETI). Therefore, resistance in disease systems involving necrotrophic fungi is usually recessive and largely due to the absence of NE recognition by the host. However, multiple NE-host gene interactions are usually present in a given system and their effects are mostly additive (Friesen and Faris 2010). Therefore, resistance/susceptibility in these systems is often best characterized as a quantitative trait.

Three NE-host gene interactions have been identified in the wheat-*Ptr* pathosystem, including Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1* (Ciuffetti et al 2010; Faris et al. 2013 for review). Among them, the Ptr ToxA-*Tsn1* interaction leads to necrosis, while the other two induce chlorosis. *Ptr* isolates have been classified into eight races based on the NEs they produce and/or their virulence toward differentials that carry individual host sensitivity genes (Lamari and Strelkov 2010; Faris et al. 2013 for review). Because the NE-host gene interaction determines race specificity in tan spot, they are considered as race-specific interactions. The host genes *Tsn1*, *Tsc1* and *Tsc2* have been mapped to wheat chromosome arms 5BL (Faris et al. 1996), 1AS (Effertz et al. 2001) and 2BS (Friesen and Faris 2004; Abeysekara et al. 2009), respectively. Among them, only the *Tsn1* gene has been cloned, and it encodes a plant resistance gene-like protein containing protein kinase, nucleotide binding, and leucine-rich repeat domains (Faris et al. 2010).

In addition to these susceptibility genes, four other qualitative genes conditioning tan spot resistance (*tsr*) were also identified, including *tsr2* (Singh et al. 2006), *tsr3* (Tedesse et al. 2006a), *tsr4* (Tedesse et al. 2006b) and *tsr5* (Singh et al. 2008). Both *tsr2* and *tsr5* were identified in tetraploid wheat and mapped to chromosome arm 3BL in a close proximity, whereas

tsr3 and *tsr4* were mapped to chromosome arms 3DL and 3AL, respectively, in hexaploid wheat. Because these resistance genes were shown to be recessive, it is possible that they also represent host susceptibility loci that interact with unidentified fungal NEs (Faris et al. 2013 for review). Manning and Ciuffetti (2015) recently demonstrated the presence of novel NE-host susceptibility gene interactions in this pathosystem, the effect of which could be masked by Ptr ToxA-*Tsn1* if they co-exist in a certain genotype.

QTL mapping using bi-parental or natural populations has also been conducted to identify genomic regions involved in tan spot resistance. All 21 wheat chromosomes except 4B and 6D have been reported to harbor QTLs conferring resistance to tan spot (Faris et al. 2013 for review; Patel et al. 2013; Kollers et al. 2014; Liu et al. 2015). Some QTLs have coincided with the locations of the three NE sensitivity loci strongly indicating these NE-host sensitivity gene interactions are important in the development of tan spot (Cheong et al. 2004; Singh et al. 2008; Faris et al. 1997, 2012; Friesen and Faris 2004; Sun et al. 2010). However, there are also many QTLs that were identified in genomic regions other than the three sensitivity loci (Faris et al. 2013 for review). One study revealed no significant role for the Ptr ToxA-*Tsn1* interaction in the development of tan spot caused by the Ptr ToxA-producing races 1 and 2 (Faris and Friesen 2005). Instead, they identified genomic regions on chromosomes 1B and 3B conferring resistance to multiple races, and these have been referred to as race-nonspecific resistance QTLs (Faris and Friesen 2005; Faris et al. 2013 for review).

Many tan spot resistant genotypes identified so far are insensitive to Ptr ToxA, and in most cases, they have been crossed with Ptr ToxA-sensitive, disease susceptible lines to develop bi-parental populations for characterizing tan spot resistance (Faris et al. 2013 for review). In our effort to screen the US spring wheat elite lines for reaction to tan spot (Liu et al. unpublished

data), we found that the soft white spring wheat (SWSW) cultivar Penawawa is sensitive to Ptr ToxA, but highly resistant to all races of tan spot. To our knowledge, no study has been done to characterize the genetics of a cultivar that is sensitive to Ptr ToxA, but highly resistant to all races including races 1 and 2, which produce Ptr ToxA. This cultivar had been crossed with another SWSW cultivar Louise to develop a recombinant inbred line (RIL) population and used to map high temperature adult plant stripe rust resistance QTLs and seed-expressed polyphenol oxidase genes (Carter et al. 2009; Beecher et al. 2012). Here, we used this population to identify Penawawa-derived tan spot resistance QTLs and to characterize the relationships between race-nonspecific resistance and race-specific interactions including Ptr ToxA-*Tsn1* and Ptr ToxC-*Tsc1*.

Materials and methods

Plant materials

The Louise/Penawawa population used in this study, hereafter referred to as the LP population, consisted of 188 RILs and has been described in Carter et al. (2009). Both Louise and Penawawa are SWSW cultivars that were highly adapted to the Pacific-Northwest region of the United States. The population was initially developed to map high temperature adult plant stripe rust resistance in Louise (Carter et al. 2009). Our preliminary data showed that Penawawa was highly resistant to tan spot while Louise was highly susceptible. The two parental lines and all RILs were used for fungal NE sensitivity and disease evaluations. Four wheat lines known as the tan spot differential lines including 'Salamouni', 'Glenlea', '6B365' and '6B662' were also included in disease evaluations. To determine the nature of resistance in Penawawa, F_1 plants of Louise/Penawawa were tested for reaction to tan spot along with the four differential lines.

Seeds of each RIL, the parental lines, F_1 and differential lines were planted in super-cell containers (Stuewe & Sons, Inc., Corvallis, OR) that were filled with Sunshine SB100 soil (Sun Grow Horticulture, Bellevue, WA). Three seeds per container and one container per line were used for planting. RL98 trays (Stuewe & Sons, Inc., Corvallis, OR) were used to hold the planted containers. Following planting, all planted containers were given equal amounts of Osmocote Plus 15-19-12 fertilizer (Scotts Sierra Horticultural Product Company, Maysville, OH). The highly susceptible (Liu et al. 2015) North Dakota hard red winter wheat cultivar 'Jerry' was planted in the containers along the borders of each RL 98 tray to reduce the edge effect. The plants were grown in a greenhouse room with the temperature ranging from 20 to 25 ºC. When the plants reached the two- to three-leaf stage (around 14 days after planting under our greenhouse conditions), the plants were used for NE infiltrations or fungal inoculations. At least three biological replications were conducted for NE and disease evaluations following a randomized complete block design (RCBD).

Necrotrophic effector infiltration

The parental lines and RILs were evaluated for reaction to NEs Ptr ToxA and Ptr ToxB. Both NE genes have been cloned and transformed into *Pichia pastori* yeast strain X33 (Liu et al. 2009; Abasakara et al. 2010). The corresponding genetically modified X33 strains were used to produce each NE. The strains were cultured in yeast potato dextrose broth for 24-48 h at 30 ºC with vigorous shaking and the resulting cultures were centrifuged to collect the culture filtrates for infiltration. Approximate 20 µl of NE culture filtrates were infiltrated into the fully expanded secondary leaf of a wheat seedling using a 1 ml syringe with the needle removed. The infiltrated areas were marked using a felt pen and plants were placed in a growth chamber at 21 ºC with a

12-h photoperiod. The reactions were scored five days after infiltration as sensitive (necrosis or chlorosis developed in the marked area) or insensitive (no reaction in the marked area).

Fungal inoculations and disease evaluation

Five *Ptr* isolates were used to evaluate the LP population, including Pti2, 86-124, 331-9, DW5 and AR CrossB10. Of the three known *Ptr* NEs, 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. Therefore, these isolates have been classified as races 1, 2, 3 and 5, respectively. Isolate AR CrossB10 does not conform to the race classification system because it causes necrosis on the differential line Glenlea even though it does not produce Ptr ToxA (Ali et al. 2010). Based on our observations, AR CrossB10 causes extensive chlorosis on 6B365, thus indicating that it likely produces Ptr ToxC (ZH Liu, unpublished). All these isolates were collected from North America (Friesen et al. 2003; Ali et al. 2010).

To further examine the role of the Ptr ToxA-*Tsn1* interaction in disease, a *ToxA* knockout strain of 86-124 (86-124Δ*ToxA*) was also used to evaluate the LP population. This knockout strain was obtained from the genetic modification of 86-124 by replacing the whole *ToxA* coding region with the hygromycin resistance gene (Rasmussen et al. unpublished data). We have confirmed that the strain does not contain the *ToxA* gene and does not produce Ptr ToxA in culture (data not shown).

Inoculum was prepared as described in Lamari and Bernier (1989). Briefly, fungal isolates were grown in the dark for 5 days on V8-potato dextrose agar at room temperature. After being flooded with sterilized distilled water and flattened using the bottom of a flame sterilized test tube, the cultures were moved to a light bank and kept under continuous light for 24 h at room temperature. Then, the cultures were incubated in the dark for 24 h at 16 ºC to induce

sporulation. Conidia were harvested by flooding the plate with sterilized distilled water followed

by gentle scraping of the surface of the culture with an inoculation loop. The harvested spore

solution was adjusted to a concentration of approximately 3,000 spores/ml and two drops of

Tween-20 per 100 ml were added before inoculations.

Plants were inoculated and then kept in a mist chamber with 100% humidity as described

in Liu et al. (2015). Plants were then placed in a growth chamber with 12-h photoperiod at 21℃

for plant growth and disease development. Disease reactions were evaluated seven days after

inoculation using a lesion type-based 1-5 rating scale with 1 being highly resistant and 5 being

highly susceptible (Lamari and Bernier 1989). If a line had equal amounts of two reaction types,

an intermediate score was given.

Statistical analysis and QTL mapping

These statistical analyses were conducted using SAS program with corresponding

command codes (SAS Institute 2011). The disease data for each isolate was first tested for

normal distribution using Shapiro-Wilk in the PROC UNIVARIATE procedure (SAS Institute

2011). Homogeneity of variance among different experiments was then performed using

Bartlett's χ^2 test if the data fit a normal distribution (Snedecor and Cochran 1989), or Levene's

test if it did not (Levene 1960). Data from homogeneous experiments was combined and used to

calculate the disease means, which were used for QTL detection and subsequent analysis.

Disease means of the LP population caused by different isolates were compared using Fisher's

protected least significant difference (LSD) at $P < 0.05$.

The linkage map of the LP population was initially developed by using 295 SSRs and one

SNP marker (Carter et al. 2009). Later, the map was reconstructed by the addition of 1,434 SNP

markers, covering all 21 chromosomes with an average marker density of 2.2 cM per marker

(Beecher et al. 2012). For QTL mapping, we removed redundant and closely linked markers, and reconstructed the linkage maps using MapDisto (Lorieux 2012). The resulting maps consisted of 21 linkage groups corresponding to the 21 wheat chromosomes and contained a total of 596 markers spanning 3163.7 cM in genetic distance. This new map was employed to identify markers associated with resistance to tan spot using QGene 4.0 (Joehanes and Nelson 2008).

A permutation test consisting of 1000 permutations yielded a significance an LOD significance threshold of 3.2 for an experiment-wise significance level of 0.05. Composite interval mapping (Zeng 1994) was also performed as described in Faris et al. (2014) to identify genomic regions significantly associated with tan spot resistance.

To dissect the genetic relationships between race-nonspecific resistance QTL with NETS caused by the Ptr ToxA-*Tsn1* and Ptr ToxC-*Tsc1* interactions, we compared the disease means of different groups of RILs in the LP population that were classified based on the presence or absence of the 3B QTL and individual host insensitivity genes. All comparisons were done using Fisher's protected least significant difference (LSD) at *P* < 0.05 (SAS Institute 2011).

Results

Reaction of parental lines and the LP population to NEs and fungal isolates

Both Louise and Penawawa were sensitive to Ptr ToxA and insensitive to Ptr ToxB (Fig. 1). However, the two lines differed in their reactions to conidial inoculations. Penawawa developed small pin-point dark spots on the leaves demonstrating high levels of resistance to all races and it had average disease reactions that ranged from 1.00 for DW5 (race 5) to 1.58 for Pti2 (race 1) (Fig. 1, Table 2). In contrast, Louise developed large necrotic and/or chlorotic lesions in reaction to all isolates indicating that it was highly susceptible to all of them (Fig. 1). The average disease score for Louise ranged from 3.08 for 86-124 (race 2) to 4.17 for AR CrossB10

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(Table 2). AR CrossB10 caused chlorosis on Louise similar to that caused by the Ptr ToxC-producing isolates Pti2 (race 1) and 331-9 (race 3) suggesting that AR CrossB10 likely produce Ptr ToxC as well.

The whole population was also tested for reaction to Ptr ToxA and as expected, all 188 RILs were sensitive. However, the LP population segregated for disease reactions from highly resistant to highly susceptible for all races (Table 2). The average disease scores of the population were 2.83, 2.32, 2.42, 2.56 and 2.89 for races 1, 2, 3, 5, and AR CrossB10, respectively. A normality test rejected the hypothesis that the disease reaction of the LP population to all isolates fit a normal distribution. Disease histograms also suggested non-normal distribution for disease reactions to all isolates (Fig. 2). In addition, the shapes of the histograms for all isolates differed with the Pti2 histogram showing relatively more susceptible RILs and the DW5 histogram showing more resistant RILs (Fig. 2).

Table 2. Lesion type means of Louise, Penawawa, and the Louise × Penawawa recombinant inbred line population to conidial inoculations of *Pyrenophora tritici-repentis races* 1, 2, 3, 5 and isolate AR CrossB10.

