MOLECULAR GENETIC CHARACTERIZATION OF PTR TOXC-*TSC1* INTERACTION AND COMPARATIVE GENOMICS OF *PYRENOPHORA TRITICI-REPENTIS*

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

By

Gayan Kanishka Kariyawasam

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

Major Department:
Plant Pathology

November 2018

Fargo, North Dakota

North Dakota State University
Graduate School

Title

MOLECULAR GENETIC CHARACTERIZATION OF PTR TOXC-*TSC1*

INTERACTION AND COMPARATIVE GENOMICS OF

PYRENOPHORA TRITICI-REPENTIS

By

Gayan Kanishka Kariyawasam

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

DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Zhaohui Liu
Chair

Dr. Shaobin Zhong

Dr. Justin D. Faris

Dr. Phillip E. McClean

Dr. Timothy L. Friesen

Approved:

November 7, 2018
Date

Jack Rasmussen
Department Chair

ABSTRACT

Tan spot of wheat, caused by *Pyrenophora tritici-repentis*, is an economically important disease worldwide. The disease system is known to involve three pairs of interactions between fungal-produced necrotrophic effectors (NEs) and the wheat sensitivity genes, namely Ptr ToxA-Tsn1, Ptr ToxB-Tsc2 and Ptr ToxC-Tsc1, all of which result in susceptibility. Many lines of evidence also suggested the involvement of additional fungal virulence and host resistance factors. Due to the non-proteinaceous nature, Ptr ToxC, has not been purified and the fungal gene (s) controlling Ptr ToxC production is unknown. The objective for the first part of research is to map the fungal gene (s) controlling Ptr ToxC production. Therefore, A bi-parental fungal population segregating for Ptr ToxC production was first developed from genetically modified heterothallic strains of AR CrossB10 (Ptr ToxC producer) and 86-124 (Ptr ToxC non-producer), and then was genotyped and phenotyped. Using the data, the gene (s) was mapped to the distal end of chromosome 2 in the reference genome of Pt-1c-BFP. The objective for the second part of my research is to develop genomic and genetic resources for the fungal pathogen. A high quality of genome sequence for AR CrossB10 and the first P. tritici-repentis genetic linkage map was generated. The AR CrossB10 genome and genetic linkage map is highly comparable to newly published reference genome except some noticeable chromosomal structural variations (SVs). Comparison of the genome sequences between parental isolates and twenty progeny isolates also revealed some SVs including deletion, insertion and inversion were detected that likely occurred during the fungal sexual reproduction. The objective for the third of my research is to characterize genetic resistance in Nebraskan winter wheat cultivar 'Wesley' using QTL mapping in a recombinant inbred line population. The results showed that resistance in Wesley is largely due to the lack of susceptibility genes *Tsc1* and *Tsn1*. My Ph.D. research provides a further

understanding of the genetics of host-pathogen interaction in wheat tan spot and contributes knowledge and tools for breeding tan spot resistant cultivars.

ACKNOWLEDGEMENTS

Foremost, I would like to express my deepest appreciation and gratitude to my advisor Dr. Zhaohui Liu for invaluable guidance and mentoring throughout my PhD study. There were many times when experiments were not working, but Dr. Zhaohui Liu's words and encouragement and advice guided me through such rough time periods.

I want to express my sincere gratitude to other members of my graduate study committee, including Drs. Timothy L. Friesen, Shaobin Zhong, Justin D. Faris, and Phillip E. McClean. Thank you all for valuable advice and helps on my Ph.D. research projects. My special thanks go to Dr. Shaobin Zhong for helping the fungal SSR project and to Dr. Timothy L. Friesen for correcting my dissertation.