Isolates [a]	Louise [b]	Penawawa[b]	LP population	LP population
Pti2 (race 1) (Ptr ToxA+, Ptr ToxC+)	4.12	1.58	2.83	1.00-4.50
86-124 (race 2) (Ptr ToxA+, Ptr ToxC-)	3.08	1.13	2.32	1.00-4.13
331-9 (race 3) (Ptr ToxA-, Ptr ToxC+)	3.83	1.16	2.42	1.00-4.67
DW5 (race 5) (Ptr ToxA-, Ptr ToxC-)	3.75	1.00	2.56	1.00-4.88
AR Cross B10 (Ptr ToxA-, Ptr ToxC+)	4.17	1.33	2.89	1.00-4.14

[a] Five isolates representing different *Pyrenophora tritici-repentis* races were used to evaluate the LP 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.
[b] Disease was scored using a 1-5 scale with 1 being highly resistant and 5 being highly susceptible.
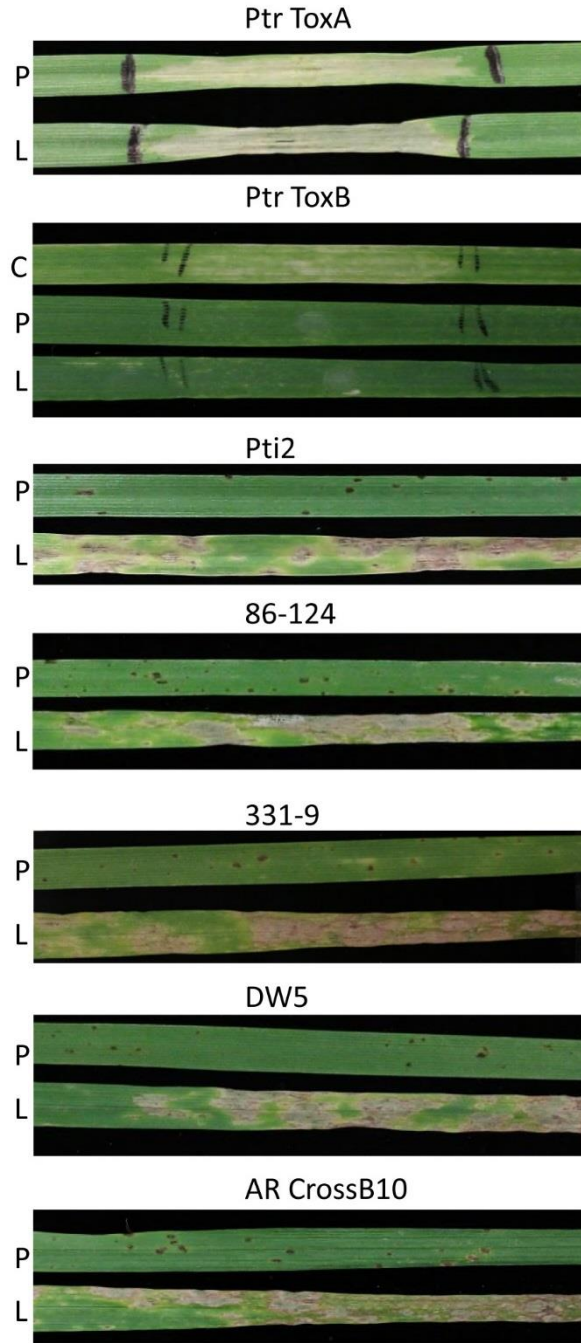
Figure 1. Reaction of Louise and Penawawa to necrotrophic effector infiltrations and individual isolate inoculations. The *Pyrenophora tritici-repentis* NEs Ptr ToxA and Ptr ToxB, and five fungal isolates representing different races including Pti2 (race 1), 86-124 (race 2), 331-9 (race 3), DW5 (race 5) and AR crossB10 (unclassified isolate) were used. P: Penawawa, L: Louise, and C: 6B662, used as a positive control for Ptr ToxB infiltration.
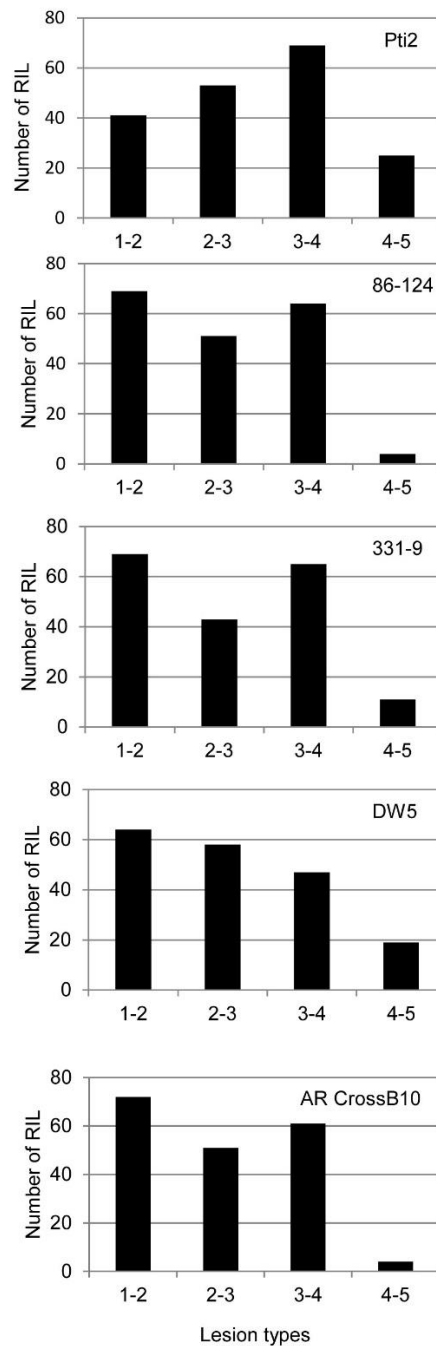
Figure 2. Histograms of disease reaction of the Louise × Penawawa population to individual isolates. The LP population was evaluated with five isolates representing different races, including Pti2 (race 1), 86-124 (race 2), 331-9 (race 3), DW5 (race 5) and AR crossB10 (unclassified isolate). The disease was scored using a 1-5 lesion type-based scale with 1 being highly resistant and 5 being highly susceptible. The x-axis is the disease scale and y-axis is the number of recombinant inbred lines.

QTL identification

Because disease reactions of the LP 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 ($P = 0.07$-0.58, $df = 2$) for all isolates. Therefore, the average disease scores of each RIL from the three experiments were computed and used in subsequent QTL analyses.

In total, four QTLs associated with tan spot resistance in the LP population were identified, and the resistance alleles at all four QTLs were contributed by the resistant parent Penawawa. These QTLs were distributed on chromosome arms 1AS, 2DL, 3BL, and 5AL and designated *QTs.zhl-1A*, *QTs.zhl-2D*, *QTs.zhl-3B*, and *QTs.zhl-5A*, respectively (Table 3). *QTs.zhl-3B* and *QTs.zhl-5A* were significantly associated with resistance to all five isolates, whereas *QTs.zhl-1A* was significantly associated with disease caused by the Ptr ToxC-producing isolates Pti2, 331-9, and AR CrossB10 and *QTs.zhl-2D* associated with disease by all isolates except DW5.

QTs.zhl-3B conferred resistance to all isolates and had the largest effect among all QTLs identified. It had a LOD value ranging from 13.6 (AR Cross B10) to 44.0 (86-124) and the effect of the QTL explained from 22 (AR CrossB10) to 53% (86-124) of the disease variation (Table 3). The genomic region harboring this QTL was flanked by the SNP markers *Xiwa1383* and *Xiwa4613*. The SSR marker *Xwmc69* was the closest to the peak position of the QTL (Fig. 3).

QTs.zhl-1A was the second-most significant QTL and it was located at a position between markers *Xiwa6644* and *Xpsp2999* on the distal end of the 1AS chromosome arm (Fig. 3). This position is near the known location of the *Tsc1* gene. As mentioned above, this QTL was

significantly associated with resistance to only the Ptr ToxC-producing isolates, for which it had

LOD values that ranged from 14.4 to 23.1 and R^2 values that ranged from 0.09 to 0.22 (Table 3).

The other race-nonspecific QTL, *QTs.zhl-5A,* was flanked by the markers *Xiwa7025* and

Xiwa5173 and explained from 6% of the disease variation for 331-9 to 14% of the disease

variation for DW5 (Fig. 3, Table 3). *QTs.zhl-2D* was located approximately on the end of the

long arm of chromosome 2D, flanked by the markers *Xwmc41* and *Xgwm608* and accounted for

3 to 9% of the disease variation

Table 3. Composite interval mapping analysis of QTLs associated with resistance to tan spot caused by *Pyrenophora tritici-repentis* races 1, 2, 3, 5 and isolate AR CrossB10 in the Louise × Penawawa recombinant inbred line population.

QTL	Interval (cM)	Flanking markers	R^{2a}					LOD^b					Source[c]
			Pti2	86-124	331-9	DW5	AR	Pti2	86-124	331-9	DW5	AR	
QTs.zhl-1A	0.0-6.0	*Xiwa6644-Xpsp2999*	0.09	NS	0.22	NS	0.14	14.7	NS	23.1	NS	14.4	P
QTs.zhl-2D	144.0-152.0	*Xwmc41-Xgwm608*	0.09	0.07	0.03	NS	0.05	8.6	6.4	4.7	NS	5.5	P
QTs.zhl-3B	72.0-78.0	*Xiwa1383-Xiwa4613*	0.30	0.53	0.41	0.46	0.22	18.4	44.0	34.3	36.3	13.6	P
QTs.zhl-5A	154.0-160.0	*Xiwa7025-Xiwa5173*	0.13	0.13	0.06	0.14	0.08	12.7	13.9	5.2	18.2	7.9	P

[a] R^2 = the coefficient of determination. The R^2 value × 100 represents the amount of phenotypic variation explained. NS indicates the QTL was not significant.
[b] LOD was determined by the execution of 1000 permutations on marker and phenotypic datasets, which yielded a value of 3.2 as the cutoff for the detection of significant QTLs.
[c] The source of each QTL indicates the resistance allele was contributed by one of the parental lines with L being Louise and P being Penawawa
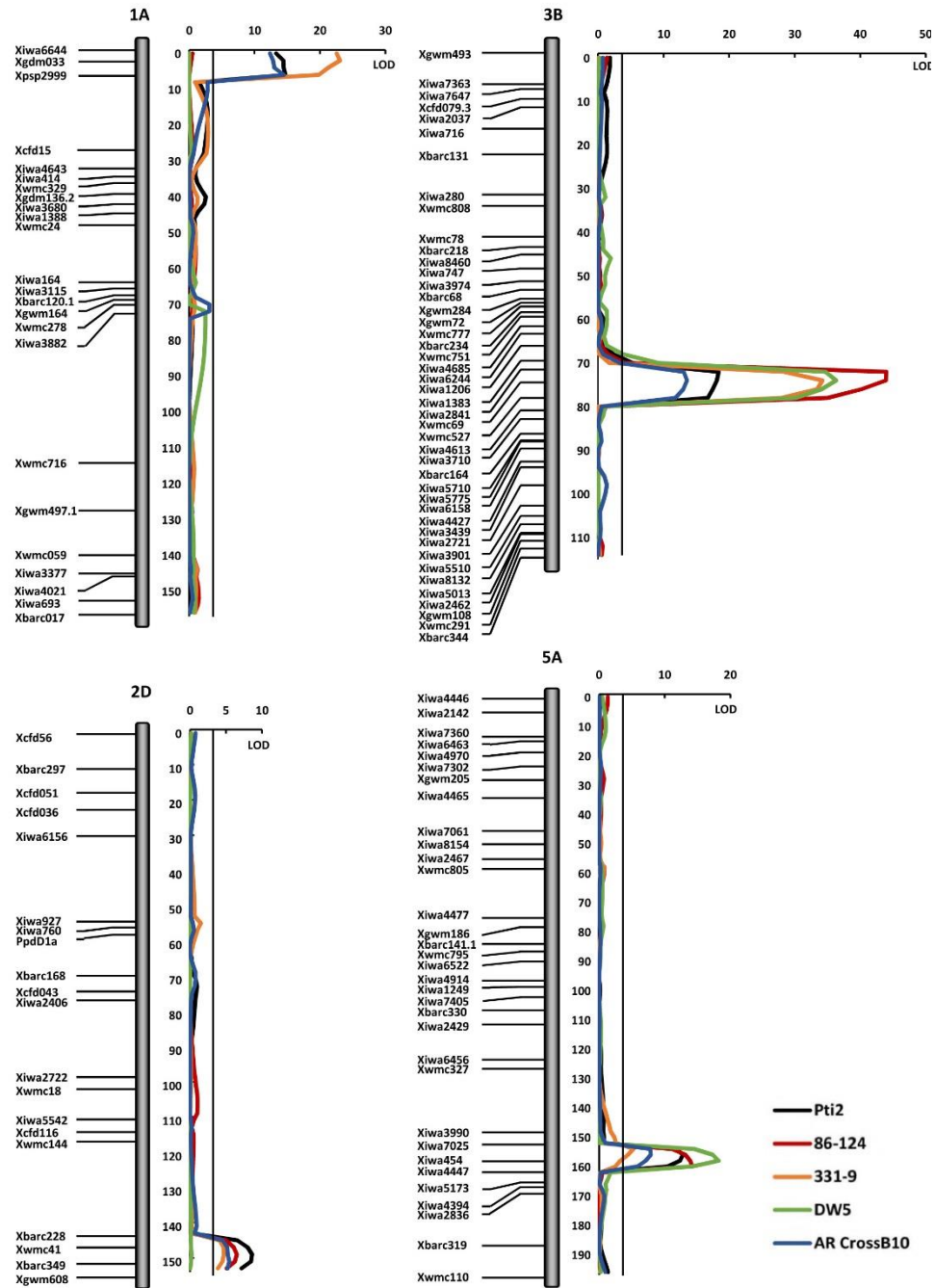
Figure 3. Composite interval regression maps of chromosomes 1A, 2D, 3B and 5A containing QTLs significantly associated with resistance to tan spot. QTL mapping was conducted on the LP population for five *Pyrenophora tritici-repentis* isolates representing different races including Pti2 (race 1, black), 86-124 (race 2, red), 331-9 (race 3, orange), DW5 (race 5, green) and AR crossB10 (unclassified isolate, blue). The positions of marker loci are shown to the left of the linkage groups and genetic scales in centiMorgan (cM) are shown along the right of each chromosome. A solid line represents the logarithm of the odds (LOD) significance threshold of 3.2. The LOD and R^2 values for each QTL are presented in Table 2.

Disease reactions of RILs for the different allelic states at *QTs.zhl-3B* and *QTs.zhl-1A*

QTs.zhl-1A and *QTs.zhl-3B* had major effects associated with disease caused by the isolates Pti2, 331-9 and AR CrossB10, which produce Ptr ToxC. To investigate the genetic relationships between the two QTLs, the RILs were grouped into four categories based their allelic state at the two loci, and the disease means of these groups were compared (Table 4). The group of RILs that had Penawawa alleles at both loci was highly resistant with mean reaction types less than 2.0, whereas RILs with Louise alleles at both loci were highly susceptible with mean reaction types greater than 3.5.

RILs with Penawawa alleles at *QTs.zhl-3B* and Louise alleles at *QTs.zhl-1A*, or vice versa, were moderately resistant to moderately susceptible. Comparisons among the mean disease reaction types of isolates Pti2 and 331-9 for these two allelic classes indicated that RILs with Louise alleles at *QTs.zhl-1A* and Penawawa alleles at *QTs.zhl-3B* were significantly more resistant than RILs with Penawawa alleles at *QTs.zhl-1A* and Louise alleles at *QTs.zhl-3B* (Table 4). However, no significant difference between these two classes was observed for average disease reactions types obtained with isolate AR CrossB10.

Table 4. Comparison of the disease means of the recombinant inbred lines grouped based on their allelic state at *QTs.zhl-1A* and *QTs.zhl-3B* in the Louise × Penawawa population.

Allele at *QTs.zhl-1A*, *QTs.zhl-3B*[a]	No. of RILs (n)	Pti2[b] (Race1)	331-9 [b] (Race 3)	AR CrossB10 [b]
L,L	50	3.50a	3.53a	3.58a
P,L	47	3.18b	2.65b	3.06b
L,P	43	2.74c	2.31c	2.94b
P,P	48	1.87d	1.18d	1.96c

[a]The allele type is indicated by L (Louise allele) and P (Penawawa allele) at the corresponding locus.
[b]Numbers in the same column followed by the same letter are not significantly different at $P = 0.05$ as determined by LSD.

Reaction of the LP population to a race 2 *ToxA* knockout strain

The LP population was also evaluated with a race 2 *ToxA* knockout strain (86-124ΔToxA), which does not produce Ptr ToxA. The average disease reaction types obtained from this modified strain were compared to those obtained from isolate 86-124 to make direct comparisons between isolates that only differ by the production of Ptr ToxA, whereby 86-124 produces Ptr ToxA and 86-124ΔToxA does not. The disease means of RILs with the *QTs.zhl-3B* resistance allele from Penawawa were 1.65 for 86-124ΔToxA and 1.71 for 86-124, and they were not significant different (Table 5). However, RILs having the Louise allele at *QTs.zhl-3B* had average disease reaction types of 2.88 for 86-124ΔToxA and 3.02 for 86-124, which were significantly different ($P = 0.03$).

Reactions of Louise × Penawawa F₁ plants to tan spot

We tested the F₁ plants derived from Louise and Penawawa along with the two parental lines for reaction to 86-124 (race 2) and 331-9 (race 3) to determine the genetic nature of resistance. For 86-124, all F₁ plants were as resistant as Penawawa and only developed pinpoint

lesions whereas Louise developed large necrotic lesions as observed before (Fig. 4). For isolate

331-9, Penawawa again had pinpoint lesions and was classified as highly resistant whereas

Louise was highly susceptible with the development of large necrotic lesions with chlorosis

(Figs. 1 and 4). However, the F_1 plants were considered moderately resistant because although

they exhibited pinpoint dark lesions they also showed chlorosis across the inoculated area (Fig.

4).

Table 5. Comparison of the recombinant inbred lines grouped on the presence or absence of the 3BL QTL in the Louise × Penawawa population for reaction to the race 2 isolate and its *ToxA* knock out strain

Allele type at *QTs.zhl-3B*[a]	No. of RILs	Isolate/strain[b]	Disease mean[c]
P	97	86-124	1.71a
		86-124ΔToxA	1.65a
L	91	86-124	3.02b
		86-124ΔToxA	2.88c

[a]The presence of allele type at *QTs.zhl-3B*, L: Louise allele and P: Penawawa allele.
[b]86-124 was used to generate the *ToxA* knockout strain 86-124ΔToxA. The knockout strain has been proved to not produce Ptr ToxA.
[c]Numbers followed by the same letter are not significantly different at the 0.05 level of probability as determined by LSD.
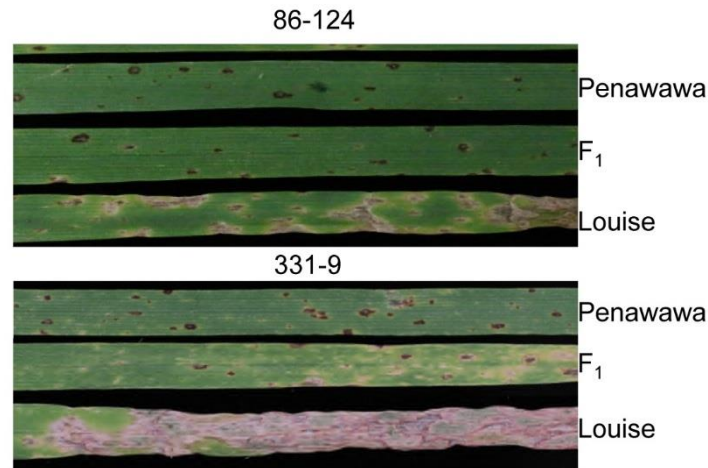
Figure 4. Reaction of F_1 plants between Louise and Penewawa to races 2 and 3. F_1 plants between Louise and Penawawa were inoculated with isolates 86-124 and 331-9 representing races 2 and 3, respectively. The leaves were photographed 7 days after inoculation.

Discussion

We identified a total of four genomic regions associated with resistance to tan spot in the LP population, all of which were derived from the resistant parent Penawawa. Among them, the QTLs on the chromosome arms 3BL (*QTs.zhl-3B*) and 5AL (*QTs.zhl-5A*) confer resistance to all races tested. Race-nonspecific resistance was first reported by Faris and Friesen (2005) in the common wheat variety 'BR34', and it was largely controlled by two QTLs with one on 1BS and the other on 3BL. Chu et al. (2008) subsequently reported chromosome arms 2AS and 5BL harboring QTLs for race-nonspecific resistance in a synthetic wheat accession. Faris et al. (2012) recently identified two QTLs on chromosome arms 5DL and 7BS also as being race-nonspecific in the wheat landrace Salamouni. Together, these results provide strong evidence that the wheat-tan spot system involves race-nonspecific resistance, and further indicates that this type of resistance may commonly occur in wheat germplasm.

QTs.zhl-3B may be the same as *QTs.fcu-3BL* identified by Faris and Friesen (2005) because both QTLs appear to exist within the same region of chromosome 3B (Fig. 3, Faris and

Friesen 2005). However, a lack of markers in common between the two 3B maps makes it difficult to draw conclusions. Mapping with more common markers within this genomic region in both mapping populations is needed to determine if *QTs.zhl-3B* and *QTs.fcu-3BL* are the same, or if different genes underlie them.

It is also interesting to note that *QTs.zhl-3B* appears to be close to the positions of the tan spot resistance genes *tsn*2 (Singh et al. 2006) and *tsn5* (Singh et al. 2008) (now designated as *tsr2* and *tsr5*, respectively; Faris et al. 2013). However, *tsr2* and *tsr5* were reported to be recessive resistance genes and specifically effective against races 3 and 5, respectively, which would suggest they are different from the gene underlying *QTs.zhl-3B* identified in the current research. It is possible that this genomic region of the chromosome 3B may contain multiple genes that have major effects on tan spot resistance/susceptibility.

Ptr ToxA was shown to be a major disease determinant for *Ptr* (Ciuffetti et al. 1997) and many QTL mapping studies have indicated that the Ptr ToxA-*Tsn1* interaction plays an important role in disease caused by races 1 and 2 (Cheong et al. 2004; Singh et al. 2008; Sun et al. 2010; Chu et al. 2008; Faris et al. 2012). However, QTL mapping by Faris and Friesen (2005) led to the identification of QTLs conferring race-nonspecific resistance with no detection of the *tsn1* locus as a significant QTL for races 1 and 2 even though the population they used segregated for *Tsn1*. It was speculated that race-nonspecific resistance QTLs might act upstream of the Ptr ToxA-*Tsn1* interaction precluding the development of necrosis. Wheat genotypes that are sensitive to Ptr ToxA, but highly resistant to races 1 and/or 2 have been reported previously (Noriel et al. 2011; Liu et al. 2015). However, genetic resistance in these genotypes has not been characterized. Using the LP population, we demonstrated that genotypes such as Penawawa carry race-nonspecific resistance. Although the entire LP population was sensitive to Ptr ToxA, most

RILs carrying *QTs.zhl-3B* Penawawa alleles were as resistant as Penawawa to race 2. In addition,

we evaluated the LP population with a race 2 *ToxA* knockout strain (86-124ΔToxA) and found

that it caused significantly less disease than wild type 86-124 only on the RILs lacking the

QTs.zhl-3B resistance allele. These results indicate that NETS from the Ptr ToxA-*Tsn1*

interaction was prohibited by the effects of the race-nonspecific resistance QTL *QTs.zhl-3B*. In

other words, *QTs.zhl-3B* has an epistatic effect on the Ptr ToxA-*Tsn1* interaction in the LP

population.

We identified a QTL on the distal end of chromosomal arm 1AS (*QTs.zhl-1A*) conferring

resistance to races 1 and 3 as well as AR CrossB10, all of which produce Ptr ToxC. Thus,

QTs.zhl-1A likely corresponds to the *Tsc1* locus which conditions sensitivity to Ptr ToxC (Effertz

et al. 2002). Faris et al. (1997) and Effertz et al. (2001) also identified QTLs for resistance to

races 1 and/or 3 on 1AS at the *Tsc1* locus. Together, these results indicate that the Ptr ToxC-*Tsc1*

interaction is important for disease caused by Ptr ToxC-producing races/isolates. Disease

dissection in the LP population showed that reactions of RILs to Ptr ToxC-producing isolates

were largely dependent on the allele types at both *QTs.zhl-3B* and *QTs.zhl-1A*. RILs with

Penawawa alleles at both loci had the lowest disease means followed by those that carried

Penawawa alleles at only one locus, and then by those that did not carry Penawawa alleles at

either locus (Table 3). This indicates that the presence of *QTs.zhl-1A* (absence of *Tsc1*) is

additive to *QTs.zhl-3B*.

Friesen and Faris (2004) were the first to map *Tsc2* and showed that the Ptr ToxB-*Tsc2*

interaction explained as much as 69% of the variation in disease caused by race 5 using the ITMI

population. Abeysekara et al. (2010) confirmed the role of the Ptr ToxB-*Tsc2* interaction in

disease caused by race 5 using a population of RILs derived from Salamouni × Kepatawa. The

LP population does not segregate at the *Tsc2* locus; therefore, it was not possible to assess the relationship of the effect of the Ptr ToxB-*Tsc2* interaction with *QTs.zhl-3B*. A population derived from Penawawa and 6B662 (sensitive to Ptr ToxB, susceptible to race 5) would serve as a good resource for this purpose.

The F_1 plants of Louise and Penawawa were highly resistant to 86-124 (race 2), but moderately resistant to 331-9 (race 3). The results indicate resistance in Penawawa, mainly conferred by the 3BL QTL, is completely dominant to susceptibility caused by race 2, but partially dominant to susceptibility by race 3. Based on the reaction to race 2, Ptr ToxA-induced necrosis in the F_1 was completely prohibited further indicating that *QTs.zhl-3B* was epistatic to the Ptr ToxA-*Tsn1* interaction. For the race 3 inoculation, F_1 plants developed mainly chlorosis across the leaves indicative of a compatible Ptr ToxC-*Tsc1* interaction indicating that the Ptr ToxC-*Tsc1* interaction is independent of the effect of *QTs.zhl-3B* as shown by the analysis of the LP population data for Ptr ToxC-producing isolates (see above).

Several lines of evidence from our research suggest the presence of additional susceptibility factors in the host besides *Tsn1*, *Tsc1* and *Tsc2*. First, the race 2 *ToxA* knock-out strain 86-124ΔToxA, which does not produce any of the three known *Ptr* NEs, was still able to cause disease in Louise and the LP population with an average lesion type of nearly 3.0 among RILs lacking the Penawawa allele at *QTs.zhl-3B*. Second, DW5, which produces only Ptr ToxB, caused average reaction types of 3.83 and 2.42 on Louise and the LP population, respectively, even though all were insensitive to Ptr ToxB. Third, Louise and some RILs developed strong necrosis (Fig. 1 and 4) after being inoculated with 331-9 which is only known to produce Ptr ToxC, a chlorosis-inducing NE. It is possible that *QTs.zhl-2D* and *QTs.zhl-5A* may represent susceptibility factors, which might consist of novel NE sensitivity genes that recognize yet

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unidentified NEs produced by these isolates. The presence of additional unidentified NE-host sensitivity interactions has been suggested in a number of other studies as well (Ciuffetti et al. 2003; Meinhardt et al. 2003; Friesen et al. 2003; Manning and Ciuffetti 2015). More work is needed to identify and characterize those potentially new interactions.

QTs.zhl-5A is the second QTL identified to confer race-nonspecific resistance in the LP population. Based on the chromosome position and common markers, we believe that *QTs.zhl-5A* is the same as *QTs.fcu-5AL* and *QTs.fcu-5A.1* that were identified in the TA4152-60/ND495 and Lebsock/PI 94749 populations, respectively (Chu et al. 2008, 2010). However, *QTs.zhl-5A* had relatively smaller effects compared to the other two. No QTL has previously been reported on 2DL using a bi-parental population, and thus *QTs.zhl-2D* might be novel.