I would also like to thank Dr. Sanzhen Liu from Kansas State University for providing the bioinformatics expertise in discovering SNPs associated with chlorosis production using genome sequence data in Chapter 2. I would also like to thank Mr. Gokul Wimalanathan of Iowa State University for teaching me the basics of bioinformatics. A very special thank goes to Mr. Nathan Wyatt for his immense support and mentoring for all the bioinformatics work done for the third chapter. Furthermore, I extend my gratitude for all the members of Friesen's Lab including Dr. Johnathan Richards, Danielle Holmes and Shaun Clare for all the support given through last few months.

I also extend my sincere thanks to Dr. Gongjun Shi for his valuable support in validating my results in various experiments. In addition, I would like to thank all the former and current lab members of Liu's lab including Jana Hansen, Gazala Ameen, Melody McConnell, Suraj Sapkota, Joseph Leard, Dr. Aimin Wen, Nelomie Galagedara, Malini Jayawardana, Jingwai Gao, and Yi Qi for their helps in research projects and for their ever-lasting friendship that makes this

journey more enjoyable. I also thank all the members in Department of Plant Pathology who have given me support, encouragement, and friendship throughout the last five years.

Very special thank goes to my best friends Ms. Nicole Bacheller and Dr. Dulan Samarappulli who have supported me in every difficult period in last five years. I am ever so grateful to these two lovely soles.

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 am grateful to my loving wife Madushika Samarasekara to expresses my deepest gratitude for love, support, dedication and understanding she provided throughout this study.

Finally, I would also like to thank my funding sources including, United States Department of Agriculture, National Institute of Food and Agriculture – Agriculture and Food Research Initiative (USDA NIFA-AFRI) and North Dakota Wheat Commission.

DEDICATION

I dedicate my disquisition to my loving parents Lalith and Chandani and my friend Nicole Bacheller.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

GENERAL INTRODUCTION

Wheat, including common wheat (*Triticum aestivum* L. 2n=6x= 42, AABBDD), and durum wheat (*T. turgidium* L., 2n=4x=28, AABB), is a major food crop in the world. The United States is the 5th largest wheat producer in the world (http://faostat.fao.org/site/339/default.aspx), and North Dakota (ND) is the leading hard red spring and durum wheat producer in the US. Hard red winter wheat (HRWW) is also produced at certain acreage in ND, and interest in growing it has been increasing.

Tan spot, caused by the necrotrophic pathogen *Pyrenophora tritici-repentis*, is a common foliar disease on all types of wheat crops in the world including ND. The disease can cause significant yield losses of up to 50% under favorable conditions (Rees et al. 1982). Infection on wheat kernels can also cause reddish discoloration, especially on durum wheat, which can downgrade the grain quality. Crop rotation, residue management, fungicide application can be integrated to control tan spot (De Wolf et al. 1998; Friskop and Liu, 2016). However, the most preferred way of managing this disease is to implement genetically resistant cultivars (Faris et al. 2013).

Genetically, wheat- *P. tritici-repentis* pathosystem follows an inverse gene-for-gene model, where a compatible or susceptible reaction interaction results from the interactions of pathogen-produced necrotrophic effector (NE) with a dominant host susceptibility gene. The fungal pathogen has been known to produce three NEs, including Ptr ToxA, Ptr ToxB and Ptr ToxC, which interacts with their corresponding susceptibility genes *Tsn1*, *Tsc2* and *Tsc1*, respectively, to induce disease (Ciuffetti et al. 2010). The fungal effectors Ptr ToxA and Ptr ToxB are a protein and their encoding genes in the fungus, designated as *ToxA* and *ToxB*, respectively, have been cloned (Ciuffetti et al. 2010). The cloning of these fungal genes has

greatly advanced our understanding of the fungal virulence mechanism and host-pathogen interaction. Ptr ToxC is an important NE produced by multiple races including race 1 which is the most predominant race worldwide. However, because Ptr ToxC is a low molecular weight secondary metabolite, the gene (s) responsible for its production is yet to be cloned.