Our work highlights the complexity of the wheat-*Ptr* pathosystem, which not only involves inverse gene-for-gene, race-specific interactions determined by the fungal-produced NEs and host sensitivity genes that leads to NETS, but also a major QTL for race-nonspecific resistance. In addition, many minor QTLs, either race-specific or race-nonspecific, might also exist to modify these two types of reactions. We provided here the first comprehensive view of how a major race-nonspecific resistance QTL is related to NETS caused by the NE and host gene interaction, which has an important application in breeding for tan spot resistance. To obtain more complete resistance, breeders should incorporate the major race-nonspecific resistance QTLs into elite lines and remove NE sensitivity genes, especially those not affected by race-nonspecific resistance, such as *Tsc1*. The molecular markers associated with the major race-nonspecific resistance QTL (*QTs.zhl-3B*) should be useful to move this QTL into breeding lines via MAS. Similarly, molecular markers linked to *QTs.zhl-1A* can be used to remove *Tsc1* from breeding lines. Nevertheless, more research is needed to investigate the genetic relationships of

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race-nonspecific resistance and NETS in a wide range of genetic backgrounds for a broad utilization of the race-nonspecific resistance genes/QTLs.

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PAPER 2: IDENTIFICATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH RESISTANCE TO TAN SPOT IN A DOUBLED HAPLOID TETRAPLOID WHEAT POPULATION

Abstract

Tan spot of wheat, caused by *Pyrenophora tritici-repentis*, is an important disease of common and durum wheat across the world. The disease not only causes significant yield losses, but also can diminish grain quality. In particular, red smudge caused by fungal infection of wheat kernels can lead to significant downgrading of durum wheat in the market. Fewer studies have been conducted to determine the chromosomal locations of tan spot resistance genes in tetraploid wheat compared to hexaploid wheat. Previously, a doubled haploid population consisting of 146 lines derived from durum cultivar 'Lebsock' and *Triticum turgidum* subsp. *carthlicum* accession PI 94749 was used to identify QTL associated with resistance to races 1 and 2. In this work, we evaluated this population for the reaction to race 3 (isolate 331-9), race 5 (isolate DW5) and a newly identified race (isolate AR CrossB10). A total of nine QTLs were identified on chromosomes 3A, 3B, 4A, 5A, 5B, 7A and 7B and explained the disease variations from 1 to 20%. The 3A QTL, which was previously shown to be associated with resistance to races 1 and 2, was also significant for all three isolates indicating it is race-nonspecific. The other QTLs were associated with resistance to one isolate or two. Lebsock contributes the resistance alleles for all QTLs except two. Three QTLs had not been detected previously and were considered to be novel. This work further indicates that both race specific and race-nonspecific resistance are also presented in tetraploid wheat genetic background.

Introduction

Tan spot can occur on both bread wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD genomes) and durum wheat (*T. turgidum* L. ssp. *durum*, 2n = 4x = 28, AABB genomes) and it is caused by the fungus *Pyrenophora tritici-repentis* (Died.) (*Ptr*) (anamorph: *Drechslera tritici-repentis* (Died.) Shoem.). With the increase of reduced or no-till farming practices that started in the 1970s, tan spot has become a significant disease in almost all wheat-growing areas of the world including USA (Hosford 1971; Rees and Platz 1992). Reduced or zero tillage practices allow pathogen to overwinter on wheat stubble as pseudothecia resulting in an increase of primary inoculum for the next growing season. Ascospores are released from pseudothecia and cause primary infections on young wheat seedlings. The infections lead to the development of the characteristic tan necrotic lesions with or without yellow halo (Lamari and Bernier 1989). Conidia are produced from the newly developed lesions and spread to other plants causing secondary infections. This cycle can be repeated multiple times and at favorable conditions, tan spot can cause yield losses of up to 50% on highly susceptible cultivars (Rees et al. 1982).

In addition to yield losses, infection of *Ptr* on wheat heads can significantly diminish grain quality by causing red smudge or pink smudge (Ferdinandez et al. 1997). Red smudge is more common in durum wheat leading to the downgrading of grain because of reddish discoloration. Based on a study done in Canada, one percent of red smudge in durum wheat can cause a drop of grain quality from grade #1 to 2, which translates into an estimated economic loss of CAN $12 per ton (Ferdinandez et al. 1998). Infected seeds usually germinate much faster than healthy seeds, and they also showed a reduction in seedling vigour, emergence and number of spikes ultimately resulting in decrease in grain yield (Ferdinandez et al. 1997). The formation of red smudge was shown to be dependent on wheat genotypes; for example, taller genotypes

usually had less percentage of kernel developing red smudge compared to shorter genotypes (Ferdinandez et al. 1998, 2002). Although crop rotation and fungicide application can be used to manage the disease, the most economical and sustainable way of managing the disease is to use genetically resistant cultivars. In order to breed tan spot resistant cultivar, genetic basis of wheat-*Ptr* interaction has to be well understood and genetic resistance has to be well characterized.

The pathosystem has been known to involve pathogen-produced host selective toxins , now referred to necrotrophic effectors (NEs), that interact with corresponding host sensitivity genes in an inverse gene-for-gene manner (Wolpert et al. 2002; Ciuffetti et al. 2010). The interaction between NE from the pathogen and the product of dominant susceptibility gene from host results in compatible interaction leading to the development of necrosis/chlorosis and thus disease. This is a mirror image of classic gene-for-gene interaction where resistance occurs upon the recognition of pathogen produced *Avr* gene product by plant resistance gene product (Flor 1956). Three such NE-susceptibility interactions have been identified in wheat-*Ptr* pathosystem, including Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1* (Faris et al. 1996; Friesen and Faris 2004; Faris et al. 1997). The dominant host susceptibility genes *Tsn1*, *Tsc2* and *Tsc1* have been mapped to chromosome arms 5BL, 2BS and 1AS, respectively (Faris et al. 1996; Faris et al. 2010; Friesen and Faris 2004; Abeysekara et al. 2009; Efferts et al. 2001). It has been shown that insensitivity to NEs (lack of dominant susceptibility genes) is highly correlated with the level of resistance in this pathosystem (reviewed by Faris et al. 2013).

Genetic analysis and mapping has also identified four other recessive resistance genes for tan spot, including *tsr2* on chromosome arm 3BL (Singh et al. 2006), *tsr3* on chromosome arm 3DL (Tedesse et al. 2006a), *tsr4* on chromosome arm 3AL (Tedesse et al. 2006b) and *tsr5* on chromosome arm 3BL (Singh et al. 2008). Genomic region associated with resistance to tan spot

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has also been investigated using genetic linkage mapping and statistical analysis, known as quantitative trait locus (QTL) mapping, in wheat natural or bi-parental populations. Many QTL have been identified significantly associated with resistance to tan spot on various chromosomes (Faris et al. 2013 for review; Patel et al. 2013; Kollers et al. 2014; Liu et al. 2015). Some QTL were located to the positions of NE sensitivity loci whereas others were not (Cheong et al. 2004; Singh et al. 2008; Faris et al. 1997, 2012, 2013; Friesen and Faris 2004; Sun et al. 2010). Interestingly, a number of QTL were found to be associated with resistance to multiple races, which has been referred to race-nonspecific resistance (Faris and Friesen 2005; Chu et al. 2008b). All these indicate the complexity of genetic interaction between wheat and *Ptr*.

So far, most of these studies were conducted on the hexaploid wheat genetic backgrounds and fewer were done on tetraploid wheat. Limited work has shown that durum wheat is generally more susceptible to tan spot disease than hexaploid wheat (Singh et al. 2006; Chu et al. 2008a). Furthermore, it was shown that race 3 and race 5 isolates which produce chlorosis on susceptible hexaploid wheat lines with *Tsc1* and *Tsc2*, respectively, produce necrosis on tetraploid wheat (Gamba and Lamari 1998). The recessive gene *tsr2* and *tsr5* that were identified in a durum wheat population conferred resistance to necrosis caused by race 3 and 5, respectively (Singh et al. 2006, 2008)

Chu et al. (2010) developed a tetraploid DH population derived from a cross between *T. turgidum* ssp. *carthlicum* (PI 94749) and durum wheat cultivar Lebsock and used it to map QTL associated with tan spot resistance for races 1 and 2 which produce Ptr ToxA. This population segregated for the reaction to Ptr ToxA, but no QTL was detected at the *Tsn1* locus. Five genomic regions were identified significantly associated with resistance to the disease caused by races 1 and 2 with two QTL on chromosome 5A, and one QTL each on chromosomes 3A, 3B

and 7B (Chu et al. 2010). Here, we use this population to map and characterize resistance to tan spot disease caused by race 3 (331-9), race 5 (DW5) and newly identified race (AR CrossB10) with the objective to gain better understanding of genetic resistance in tetraploid wheat.

Materials and methods

Plant materials

The doubled haploid population LP749 was derived from Lebsock and *T. turgidum* ssp. *carthlicum* (PI 94749) (Chu et al. 2010) was used in this study. Lebsock is a durum wheat cultivar released by the North Dakota Agricultural Experiment Station in collaboration with the USDA-ARS and it possesses resistance to stem rust and leaf rust, but is susceptible to Fusarium head blight (FHB) (Elias et al. 2001). PI 94749 is resistant to FHB and Septoria nodorum blotch (SNB) (Chu et al. 2008a). Lebsock was shown to be moderately resistant and PI 94749 was moderately susceptible to tan spot disease caused by races 1 and 2 (Chu et al. 2010). The population consists of 146 DH lines that were subjected to disease evaluations. For all the inoculations, tan spot differential lines were used including 'Salamouni', 'Glenlea' , '6B365' and '6B662' as being universal resistant, sensitive to Ptr ToxA(*Tsn1*), sensitive to Ptr ToxC (*Tsc1*) and sensitive to Ptr ToxB (*Tsc2*), respectively (Faris et al. 2013)

The seeds of parental lines, DH lines and differential lines were planted in small containers (Stuewe and Sons, Inc., Corvallis, OR) and the containers were then arranged on RL98 trays (Stuewe and Sons, Inc., Corvallis, OR). All the experiments were performed using the randomized complete block design (RCBD) with three replications and one container was treated as an experimental unit. Each super-cell container was filled with Sunshine SB100 soil (Sun Grow Horticulture, Bellevue, WA) and planted with three seeds of each entry. Each tray included sixty DH lines bordered by 38 containers planted with highly susceptible North Dakota

hard red winter wheat cultivar 'Jerry' to reduce the edge effect. Each container was treated with same amount of Osmocote Plus 15-19-12 fertilizer (Scotts Sierra Horticultural Product Company, Maysville, OH) upon planting and plants were grown for 12 to 14 days in a greenhouse room with a temperature of 20 to 25 ℃ before disease inoculation.

Fungal inoculation and disease evaluation

The population has been evaluated for reaction to tan spot caused by isolate Pti2 (race 1) and 86-124 (race 2) (Chu et al. 2010). Therefore, we evaluated this population for reaction to tan spot disease caused by three isolates that represents three more races, including 331-9 (race 3), DW5 (race 5) and AR CrossB10 (newly identified). The isolate 331-9 was known to produces Ptr ToxC whereas race 5 isolate DW5 produces Ptr ToxB. Isolate AR CrossB10 lacks *ToxA* gene and does not produce Ptr ToxA, but produce necrosis on Glenlea, which does not conform to the current race classification system (Ali et al. 2010). Our inoculation showed it produces extensive chlorosis and causes disease on 6B365 suggesting that this isolate likely produces Ptr ToxC.

Inoculum was prepared according the standard method described in Lamari and Bernier (1989). Inoculum was produced by culturing one mycelial plug at the center of petri plate containing V8-potato dextrose agar. The fungus was grown in dark for 5 days at room temperature and flooded with sterilized distilled water prior to flatten the mycelia with the bottom of a heat sterilized test tube. The water was discarded and cultures were subjected for 24 h light period at room temperature, followed by a 24 h dark period at 16 ℃ to induce conidiophore formation and sporulation, respectively. To harvest the spore for inoculation, the plates were flooded with sterilized distilled water and the culture was scratched using a flame sterilized inoculation loop. Concentration of the inoculum was adjusted to approximately 3,000

spores/ml and Tween-20 was added to the spore suspension at two drops per each 100 ml prior to the inoculation.

Plants were inoculated with the prepared spore suspension by using a paint sprayer (Husky; Home Depot) connected to an air supply with air pressure at 1.0 bar. After spray, the plants kept in a mist chamber with 100% relative humidity for 24 h and continuous light. The inoculated plants were then incubated in a growth chamber with a temperature at 21 ºC and a 12 h photoperiod for 6 days prior to disease evaluation (Liu et al. 2015). Disease reading followed a lesion-type based scale described in Lamari and Bernier (1989) with 1 being highly resistant and 5 being highly susceptible. Lines with equal amount of two reaction types were given an intermediate score.

Statistical analysis and QTL mapping

A genetic linkage map based on LP749 was previously developed using 280 SSR markers that distributed over 14 chromosomes (Chu et al. 2010). Genetic map covered a genetic distance about 2034.1 cM with an average of one marker of 7.2 cM. This map covered 75% of the whole genome (Chu et al. 2010). For QTL mapping, we drew the map using Mapdisto (Lorieux 2012) and the resulting map was used to identify QTL associated with tan spot.

Normality of the distribution of disease evaluation data was evaluated using Shapiro-Wilk in the PROC UNIVARIATE procedure (SAS Institute 2011). Homogeneity of variance of the data sets was tested using Bartlett's χ^2 test (Snedecor and Cochran 1989), or Levene's test (Levene 1960) based on the normality of the data. Disease means obtained from combining the data of homogeneous replications were used to carry out QTL mapping and analysis. QTL analysis was carried out using QGene 4.0 (Joehanes and Nelson 2008) with interval mapping function. Simple interval mapping (SIM) was used to identify significant genomic regions

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associated with disease resistance and to perform permutation test to determine the LOD threshold. Permutation test with 1000 times yielded LOD threshold values ranging from 2.8 to 3.0 for different isolates at the $p < 0.05$ significance level. Composite interval mapping (CIM) was then performed to calculate the LOD values. SIM was used to estimate the disease variation (R^2) explained by each QTL associated with resistance. In CIM, additive effect values were used to determine the origin of each resistance QTL.

Results

Reaction of parental lines and the LP749 population

Lebsock showed moderately resistant to susceptible reaction to conidia inoculation of three isolate with average disease score of 2.67 for all three isolates, whereas PI 94749 exhibited susceptible to highly susceptible reaction with average disease score ranging from 3.33 to 4.17 (Table 2.1). Lebsock developed small dark spots or isolated small size of necrotic lesions without obvious chlorosis for three isolate inoculations. In contrast, PI 94749 developed bigger necrotic lesions and in most cases these lesions coalesced to form a large area of dead tissue. However, there was also no obvious chlorosis symptom developed on PI 94749 after inoculated with races 3 and 5 (Fig. 5, Table 6). Therefore, it is likely neither Lebsock nor PI 94749 carry sensitivity gene to Ptr ToxB and Ptr ToxC.

Although the parental lines were moderately susceptible and highly susceptible to three races, the LP749 population segregated for disease reaction from highly resistant reaction (1.0) to highly susceptible reaction (5.0) for all three races suggesting the transgressive inheritance. This was also reported in Chu et al. (2010) when the population was evaluated for reaction to races 1 and 2. Average disease means of the entire population for 331-9, DW5 and AR CrossB10 were 2.86, 3.36 and 3.13, respectively (Table 6). These numbers are in the category of being

moderately susceptible and susceptible, which indicates that the entire population skewed

towards the side of susceptibility. The histograms of disease reaction of the LP749 population

also showed that the majority of DH lines have the disease reactions in the category of 3-4 and

the population shifted towards susceptible reaction (Fig. 6). Normality test revealed that disease

reaction of LP749 for all three isolates does not fit to a normal distribution ($P < 0.0001$-0.0005).

QTL analysis

Because the LP749 population was not normally distributed for reaction to all *Ptr* races,

Levene's test was used to determine the homogeneity of variances of disease evaluation among

the three replications for the each isolate. The results showed that the three replications were

homogeneous ($P = 0.06$-0.52, *df* $=2$) for all three isolates. Therefore, the average disease reaction

of the three replications for each isolate was used in the subsequent QTL analysis. A total of nine

QTL were identified associated with tan spot resistance in LP749 population, each of which

explained the disease variation from 1 to 20% (Table 7). Seven QTLs had the resistance alleles

derived from the parental line Lebsock and were located on chromosome arms 3AS, 3BS, 4AL,

5AS, 5BL and 7AL, which were designated as *QTs.zhl-3A*, *QTs.zhl-3BS*, *QTs.zhl-4A.1*, *QTs.zhl-*

4A.2, *QTs.zhl-5A.1*, *QTs.zhl-5B* and *QTs.zhl-7A*, respectively (Table 7). The parental line PI

94749 contributed the resistance allele for the remaining two QTL that were designated as

QTs.zhl-5A.2 and *QTs.zhl-7B*. *QTs.zhl-5A.2* was located adjacent to *QTs.zhl-5A.1* on

chromosome arm 5AL and *QTs.zhl-7B* was located at the distal end of the chromosome arm 7BL

(Fig. 9, Table 7).

Table 6. Lesion type means of Lebsock, PI 94749, and the Lebsock × PI 94749 doubled haploid population to conidial inoculations of *Pyrenophora tritici-repentis* races 3, 5 and isolate AR CrossB10.

Isolates [a]	Lebsock [b]	PI 94749 [b]	LP population	LP population
331-9 (race 3) (Ptr ToxC+)	2.67	3.33	2.86	1.00-4.33
DW5 (race 5) (Ptr ToxB+)	2.67	4.17	3.36	1.17-4.67
AR Cross B10 (Ptr ToxA-, Ptr ToxC+)	2.67	4.00	3.13	1.33-4.83

[a] Three isolates representing different *Pyrenophora tritici-repentis* races were used to evaluate the LP 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.
[b] Disease was scored using a 1-5 scale with 1 being highly resistant and 5 being highly susceptible.
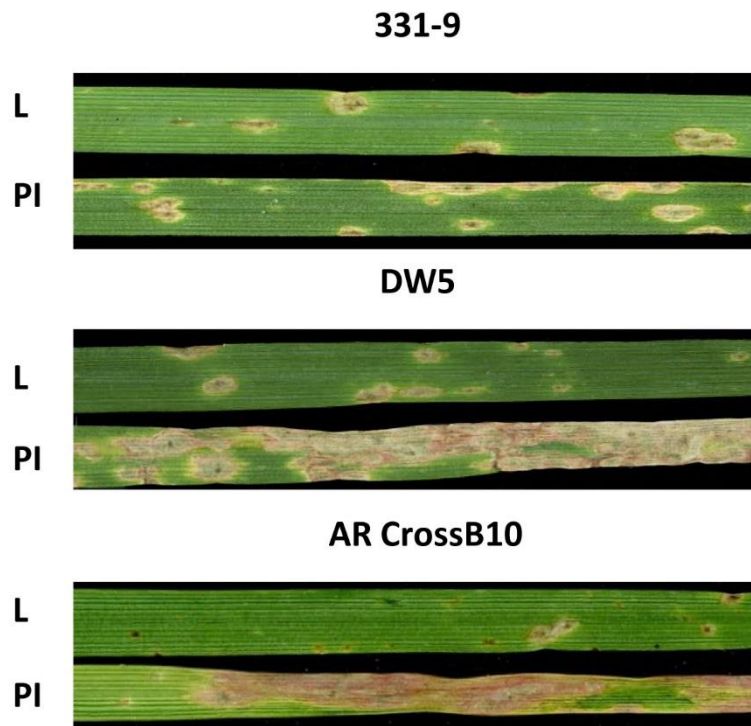
Figure 5. Reaction of parental lines Lebsock and PI 94749 to three isolates that represented different races including 331-9 (race 3), DW5 (race 5) and AR CrossB10 (unclassified race). L: Lebsock, PI: PI 94749.
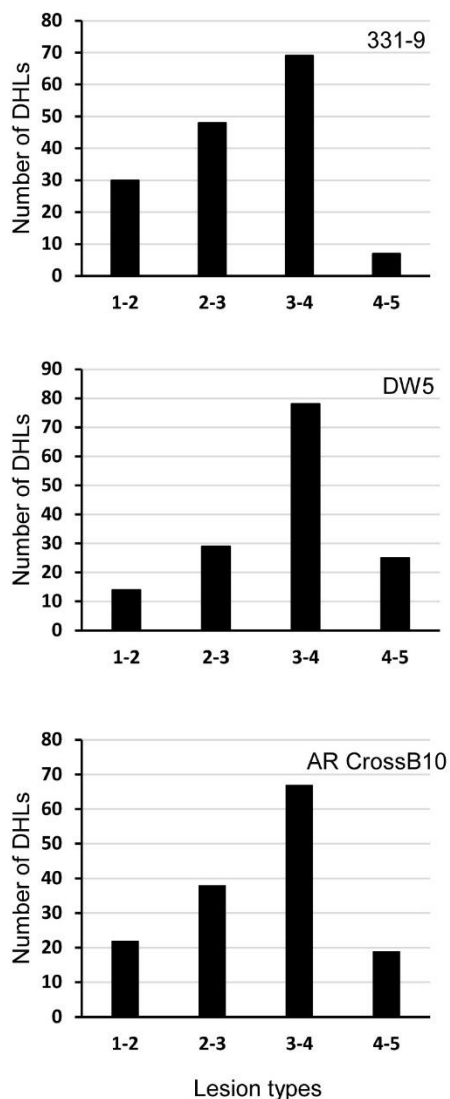
Figure 6. Histograms of disease reaction of the Lebsock × PI 94749 population to individual isolate. Population was evaluated with three isolates representing different races including 331-9 (race 3), DW5 (race 5) and AR CrossB10 (unclassified race). Disease was evaluated using 1-5 rating scale with 1 highly resistant and 5 highly susceptible. The x-axis is the lesion type based on disease scale, and y-axis is the number of double haploid lines.Histograms for disease caused by all three isolates showed high number of susceptible DH lines and less number of resistant lines.

Among the QTL identified, only *QTs.zhl-3A* associated with resistance to all three isolates tested: 331-9, DW5 and AR CrossB10, and the LOD values were 7.8, 4.7, and 5.8, respectively. This QTL spanned the genomic region flanked by *Xbarc321* and *Xwmc11* and explained the disease variations from 12 to 13%. The peak of QTL was underlined by the SSR marker *Xbarc321* that was located right on the end of chromosome arm 3AS (Fig. 7, Table 7).

QTs.zhl-7B had similar or slight higher R^2 values compared to *QTs.zhl-3A*, but was only associated with resistance to 331-9 and DW5 with LOD values of 8.1 and 6.0 respectively. R^2 values for this QTL was 20 and 12% for 331-9 and DW5, respectively. This QTL was identified between SSR markers *Xwmc273* and *Xbarc182* on chromosome 7B and it was peaked on the marker *Xbarc32* (Fig. 9, Table 7).

The chromosome arm 4AL harbored two QTL, *QTs.zhl-4A.1* and *QTs.zhl-4A.2*. *QTs.zhl-4A*.1 was located between *Xwmc707* and *Xcfd88* and *QTs.zhl-4A*.2 spanned the genomic region between *Xwmc232* and *Xwmc723,* which is about 20 cM away. *QTs.zhl-4A.1* was only significant for disease caused by DW5 while *QTs.zhl-4A.2* was significant for both DW5 and AR CrossB10. *QTs.zhl-4A.1* had a LOD value of 3.0 and explained 11% of disease variation. *QT.zhl-4A.2* explained 12 and 14% of the genetic variation in disease by AR CrossB10 and DW5, respectively (Fig. 8 and Table 7).

There were also two resistance QTL identified on chromosome 5A with one likely on short arm between SSR markers *Xbarc360* and *Xgwm6.2* (*QTs.zhl-5A.1*) and the other on the long arm between *Xwmc110* and *Xgwm595* (*QTs.zhl-5A.2*) (Fig. 2.4). *QTs.zhl-5A.1*accounted for 10% of the disease caused by AR CrossB10 and *QTs.zhl-5A.2* explained around 6% of the disease for both 331-9 and AR CrossB10 (Table 7). Another two small QTLs: *QTs.zhl-5B* and *QTs.zhl-7A* were identified on chromosome arms of 5BL and 7BL, respectively (Fig. 9). *QTs.zhl-*

5B was close to distal side of the *Tsn1* locus and accounted for disease variation of 6% and 10% for DW5 and AR CrossB10, respectively (Table 2.2). The 7A QTL had a similar size of effect but conferred resistance to 331-9 and DW5. Lastly, *QTs.zhl-3B.1* on chromosome arm 3BL is a very minor QTL that explained only 1% of the disease variation for 331-9 (Fig. 7, Table 7).

Table 7. Composite interval mapping analysis of QTLs associated with resistance to tan spot caused by *Pyrenophora tritici-repentis* races 3, 5 and isolate AR CrossB10 in the Lebsock × PI94749 doubled haploid line population.

QTL	Interval (cM)	Flanking Markers	Closest Marker	R^{2a}			LOD[b]			Source[c]
				331-9	DW5	AR CrossB10	331-9	DW5	AR CrossB10	
QTs.zhl-3A	0-2.0	*Xbarc321-Xwmc11*	*Xbarc321*	0.13	0.12	0.12	7.8	5.0	5.8	L
QTs.zhl-3B.1	36.0-52.0	*Xbarc147-Xwmc78*	*Xbarc101.1*	0.01	NS	NS	3.2	NS	NS	L
QTs.zhl-4A.1	82.0-114.0	*Xwmc707-Xcfd88*	*Xwmc718*	NS	0.11	NS	NS	3.7	NS	L
QTs.zhl-4A.2	132.0-150.0	*Xwmc232-Xwmc723*	*Xbarc78*	NS	0.14	0.12	NS	5.1	3.5	L
QTs.zhl-5A.1	26.0-44.0	*Xbarc425-Xgwm6.1*	*Xbarc360*	NS	NS	0.10	NS	NS	4.0	L
QTs.zhl-5A.2	138.0-152.0	*Xwmc110-Xgwm595*	*Xgwm6.2*	0.06	NS	0.07	3.9	NS	4.5	P
QTs.zhl-5B	96.0-114.0	*Tsn1-Xbarc140*	*Xwmc75*	NS	0.06	0.09	NS	3.3	5.2	L
QTs.zhl-7A	130.0-140.0	*Xbarc174-Xbarc121*	*Xbarc174*	0.09	0.10	NS	3.0	4.4	NS	L
QTs.zhl-7B	132.0-140.0	*Xwmc273-Xbarc182*	*Xbarc32*	0.20	0.12	NS	8.1	6.0	NS	P