The fungal pathogen has been classified into eight races and new races which cannot fit the current classification system have also been identified (reviewed in Ciuffetti et al. 2014). Understanding of the genome for all the races would provide important insights into the diversity of pathogen virulence. A high quality of reference genome sequence has been available for two *P. tritici-repentis* race 1 isolates: Pt-1C-BFP (from U.S.A.), and M4 (from Australia) (Manning et al. 2013; Moolhuijzen et al. 2018). Draft genome sequences derived from short-reads have also been available for other races (Manning et al. 2013; Moolhuijzen et al. 2018). However, there is no a high-quality reference genome sequence for other races.

Host resistance to wheat tan spot is a complex, which has been shown to involve three host NE sensitivity genes, major recessive resistance genes, race-nonspecific resistance QTL and other minor QTL (Faris et al. 2013; Kariyawasam et al. 2016; Virdi et al. 2016). In addition, the effect of each NE-host sensitivity gene interaction on disease development have been shown to be genetically background-dependent (Cheong et al. 2004; Faris and Friesen, 2005; Chu et al. 2008, 2010; Kariyawasam et al. 2016; Virdi et al. 2016). Genetic characterization of host resistance is needed to be conducted in broader genetic backgrounds. The major HRWW cultivar 'Jerry' in ND is highly susceptible to tan spot and need to be improved for tan spot resistance (Liu et al. 2015). Previously, the winter wheat variety 'Wesley' from Nebraska has been shown to be resistant to multiple races of *P. tritici-repentis*. To better utilization of source of resistance in Wesley for NDSU breeding programs, resistance in this cultivar needs to be characterized.

Therefore, the objectives of my Ph.D. study were to 1) map the fungal gene (s) conditioning Ptr ToxC production using a bi-parental fungal population, 2) development of the first genetic linkage map in *P. tritici-repentis* and identify chromosomal structural variation through comparative genome analysis, 3) characterize genetic resistance in in Wesley using QTL mapping.

CHAPTER 1. LITERATURE REVIEW

1.1. Wheat

1.1.1. Wheat classification

Wheat is currently classified under the family Poaceae which is one of the largest families of plants and consists of 11,506 grass species. The Poaceae family are further classified into 12 subfamilies, 52 tribes, and 90 subtribes. Out of the 52 tribes wheat is classified under the tribe Triticeae, subtribe Triticinae, and genus *Triticum* (Soreng et al. 2015, 2017). Triticeae is an important tribe to mankind consisting of 501 of annual and perennial species that are classified under 27 genera including barley, rye and triticale (Lu and Ellstrand 2014). Therefore, Triticeae is one of the most intensively researched tribes (Soreng et al. 2015).

1.1.2. Wheat 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 progenitors of *Triticum* and *Aegilops* were derived from a common ancestor with 7 chromosomes about 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 the Fertile Crescent of the Middle East (Luo et al. 2007).

The first amphiploidization events took place about a half million years ago between wild diploid wheat *T. uratu* Tumanian ex Gandylian (2n = 2x = 14, AA genome) that donated the A genome and *Ae. speltoides* ssp. *lingustica* (2n = 2x = 14, SS genome) that donated the B genome, to form the tetraploid wheat *T. turgidum* ssp. *dicoccoides* (Korn.) Thell (2n = 4x = 28,

AABB genomes) (Dvorak et al. 1993). *T. turgidium* ssp. *dicoccoides*, commonly known as wild emmer 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 (Charmet 2011). The second amphiploidization event occurred about 8000 years ago where a sub species of *T. turgdium* (AABB) hybridized with the diploid goatgrass *Ae. tauschii* Coss. (2n = 2x = 14, DD genome). This event likely gave rise to the hexaploid wheat *T. aestivum* ssp. *spelta* (Asian or Asian like) (2n = 6x =42, AABBDD genome), which was then evolved through gaining the free-threshing character to form the modern cultivated bread wheat (Lelley et al. 2000; Faris 2014). In addition to free-threshing, traits such as brittle rachis and tenacious glume are also associated with domestication of wheat (Faris 2014).