[a] R^2 = the coefficient of determination. The R^2 value × 100 represents the amount of phenotypic variation explained. NS indicates the QTL was not significant.
[b] LOD was determined by the execution of 1000 permutations on marker and phenotypic datasets, which yielded a value of 2.8 as the cutoff for the detection of significant QTLs.
[c] The source of each QTL indicates the resistance allele was contributed by one of the parental lines with L being Lebsock and P being PI 94749.
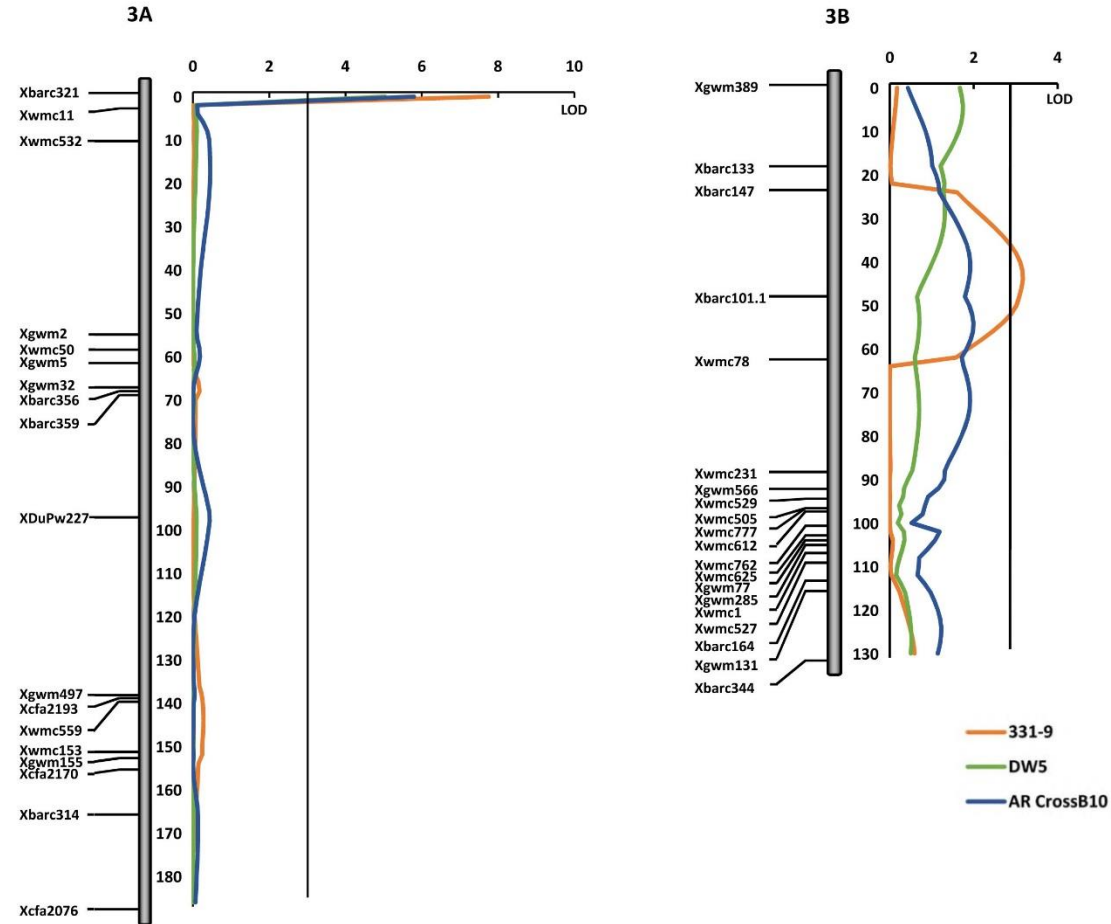
Figure 7. Composite interval maps of chromosomes 3A and 3B containing significant QTL associated with resistance to tan spot. QTL mapping was conducted on the LP749 population using *Pyrenophora tritici-repentis* isolates 331-9 (race 3, orange), DW5 (race 5, green) and AR CrossB10 (unclassified isolate, blue). Positions of the marker loci are shown in left side and genetic scale in centiMorgans (cM) is shown in the right side of the linkage group. Solid black line represents the LOD threshold of 2.8.
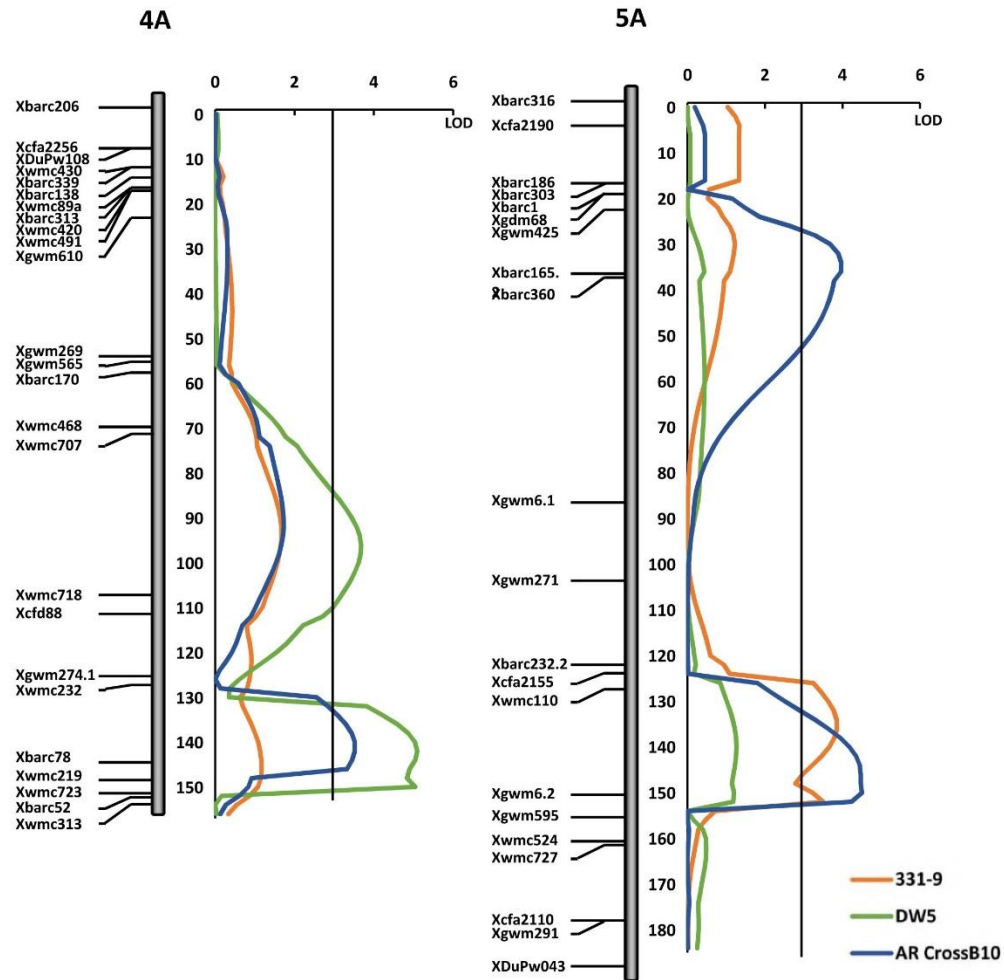
Figure 8. Composite interval maps of chromosomes 4A and 5A containing significant QTL associated with resistance to tan spot. QTL mapping was conducted on the LP749 population using *Pyrenophora tritici-repentis* isolates 331-9 (race 3, orange), DW5 (race 5, green) and AR CrossB10 (unclassified isolate, blue). Positions of the marker loci are shown in left side and genetic scale in centiMorgans (cM) is shown in the right side of the linkage group. Solid black line represents the LOD threshold of 2.8.
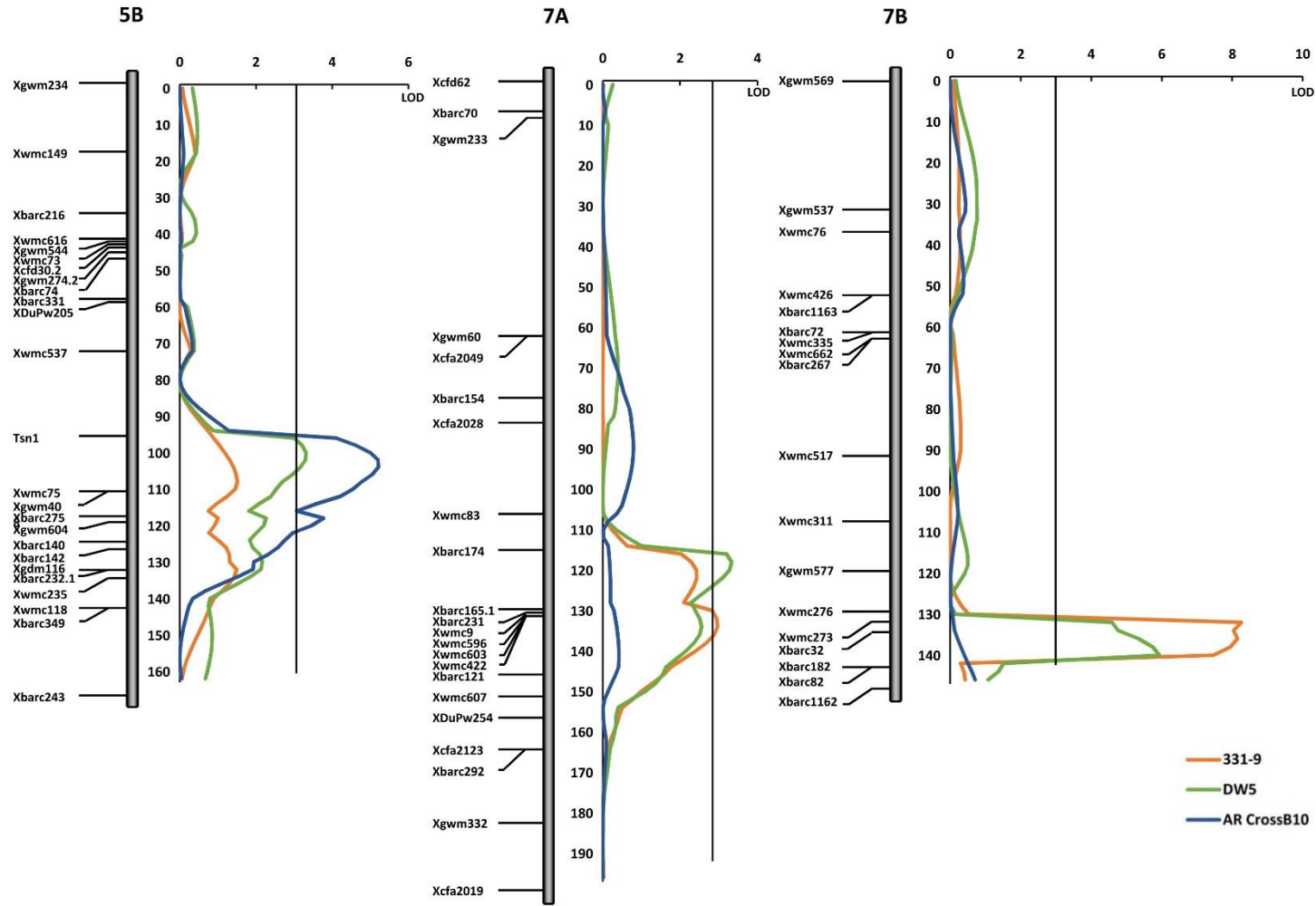
Figure 9. Composite interval maps of chromosomes 5B, 7A and 7B containing significant QTL associated with resistance to tan spot. QTL mapping was conducted on the LP749 population using *Pyrenophora tritici-repentis* isolates 331-9 (race 3, orange), DW5 (race 5, green) and AR CrossB10 (unclassified isolate, blue). Positions of the marker loci are shown in left side and genetic scale in centiMorgans (cM) is shown in the right side of the linkage group. Solid black line represents the LOD threshold of 2.8.

Discussion

P. tritici-repentis is a diverse fungal pathogen with more than eight races having been described (Lamari and Strelkov 2003; Faris et al. 2013; Ali et al. 2010) and genetic resistance in wheat germplasm should be evaluated and characterized for reaction to all races possible. Previously, Chu et al. (2010) evaluated the LP749 population for reaction to races 1 and 2 isolates and identified QTL associated with resistance to these two races. In this work, we evaluated the population for reaction to other three important virulent *Ptr* races and identified corresponding genomic regions for these three races. Therefore, this research provides the first more complete view on the genetic architecture governing resistance/susceptibility to tan spot, particularly in the tetraploid wheat background.

We identified a total of nine chromosomal regions that are associated with resistance to tan spot disease caused by three *Ptr* isolates, including 331-9 (race 3), DW5 (race 5) and AR CrossB10 (unclassified isolate). Among them, four QTL were identified on the similar regions as those identified by Chu et al. (2010) that are associated with resistance for races 1 and 2, including *QTs.zhl-3A*, *QTs.zhl-5A.1*, *QTs.zhl-5A.2* and *QTs.zhl-7B* and they are considered to be same as *QTs.fcu-3A*, *QTs.fcu-5A.1*, *QTs.fcu-5A.2* and *QTs.fcu-7B*, respectively (Chu et al. 2010).

Among all QTL identified, only *QTs.zhl-3A* was found to confer resistance to all races tested indicating it is race-nonspecific. Race-nonspecific resistance in wheat tan spot was first reported by Faris and Friesen (2005) on chromosomes 1B and 3B in a hexaploid wheat population derived from the resistant parent cultivar 'BR34'. Later, race-nonspecific resistance QTL were also identified in other hexaploid populations and found to involve chromosome arms 2AS, 5BL, 5DL and 7BS (Chu et al. 2008b; Faris et al. 2012). Recently, we also mapped a major QTL on 3BL conferring race-nonspecific resistance in hexaploid wheat cultivar 'Penawawa' (see

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Chapter 1). However, no race-nonspecific resistance QTL has been mapped to chromosome arm 3AS, and thus *QTs.zhl-3A* is a novel QTL conferring race-nonspecific resistance. Furthermore, this is also the first report of race-nonspecific resistance QTL in tetraploid wheat. Our research indicates that race-nonspecific resistance to *Ptr* different races is also presented in tetraploid wheat.

QTs.zhl-3A is unlikely homoeologous to the ones reported on 3B, including *QTs.zhl-3B* (chapter 1) and *QTs.fcu-3B* (Faris and Friesen 2005) because *QTs.zhl-3A* mapped to the most distal end of 3AS while the other two were located to the long arm of chromosome 3B. Singh et al. (2008) reported a QTL at the distal end of 3AS for resistance to tan spot in a hexaploid wheat population derived from resistant spring wheat cultivar 'WH542' and a moderately susceptible cultivar 'HD29'. Because the study only used a race 1 isolate, it is unclear whether it is race-nonspecific or not. However, this QTL is located very closely to that of *QTs.zhl-3A* based on the relative position of the common SSR *Xwmc11*. Evaluating the WH542 × HD29 population with more races will allow us to know race specificity of this 3AS QTL. It is possible that this 3AS QTL conferring race-nonspecific resistance is presented in both hexaploid and tetraploid levels. The resistance gene in winter wheat cultivar 'Red Chief', designated as *tsr4*, was also mapped to chromosome 3A close to SSR markers *Xgwm2* and *Xgwm5* (Tadesse et al. 2010). Very limited number of markers was available in the mapping of *tsr4* gene, therefore, it is difficult to compare the relationship between *QTs.zhl-3A* and *tsr4* gene.

Ptr ToxA-*Tsn1* interaction has been shown to unimportant in disease development in several hexaploid populations where race-nonspecific resistance has been detected (Faris and Friesen 2005, chapter 1) indicating that race-nonspecific resistance is able to genetically mask the effect from the Ptr ToxA-*Tsn1* interaction. It was speculated that reaction from race-

nonspecific QTL might completely prohibit the development of necrosis due to Ptr ToxA-*Tsn1* interaction. Although the LP749 population segregates for reaction to Ptr ToxA, no QTL was detected at the *Tsn1* locus for races 1 and 2 which produce Ptr ToxA. It is possible that the effect of Ptr ToxA-*Tsn1* interaction in this tetraploid population is also masked by reaction from the 3AS race-nonspecific resistance QTL. In chapter 1, we also showed that the race-nonspecific resistance QTL has no epistatic effect on Ptr ToxC-*Tsc1* interaction. Both parental lines did not show chlorosis symptoms after inoculated with race 5 and 331-9 which are known to produce Ptr ToxB and Ptr ToxC, respectively. This suggests that Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1* interaction are not presented in the LP749 population. Therefore, effect of race-nonspecific resistance QTL on these two NE-sensitivity interactions remains unknown in this tetraploid population.

QTs.zhl-7B was significant for all races except AR CrossB10. The QTL accounted for disease variation of 6 to 7% reported by Chu et al. (2010) for races 1 and 2, but 12 to 20% in this study for races 3 and 5. This QTL has not been identified in other published hexaploid wheat populations. Similarly, *QTs.zhl-5A.2* was detected as a significant QTL for four out of five isolates in LP749 and has not been identified elsewhere. Therefore, these two QTLs might be unique to tetraploid wheats. Interestingly, both QTLs were derived from the susceptible parent PI 94749.

The second QTL identified on chromosome arm 5AS may be the same QTL identified by Faris and Friesen (2005), Chu et al. (2008b) and Chu et al. (2010). We also observed a QTL chromosome arm 5AS at a similar location in Louise/Penawawa population in the previous study for all used races (see Chapter 1). Therefore, this QTL may be commonly occurred in wheat.

The four QTLs identified in study were not detected for races 1 and 2 by Chu et al. (2010), including *QTs.zhl-4A.1*, *QTs.zhl-4A.2*, *QTs.zhl-5B* and *QTs.zhl-7A* and they were associated with resistance to one isolate or two. *QTs.zhl-4A.2* had the largest R^2 value (12-14%) among them and were significant for DW5 and AR CrossB10. The QTL was delimited to the genetic interval flanked by *Xwmc232* and *Xwmc723*. Therefore, we believe that it is different from the previously reported QTL on chromosome 4A (Faris et al. 1997; Friesen and Faris 2004; Faris and Friesen 2005; Chu et al. 2008b), thus likely a novel QTL. However, the second QTL on chromosome 4AL (*QTs.zhl-4A.1*) which was only significant for DW5 could be the same as the one identified by Chu et al. (2008) on chromosome arm 4AL because both QTL mapped same side of the common marker *Xwmc232*. However, *QTs.fcu-4AL* reported by Chu et al. (2008) was observed for race 3 isolate OH99 suggesting the two could also be different.

A number of tan spot resistance QTL have been identified on chromosome arm 5BL, including some undesignated QTLs (Cheong et al. 2004; Li et al. 2011) and designated QTLs such as *QTs.fcu-5BL.1*, *QTs.fcu-5BL.2* (Chu et al. 2008), *QTs.ksu-5BL* (Singh et al. 2008), and *QTs.fcu-5B* (Faris et al. 2012), all of which except *QTs.fcu-5BL.1* corresponded to the *Tsn1* locus. The 5BL QTL (*QTs.zhl-5B*) we identified should not be the *Tsn1* locus because the QTL associated with disease caused by DW5 and AR CrossB10 which does not produce Ptr ToxA. *QTs.zhl-5B* and *QTs.fcu-5BL.1* could be different because they were located different side of the *Tsn1* gene; therefore, we believe *QTs.zhl-5B* is a new one. The QTL on chromosome 7A which confers resistance to 331-9 and DW5 has not been reported by using bi-parental population mapping. However, two association mapping studies detected tan spot resistance QTL on chromosome 7A (Patel et al. 2013; Liu et al. 2015).

In summary, we identified a total of nine QTL in the LP749 populaiton associated with tan spot disease caused by 331-9, DW5 and AR CrossB10. Based on our results and these from Chu et al. (2010), we found *QT.zhl-3A* was only one conferring resistance to all races tested providing the first evidence that race-nonspecific resistance is also presented in tetraploid wheat. We also identified QTLs that might be new and/or unique to durum wheat. These QTLs as well as the close markers to them can be used to facilitate the development of durum cultivars with resistance to tan spot.

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APPENDIX A. PHENOTYPIC DATA FOR THE TAN SPOT DISEASE CAUSED BY PTI2 (RACE 1), 86-124 (RACE 2), 86-124ΔTOXA, 331-9 (RACE 3), DW5 (RACE5) AND AR CROSSB10 (NEW RACE) ON LOUISE×PENAWAWA POPULATION

RIL ID.	RIL No.	Pti2	86-124	86-124ΔToxA	331-9	DW5	AR CrossB10
Louise-1	Ind189	4.12	3.08	2.33	3.83	3.75	4.17
Penawawa-2	Ind195	1.58	1.13	1.25	1.16	1.00	1.33
RIL-001	Ind1	2.83	2.67	3.25	2.67	3.00	2.83
RIL-002	Ind2	3.00	1.67	2.50	3.17	2.83	3.67
RIL-004	Ind3	3.67	2.33	2.38	3.33	4.33	4.67
RIL-006	Ind4	3.33	1.00	1.00	1.33	2.33	2.00
RIL-007	Ind5	3.50	2.33	2.63	3.83	3.50	4.17
RIL-008	Ind6	2.50	1.83	1.88	1.00	1.33	2.50
RIL-009	Ind7	1.83	1.83	2.25	1.00	2.83	2.67
RIL-010	Ind8	2.50	2.33	2.17	1.25	1.00	1.67
RIL-011	Ind9	4.17	3.50	3.88	4.33	3.67	4.50
RIL-013	Ind10	2.50	1.83	2.25	1.33	1.67	2.33
RIL-014	Ind11	2.33	2.00	1.88	1.83	2.83	1.67
RIL-015	Ind12	1.67	1.17	1.00	1.83	1.00	2.50
RIL-016	Ind13	3.50	1.83	2.88	3.17	2.50	4.50
RIL-017	Ind14	2.33	1.00	1.63	2.00	1.67	3.33
RIL-018	Ind15	3.00	1.17	2.00	2.33	1.33	2.83
RIL-019	Ind16	4.17	3.67	3.88	3.00	4.33	4.50
RIL-020	Ind17	2.83	2.83	3.00	1.67	2.83	2.75
RIL-021	Ind18	3.17	1.67	1.88	1.83	2.83	2.17
RIL-022	Ind19	2.83	1.67	1.38	1.17	2.50	2.83
RIL-023	Ind20	1.83	1.33	2.17	1.00	2.17	2.33
RIL-024	Ind21	4.50	3.33	3.13	4.67	4.17	4.50
RIL-025	Ind22	2.83	1.17	1.00	1.83	1.50	3.00
RIL-026	Ind23	2.17	1.33	1.88	ND	1.33	2.67
RIL-028	Ind24	3.50	3.50	3.63	3.67	4.83	3.50
RIL-029	Ind25	1.50	1.33	1.50	1.17	2.00	1.17
RIL-030	Ind26	1.33	1.33	1.50	1.00	1.00	2.17
RIL-031	Ind27	3.67	2.17	2.38	3.83	2.83	2.83
RIL-032	Ind28	1.83	1.17	1.13	2.33	1.00	2.50
RIL-033	Ind29	3.83	1.67	1.25	2.83	1.00	3.50
RIL-034	Ind30	4.00	3.17	3.13	3.83	3.17	2.83
RIL-035	Ind31	2.33	2.00	2.38	3.33	3.75	3.50
RIL-036	Ind32	3.00	3.00	2.88	3.17	2.33	4.33
RIL-037	Ind33	2.83	2.83	3.00	3.17	3.50	3.50
RIL-038	Ind34	1.25	1.17	1.00	2.00	1.00	2.17
RIL-039	Ind35	4.17	2.17	2.25	3.83	2.17	2.67
RIL-040	Ind36	3.50	3.67	3.38	2.50	3.50	4.17
RIL-041	Ind37	4.50	3.00	2.88	3.33	3.17	4.67
RIL-042	Ind38	2.00	1.67	1.38	1.50	2.67	1.67
RIL-044	Ind39	3.17	3.00	2.63	3.67	3.50	3.17
RIL-045	Ind40	3.83	3.17	2.88	3.67	3.50	2.00
RIL-048	Ind41	2.50	1.67	1.25	1.83	2.00	2.83
RIL-050	Ind42	1.33	2.00	1.50	1.17	2.33	3.17
RIL-051	Ind43	3.83	3.33	3.75	3.50	4.00	4.00
RIL-052	Ind44	3.33	2.00	1.83	2.83	2.83	2.33
RIL-053	Ind45	3.83	3.00	2.00	4.00	2.83	2.67
RIL-055	Ind46	3.83	2.83	3.00	2.83	3.83	4.00
RIL-056	Ind47	3.33	3.83	3.25	3.17	3.33	3.33
RIL-058	Ind48	2.50	2.67	2.63	2.17	3.00	3.00
RIL-059	Ind49	3.33	3.00	3.25	2.50	4.17	3.33
RIL-060	Ind50	1.17	1.50	1.25	1.00	1.17	1.17

RIL ID.	RIL No.	Pti2	86-124	86-124ΔToxA	331-9	DW5	AR CrossB10
RIL-062	Ind52	2.83	2.83	3.50	3.33	3.17	3.67
RIL-063	Ind53	2.00	1.17	1.13	2.00	1.33	2.67
RIL-064	Ind54	1.17	1.50	1.00	1.00	1.00	1.50
RIL-065	Ind55	1.33	1.17	1.00	1.33	1.00	1.67
RIL-066	Ind56	2.83	3.00	3.00	2.33	2.67	3.17
RIL-067	Ind57	3.17	1.67	1.25	3.00	2.67	4.33
RIL-069	Ind58	3.17	3.00	2.13	3.33	2.83	3.50
RIL-070	Ind59	1.50	1.17	1.50	1.00	1.17	2.17
RIL-071	Ind60	4.00	2.67	2.88	3.17	2.67	4.17
RIL-072	Ind61	3.67	2.50	2.50	3.17	3.67	4.50
RIL-073	Ind62	3.00	1.33	2.00	2.00	2.17	2.33
RIL-074	Ind63	3.67	3.50	3.88	3.33	4.17	3.25
RIL-076	Ind64	4.50	3.67	3.75	3.67	4.67	4.75
RIL-077	Ind65	1.00	1.00	1.13	1.00	1.33	1.33
RIL-078	Ind66	1.17	1.33	1.25	1.00	1.67	2.00
RIL-079	Ind67	2.33	1.50	1.63	1.00	1.67	2.00
RIL-080	Ind68	1.17	1.00	1.25	1.00	1.00	2.17
RIL-081	Ind69	1.33	1.67	1.25	1.00	2.17	1.50
RIL-082	Ind70	2.33	2.00	1.75	2.50	1.83	3.33
RIL-083	Ind71	2.00	3.17	1.75	1.50	3.00	3.00
RIL-084	Ind72	1.83	2.83	2.25	1.33	1.67	2.83
RIL-085	Ind73	3.67	1.67	2.88	3.33	2.50	4.17
RIL-086	Ind74	4.50	3.50	3.50	4.17	4.50	3.67
RIL-087	Ind75	3.17	3.17	3.25	3.33	3.67	4.00
RIL-088	Ind76	4.00	2.83	3.13	3.17	4.50	4.33
RIL-089	Ind77	3.33	2.33	2.50	1.67	3.50	3.00
RIL-090	Ind78	1.50	1.33	1.63	1.00	1.00	1.33
RIL-091	Ind79	2.67	2.67	2.75	3.67	3.00	3.33
RIL-092	Ind80	2.50	2.67	2.75	1.33	3.00	2.33
RIL-094	Ind81	4.33	3.00	2.75	3.17	3.50	4.33
RIL-095	Ind82	2.33	1.00	1.50	1.00	1.83	2.00
RIL-096	Ind83	4.50	3.67	3.63	4.50	3.50	4.50
RIL-097	Ind84	1.83	1.83	2.25	1.00	2.00	2.17
RIL-098	Ind85	3.00	2.83	2.75	3.17	3.83	4.33
RIL-099	Ind86	1.33	1.17	1.50	1.17	2.67	2.17
RIL-100	Ind87	1.50	1.00	1.25	1.00	2.33	1.33
RIL-102	Ind88	3.33	2.67	2.88	2.33	4.67	3.17
RIL-103	Ind89	3.67	2.33	1.88	1.33	1.83	3.33
RIL-104	Ind90	2.83	2.83	2.38	2.50	3.83	2.33
RIL-105	Ind91	2.83	3.17	2.75	2.00	4.17	3.33
RIL-107	Ind92	2.50	3.00	2.25	1.67	3.00	2.67
RIL-108	Ind93	3.67	3.67	3.63	3.00	4.50	3.67
RIL-109	Ind94	4.33	3.67	3.63	3.50	5.00	4.33
RIL-110	Ind95	1.67	1.50	1.50	1.00	1.17	1.50
RIL-111	Ind96	2.50	1.00	1.38	1.67	2.00	2.50
RIL-112	Ind97	3.50	2.50	2.88	3.00	4.33	3.83
RIL-114	Ind98	4.17	3.00	3.38	2.83	4.00	3.50
RIL-116	Ind99	3.83	4.17	3.63	3.33	4.17	4.17