1.1.3. Wheat production and diseases in North Dakota

1.1.3.1. Wheat production and classes

Wheat is one of the most important stable food crops in the world and accounts for at least 20% of the daily caloric consumption of humans (Faris et al. 2014). In the 2017/18 season, wheat was grown in 219.70 million hectares globally, accounting for 758.74 million metric tons (Foreign Agricultural Service, USDA, 2018). The United States (US) is the 5th largest wheat producer in the world behind the European Union, China, India and Russia where US produced $47.3 million tons in the 2017/18 season (https://www.statista.com/statistics/237912/global-top-wheat-producing-countries/, 10/15/2018).

Six major classes of wheat are grown in 42 states of the US including hard red winter wheat (HRW), hard red spring wheat (HRS), soft red winter wheat (SRW), soft white wheat (SW), hard white (HW) wheat and durum wheat. HRW wheat, which is mainly used for making bread, is commonly grown in the Southern and Central Great Plains and California, accounting

for 40% of the total US wheat production. HRS wheat is primarily cultivated in the Northern Great Plains of the US which makes up 20% of the total US wheat production. HRS wheat is known to contain high protein content and therefore, it is used to make specialty breads such as pan breads, hearth breads, and other bakery products such as bagels, hamburger buns, rolls etc. In addition, HRS has been also used to blend with wheats that have low protein content. SRW wheat is grown in Eastern states of the US making up for 15-20% of the total wheat production whereas white wheat is grown predominately in the Pacific Northwest, Michigan and New York and accounts for 10-15% of total US production. SRW wheats are used in the production of snack food, pastries, crackers and cakes. White wheat has been used to produce white crusted bread, noodle products and crackers. Durum is the smallest class of wheat in US which accounts for 3-5% of the total wheat production. Durum is mainly used for making pasta and is primarily grown in the North Central and Southwest regions of the US. (https://www.ers.usda.gov/topics/crops/wheat/background/#classes; https://www.ndsu.edu/faculty/simsek/wheat/production.html).

1.1.3.2. ND wheat production

Wheat is the second most grown crop in North Dakota behind soybeans. In 2017, wheat was grown over 6,260,000 acres which included 5,050,000 acres of spring wheat, 1,175,000 acres of durum and 35,000 acres of winter wheat. A total of 237,133,000 bushels of wheat were produced in 2017 with an average yield of 37.9 bu/acre in harvested land. Production included 207,050,000 bushels of spring wheat, 28,788,000 and 1,295,000 bushels of winter wheat. In 2017, the US wheat crop was valued as $1.3 billion with an average of $5.74 /bu. Durum wheat received the highest value of $6/bu whereas spring wheat received a price of $5.72/bu (https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=NORTH%20D

akota, 10/15/2018). ND produces three classes of wheat, including HRS wheat, durum and HRW wheat, which account for approximately 87.3%, 12.1% and 0.6% of the total wheat production in ND respectively, In general, approximately 50% of HRS wheat and 33% of durum wheat produced in ND is exported.

1.1.4. Wheat diseases in ND

Wheat production in ND is challenged by several fungal and bacterial diseases. Bacterial leaf streak is the bacterial wheat disease in ND caused by *Xanthomonas translucens* pv. *undulosa*. Recently, it has been commonly found in each growing season. Among the fungal diseases, rust diseases have a major focus due to the historical devastating epidemics related to wheat stem rust caused by *Puccinia graminis* f. sp. *tritici*. However, stem rust has been rarely observed due to the implementation of genetic resistance. However, leaf rust caused by *P. triticina* is commonly found in ND whereas strip rust caused by *P. striiformis* f. sp. *tritici* can be observed under cool conditions in growing seasons (Friskop and Acevedo, 2015). Fusarium head blight (FHB) caused by *Fusarium graminearum* is considered as the most economically important disease of wheat, since the devastating epidemics occurred in 1990s in the Northern Great Plains including ND (Windels 2000). Wheat is susceptible for the disease during the flowering stage, and disease can occur under prolonged periods of high humidity during the flowering stage (McMullen et al. 2008).