RIL ID.	RIL No.	Pti2	86-124	86-124ΔToxA	331-9	DW5	AR CrossB10
RIL-117	Ind100	3.17	2.50	2.25	1.50	2.83	3.33
RIL-119	Ind101	2.00	1.33	1.25	1.00	1.00	1.83
RIL-120	Ind102	2.83	1.50	1.00	1.00	2.00	3.00
RIL-121	Ind103	3.33	2.33	3.00	2.50	4.00	2.33
RIL-122	Ind104	3.50	3.00	3.38	3.17	3.17	2.83
RIL-123	Ind105	2.50	2.33	1.88	2.67	1.50	3.50
RIL-124	Ind106	3.83	3.00	2.50	3.83	4.33	3.67
RIL-125	Ind107	4.50	3.83	3.50	3.83	4.33	3.17
RIL-126	Ind108	3.67	3.67	3.50	2.33	2.67	3.67
RIL-127	Ind109	1.67	1.50	1.25	1.00	1.17	1.17
RIL-128	Ind110	2.83	2.67	2.25	2.00	2.50	2.67
RIL-129	Ind111	3.50	1.67	2.13	1.17	1.83	3.17
RIL-131	Ind112	3.17	1.83	1.75	3.17	2.33	2.67
RIL-132	Ind113	4.00	2.67	2.67	3.33	4.33	4.17
RIL-133	Ind114	4.00	3.17	3.13	2.50	3.67	3.50
RIL-134	Ind115	3.33	2.50	2.50	2.83	2.17	3.33
RIL-136	Ind116	4.17	3.67	2.88	3.17	3.00	3.50
RIL-137	Ind117	2.83	1.00	1.25	1.00	1.33	1.50
RIL-138	Ind118	1.33	1.00	1.13	1.00	1.83	1.00
RIL-139	Ind119	1.50	1.67	1.50	1.00	1.00	1.33
RIL-140	Ind120	3.00	2.83	2.63	2.33	4.00	2.17
RIL-142	Ind121	2.00	2.33	2.00	1.33	2.00	2.67
RIL-143	Ind122	2.33	2.33	2.38	2.00	2.50	2.67
RIL-144	Ind123	3.83	3.00	3.13	3.17	2.50	3.33
RIL-145	Ind124	3.00	2.67	2.00	2.50	2.33	3.00
RIL-146	Ind125	2.50	1.00	1.00	1.00	1.17	1.83
RIL-147	Ind126	3.33	3.17	3.38	2.83	3.33	4.00
RIL-148	Ind127	3.83	3.50	3.25	3.17	3.67	3.67
RIL-150	Ind128	3.83	3.33	2.88	4.00	3.00	3.17
RIL-151	Ind129	2.83	1.50	1.88	2.67	1.33	4.33
RIL-152	Ind130	3.67	3.83	3.88	3.50	3.67	3.83
RIL-153	Ind131	3.17	3.00	3.25	2.50	3.67	4.50
RIL-155	Ind132	2.67	1.67	1.50	2.00	2.00	1.83
RIL-156	Ind133	2.67	1.17	1.13	1.17	1.17	2.33
RIL-157	Ind134	1.67	1.33	1.13	2.17	1.00	2.17
RIL-158	Ind135	1.83	1.33	1.50	1.00	1.00	1.33
RIL-159	Ind136	2.00	2.00	1.75	1.17	1.00	1.67
RIL-161	Ind137	1.33	1.83	2.25	2.00	3.33	2.00
RIL-163	Ind138	1.67	1.83	1.50	2.50	2.83	3.00
RIL-164	Ind139	2.33	2.83	2.63	1.33	3.00	2.75
RIL-166	Ind140	4.00	3.67	3.63	3.67	3.83	4.17
RIL-167	Ind141	1.17	1.00	1.00	1.00	1.17	1.50
RIL-169	Ind142	3.33	3.17	3.00	3.33	3.17	2.67
RIL-170	Ind143	3.17	3.00	2.38	3.00	2.83	4.17
RIL-171	Ind144	3.17	2.00	2.25	1.17	1.83	2.50
RIL-172	Ind145	1.00	1.00	1.17	1.00	1.17	1.50
RIL-173	Ind146	4.50	2.83	3.50	3.17	3.00	2.83
RIL-174	Ind147	3.83	3.00	3.00	3.83	3.33	2.83
RIL-175	Ind148	1.83	1.67	2.00	1.50	1.50	2.50

RIL ID.	RIL No.	Pti2	86-124	86-124ΔToxA	331-9	DW5	AR CrossB10
RIL-176	Ind149	1.50	1.50	1.38	2.67	2.00	3.00
RIL-177	Ind150	3.17	3.17	2.50	3.17	3.50	3.83
RIL-178	Ind151	2.83	2.33	1.88	3.67	3.83	3.33
RIL-179	Ind152	2.50	3.00	2.75	3.38	3.17	2.50
RIL-181	Ind153	4.00	3.17	2.88	4.00	3.00	3.00
RIL-182	Ind154	3.33	3.00	3.13	4.50	3.67	3.67
RIL-183	Ind155	3.50	3.67	4.13	2.83	3.17	2.83
RIL-185	Ind156	1.50	1.67	2.38	1.50	1.33	2.33
RIL-186	Ind157	2.67	2.00	2.00	1.67	2.83	2.33
RIL-187	Ind158	3.17	2.83	1.88	3.50	2.33	3.50
RIL-188	Ind159	2.83	2.83	2.13	3.67	2.83	3.50
RIL-190	Ind160	1.17	1.00	1.13	1.17	1.00	1.50
RIL-193	Ind161	2.67	2.33	1.67	3.33	3.83	3.67
RIL-194	Ind162	1.33	1.00	1.13	1.50	1.00	1.50
RIL-195	Ind163	2.00	3.00	1.63	1.50	1.00	1.67
RIL-196	Ind164	2.33	1.83	2.00	2.17	1.33	2.50
RIL-197	Ind165	3.00	3.33	3.13	2.83	3.33	3.67
RIL-198	Ind166	2.17	2.50	2.38	3.33	2.00	3.17
RIL-201	Ind167	2.83	3.50	2.88	2.50	2.33	2.50
RIL-203	Ind168	4.00	3.17	2.38	3.17	1.83	3.33
RIL-204	Ind169	1.33	1.33	1.38	1.00	1.50	1.67
RIL-205	Ind170	4.17	3.83	4.00	4.50	3.83	3.67
RIL-206	Ind171	4.00	3.33	2.38	4.00	3.83	4.00
RIL-208	Ind172	3.50	2.17	2.75	2.50	1.50	3.00
RIL-209	Ind173	3.00	2.00	2.17	3.67	2.50	2.75
RIL-210	Ind174	1.83	1.17	1.25	1.00	1.00	2.33
RIL-211	Ind175	2.00	1.67	1.88	1.83	1.33	2.50
RIL-212	Ind176	4.00	4.00	3.75	3.67	3.67	3.33
RIL-213	Ind177	3.33	2.17	2.13	3.83	1.67	3.17
RIL-214	Ind178	3.33	3.00	2.75	3.67	3.50	4.00
RIL-217	Ind179	2.17	1.67	1.88	1.17	1.67	1.83
RIL-218	Ind180	3.83	3.67	3.63	3.17	4.17	2.83
RIL-220	Ind181	3.67	3.50	2.83	3.67	2.83	3.00
RIL-221	Ind182	1.00	1.00	1.00	1.00	1.00	1.17
RIL-222	Ind183	3.00	1.83	1.67	3.17	1.67	2.83
RIL-223	Ind184	2.50	1.17	2.17	2.33	1.00	2.00
RIL-224	Ind185	3.00	2.17	2.33	3.17	3.33	2.33
RIL-225	Ind186	3.50	2.83	2.00	2.67	2.67	3.83
RIL-228	Ind187	3.17	3.50	2.75	3.33	2.67	2.83
RIL-230	Ind188	1.17	1.33	1.00	1.00	1.00	1.00
Differential	Salamouni	1.00	1.00	1.00	1.00	1.00	1.00
Differential	Glenlea	2.75	3.67	2.12	1.50	3.00	3.33
Differential	6B365	3.25	2.83	2.67	4.30	2.00	4.50
Differential	6B662	3.00	1.50	2.50	2.50	3.17	2.17

APPENDIX B. PHENOTYPIC DATA FOR THE TAN SPOT DISEASE CAUSED BY 331-9 (RACE 3), DW5 (RACE5) AND AR CROSSB10 (UNCHARACHTERIZED RACE) ON LEBSOCK×PI 94749 POPULATION

RIL No.	331-9	DW5	AR CrossB10
Lebsock	2.67	2.50	2.67
PI 94749	3.33	4.17	4.00
LP1	3.17	3.50	2.17
LP2	3.00	3.50	3.33
LP3	3.00	3.17	3.33
LP4	3.67	3.50	3.67
LP5	3.67	4.00	4.17
LP6	3.33	3.83	3.33
LP7	3.83	4.00	4.67
LP8	3.67	4.00	3.00
LP9	4.00	4.33	4.00
LP10	3.00	4.00	3.33
LP11	3.67	3.33	3.67
LP12	3.33	3.17	2.83
LP13	3.67	4.00	3.67
LP14	1.67	2.33	1.67
LP15	2.67	2.50	1.67
LP16	4.00	4.00	3.67
LP17	3.17	4.00	3.33
LP18	3.00	4.17	2.83
LP19	3.83	4.00	3.83
LP20	2.17	1.67	2.00
LP21	1.50	2.33	1.83
LP22	3.17	3.83	3.33
LP23	3.33	3.33	3.83
LP24	3.50	3.67	2.83
LP25	2.50	3.17	1.50
LP26	2.83	3.00	2.67
LP27	2.00	1.67	2.50
LP28	3.17	3.33	2.67
LP29	3.50	4.50	3.33
LP30	1.83	2.33	2.17
LP31	3.33	3.50	3.67
LP32	1.67	1.83	2.33
LP33	2.00	3.00	2.83
LP34	3.67	4.17	3.83
LP35	4.00	4.33	4.67
LP36	3.50	3.33	3.33
LP39	2.83	3.67	3.67
LP40	2.00	3.33	2.33
LP41	3.00	2.75	2.17
LP42	3.17	3.00	3.17
LP43	3.67	4.33	4.00
LP44	4.33	4.17	4.17
LP45	2.17	3.33	2.17
LP46	3.67	3.67	4.33

RIL No.	331-9	DW5	AR CrossB10
LP47	1.67	1.50	1.67
LP48	1.83	2.33	2.00
LP49	3.67	4.00	3.33
LP50	1.17	1.17	1.50
LP51	3.00	3.50	3.00
LP52	4.17	4.50	4.33
LP53	3.33	4.00	4.00
LP54	2.50	2.75	3.33
LP55	3.50	2.17	3.33
LP56	4.33	3.67	3.83
LP57	3.00	2.83	3.17
LP58	3.83	3.33	3.33
LP59	3.17	2.67	2.33
LP60	1.17	1.50	2.50
LP61	1.67	2.00	2.33
LP62	1.00	1.83	1.50
LP63	3.83	4.33	4.33
LP64	3.00	4.17	3.75
LP65	2.50	4.50	4.00
LP66	3.67	4.17	4.50
LP67	2.83	3.50	3.17
LP68	4.17	3.17	3.83
LP69	3.83	3.83	4.33
LP70	2.33	2.50	1.50
LP71	2.33	2.67	2.67
LP72	1.17	2.00	1.67
LP73	2.17	3.83	3.33
LP74	1.67	2.33	1.33
LP75	3.17	4.17	3.83
LP76	2.75	3.50	2.83
LP77	2.67	3.33	3.00
LP78	3.50	4.33	4.50
LP79	3.67	3.67	3.83
LP80	2.33	3.50	3.50
LP81	2.50	3.83	2.83
LP82	3.50	4.17	3.33
LP83	2.33	3.33	2.17
LP84	1.33	2.00	1.33
LP85	4.00	4.50	4.33
LP86	3.50	3.67	2.33
LP87	3.50	4.50	4.00
LP88	2.67	3.50	4.00
LP89	2.00	2.75	3.00
LP90	4.17	4.67	4.17
LP91	3.50	4.17	4.00
LP92	3.17	4.33	2.83
LP93	3.17	4.00	3.50
LP94	1.50	1.83	1.00
LP95	3.17	3.83	3.67
LP96	3.50	3.67	3.67

RIL No.	331-9	DW5	AR CrossB10
LP97	3.67	3.67	3.17
LP98	1.67	2.17	2.00
LP99	2.50	3.50	3.50
LP100	3.00	3.67	3.67
LP101	1.17	3.00	1.33
LP102	3.33	3.67	3.17
LP103	3.83	4.17	3.83
LP104	3.67	4.67	4.17
LP105	2.00	2.83	2.50
LP106	1.17	1.50	1.00
LP107	1.83	3.00	3.17
LP108	3.67	3.67	4.00
LP109	2.00	3.17	3.67
LP110	3.50	3.17	2.83
LP111	1.67	3.00	3.00
LP112	1.17	1.33	1.67
LP113	3.17	3.00	3.17
LP114	2.50	3.83	4.33
LP115	2.17	4.00	3.67
LP116	2.17	4.17	3.17
LP117	2.67	3.67	4.00
LP118	2.67	3.00	1.67
LP119	2.50	3.00	3.00
LP120	4.00	4.00	4.00
LP121	2.67	2.50	1.33
LP122	3.17	3.75	3.33
LP123	2.83	3.33	4.17
LP124	3.33	3.50	3.33
LP125	1.17	2.00	3.00
LP126	1.83	2.00	2.17
LP127	3.00	3.83	3.17
LP128	3.00	4.00	3.17
LP129	2.00	3.83	2.50
LP130	3.67	3.83	4.17
LP131	2.67	3.33	1.67
LP132	3.00	3.50	4.83
LP133	2.17	3.33	3.67
LP134	1.33	2.00	2.00
LP135	2.33	2.33	2.83
LP136	3.25	3.67	3.67
LP137	3.50	3.17	3.00
LP138	3.17	3.83	3.50
LP139	2.17	3.33	2.33
LP140	4.00	4.17	4.17
LP141	2.33	3.50	4.00
LP142	3.50	4.17	3.67
LP143	2.17	3.50	4.00
LP144	2.83	3.33	2.67
LP145	2.67	3.83	4.33
LP146	4.00	3.83	3.50

RIL No.	331-9	DW5	AR CrossB10
Salamouni	1.00	1.00	1.00
Glenlea	2.50	3.50	3.50
6B365	3.67	2.75	4.67
6B662	2.00	3.75	2.17