However, leaf spotting diseases such as tan spot, septoria tritici blotch and septoria nodorum blotch, caused by *Pyrenophora tritici-repentis*, *Zymoseptoria tritici*, and *Parastagonospora nodorum* respectively, commonly occur as a complex and are the most destructive foliar diseases of wheat in ND. Differentiation of these three diseases under field conditions based on leaf lesions is difficult. However, characteristics and occurrence of

pseudothecia and pycnidia gives a better chance of diagnosing them in field conditions (Friskop and Liu, 2016). In ND, tan spot is very common in every growing season of the last two decades and the annual yield loss is estimated to range from 5 to 15%, which translates into millions of dollars in economic loss to the state (Marcia McMullen, personal communication).

1.2. Tan spot of wheat

Tan spot disease is a devastating foliar disease of wheat in all wheat growing regions worldwide and is caused by the necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoem. Furthermore, it can infect all forms of cultivated wheat including hexaploid wheat and tetraploid wheat making it a fungal pathogen with a global impact (Faris et al. 2013; Ciuffetti et al. 2014). Therefore, this pathogen has been observed and studied for over 150 years.

P. tritici-repentis is an ascomycete fungus classified under the class Dothideomycetes, order Pleosporales, and in the family Pleosporaceae. Pleosporaceae includes, the genus *Pyrenophora* together with other genera that contain important plant fungal pathogens including, *Parastagonospora*, *Cochliobolus*, *Alternaria* and *Leptosphaeria*. (Ohm et al. 2012). The word pyrenophora is defined as the presence of seed, kernel and pit within the ascocarp (reviewed in De Wolf et al. 1998). In addition to *P. tritici-repentis*, this genus also includes *P. teres* a devastating pathogen of barley (Ohm et al. 2012).

1.2.1. History

P. tritici-repentis was first identified as a saprotroph on grass in the 1850s and was not initially characterized as a pathogen. In 1902, the fungus was first isolated by Diedicke from *Agropyron repens*, a grass species and initially named it *Pleospora trichostoma* which was subsequently renamed by him as *Pleospora tritici-repentis* (Mitra 1934). Drechsler (1923)

identified and renamed the fungus to its current name, *Pyrenophora tritici-repentis.* The fungus was first reported on wheat in Japan. However, in Japan the fungus was described as *Helminthosporium tritici-vulgaris* based on the conidial state (Nisikado, 1928). Ito (1930) established the group of *Drechslera* including four *Helminthosporium* sp. with *H. tritici-vulgaris* and renamed the conidial state of the fungus to *D. tritici-vulgaris*. Shoemaker (1962) showed 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 a synonym to *P. tritici-vulgaris* and *P. tritici-repentis* (Hosford 1971). Today, the sexual stage of the tan spot fungus is named as *Pyrenophora tritici-repentis* (Died.) Drechs. and the asexual stage is named as *Drechslera tritici-repentis* (Died.) Shoem (reviewed in De Wolf et al. 1998).

Even though some reports suggest *P. tritici-repentis* was first described as early as 1823, it was not considered to be pathogenic to wheat until 1928 (Hosford 1971). From the early 1930s, the fungus was frequently identified and known to cause disease on wheat (Conners 1937; Mitra 1934). Due to the production of chlorosis, the disease was originally called yellow spot or yellow leaf blotch (Conners 1940; Hosford 1971; Friesen et al. 2006). Outbreaks of tan spot started to occur worldwide since the 1940s, and the disease was associated with the development of light brown and tan-colored necrotic lesions in addition to the chlorosis symptom on leaves (Barrus 1942; Johnson 1942; Hosford 1971; Friesen et al. 2006). The disease may have gained the name of "tan spot" at that time because of this severe form of symptoms. The study from Friesen et al. (2006) strongly suggested that *P. tritici-repentis* may have acquired the *ToxA* gene from another wheat pathogen, *Parastagonospora nodorum* which made the pathogen to form the tan colored necrotic symptoms. By the 1970s, the severe epidemics of tan spot had been reported in many countries around the world including Canada, USA, and Australia (Hosford 1982, Rees

and Platz, 1992). Occurrence of tan spot appeared to coincide with the introduction of no-tillage farming practices that adapted for the conservation of soil moisture, organic compounds and other beneficial microbes. However, unintentionally it also increased the inoculum level of stubble-born disease, such as tan spot.

1.2.2. Economic importance

Currently, tan spot has been found in all the major wheat growing regions of the world. Tan spot is considered the most prevalent disease of wheat in Canada and US, whereas tan spot is considered as the most devastating wheat disease in Australia (Ciuffetti et al. 2014). In the US, it is known to cause yield losses from 2 to 15% and under favorable conditions it can cause yield losses of up to 49% (Evens et al. 1999; Hosford, 1982). In Australia, tan spot annually causes direct yield losses of $212 million whereas $461 million have been spent on controlling the disease (Moolhuijzen et al. 2018). Tan spot has also been found in the Southern Cone Region of South America and caused yield losses ranging from 20-70% (Gamba et al. 2012).

Studies showed that the loss of yield due to tan spot is governed by the amount of primary inoculum, wet period following the infection, host genotype and growth stage of the wheat plant at the infection (Rees and Platz 1982; Hosford and Busch 1974; Raymond et al. 1985; Shabeer and Bockus 1988). It was shown that yield losses would be higher if the infection occurred after jointing stage of the plant compared to the seedling stage (Rees and Platz, 1983). A few years later it was found that yield losses could be high if the infection occurred in booting and flowering stage (Shabeer and Bockus, 1988). Reductions in kernel weight, number of grains per head, number of tillers, grain size and leaf area (Shabeer and Bockus, 1988; Rees and Platz, 1983) are affected by tan spot. In addition, infection on wheat kernels during the filling stage results in pink/red color discoloration and is known as red smudge. Red smudge is commonly

observed in durum wheat and it will result in the downgrading of the grain quality of the wheat (Fernandez et al. 1998).

1.2.3. Host range

Host range includes the list of species that can be infected by a pathogen (Dinoor, 1974). Identification of the secondary host can be important for disease management since these hosts can harbor *P. tritici-repentis* on the off-season and act as a source of primary inoculum. Secondary host can act as source of genetic variation as well as it can facilitate the events such as horizontal gene transfers between co-existing fungal communities (De Wolf et al. 1998; Friesen et al .2006). Early studies showed that *P. tritici-repentis* can infect other grass species. In fact, it was first isolated from a grass species known as *Agropyron repens* (Diedicke 1902). Hosford (1971) reported that *A. desertorum*, *A. intermedium, A. smithii*, and *Bromus inermis*, were susceptible to *P. tritici-repentis.* In addition, Krupinsky (1992) showed several other grass species such as *Dactylis glomerate*, *Lemus angustus*, *L. cinerus*, *L. triticoides*, *Pascopyrum smithii*, *Stipta viridula* and *Thinopyrum intermedium* also act as secondary host to *P. tritici-repentis*. A recent study also collected *P. tritici-repentis* from non-cereal grasses and showed that almost all the isolates recovered from them belonged to race 4, which are non-pathogenic to wheat (Ali et al. 2003).

In addition to these grass species, economically important cereal grasses such as barley and rye also act as host for *P. tritici-repentis* (Hosford 1971, Ali et al. 2001). Ali et al. (2001) isolated race 1 isolates from barley whereas Aboukhaddour et al. (2016) showed that some barley genotypes are highly susceptible to Ptr ToxB producing isolates. Recent study showed that races 1 and 5 of *P. tritici-repentis* have the ability to infect rye with the use of 211 rye accessions (Abdullah et al. 2017b). Even though these races caused the disease study also

showed that all the accessions were insensitive to Ptr ToxA and Ptr ToxB that suggest different NEs are at play. However, they concluded that rye plays a lesser role in disease epidemiology, since the majority of isolates collected from rye fields belonged to race 4 (Abdullah et al. 2017b).

1.2.4. Disease cycle and symptoms

Tan spot is a polycyclic disease. The fungal pathogen overwinters on wheat stubble as the black pinhead-sized fruiting body called pseudothecia. In spring or early summer, the mature ascospores released from pseudothecia or conidia serve as the primary inoculum and infect the leaves of wheat seedlings to cause primary infection (Friskop and Liu, 2016). High humidity and temperatures above 10℃ favor the ascospore discharge and the infection process (Wegulo, 2011).

Early histological studies showed that once the discharged spores landed on the leaf surface, infection occured within 6 -24 hours, and conidia of *P. tritici-repentis* produced multiple germ tubes followed by appressoria and penetration pegs (Larez et al. 1986). These penetration pegs penetrate into the epidermal cells and form a vesicle and complete process of penetration can take up to 24 hours (Larez et al. 1986; Loughman and Deverall 1986; Ciuffetti et al. 2014). *P. tritici-repentis* grow intracellularly from the vesicles in the epidermal cells and then get in to the mesophyll cell layer and grows intercellularly (Ciuffetti et al. 2014). However, fungal hyphae do not penetrate the mesophyll cells (Loughman and Deverall 1986) and it was recently shown that *P. tritici-repentis* released necrotrophic effector (NE), Ptr ToxB secreted in to the apoplast only (Figoura et al. 2015).

Upon pathogen infection, tan-colored, elliptical-shaped necrotic lesions surrounded by chlorotic halos form on susceptible cultivars (Weise 1987). Some races of the pathogen cause extensive and localized chlorosis on infected leaves. Large numbers of conidia are then produced

on these lesions, which act as the secondary inoculum to cause infections on new leaves of the same plant or neighboring plants in the field (McMullen and Adhikari 2009). The cycle of conidial production and infection can be repeated multiple times in a growing season, thus increase disease incidence and severity in the field.

1.2.5. Disease management

Epidemics of tan spot have occured since the 1970s when most of the wheat growing regions widely adopted no or reduced tillage practices to prevent soil erosion. Therefore, Rees and Platz (1992) suggested that lack of tilling might be the reason for the frequent tan spot epidemics since such farming practices lead to the buildup of initial inoculum. Appropriate crop rotation and residue management are two cultural practices that can reduce the initial inoculum, and thus they are effective in controlling tan spot. Surveys in North Dakota tan spot was less prevalent in the areas where broadleaf crops had been grown in previous seasons (Friskop and Liu, 2016). Therefore, rotation with crops such as soy bean, flax, crambe, and mustard can reduce the disease (Wegulo, 2011). However, planting wheat in to corn residue is not recommended due to the risk of Fusarium head blight because *Fusarium graminearum* can infect both crops. Chisel plowing has been often used to reduce residue covers in North Dakota, but it could still leave sufficient residues that can become a significant source of inoculum (McMullen and Adhikari 2009).

Fungicide application is one of the most common ways of managing tan spot. Most recent trials conducted by NDSU showed that strobilurins such as Picoxystrobin, Pyraclostrobin, and triazoles such as Metconazole, Propiconazole and mixtures of fungicides belongs to these two classes have good to excellent efficacy against tan spot (https://www.ag.ndsu.edu/extplantpath/publications-newsletters/crop-disease-

control/NCERA184Wheatfungicidetable2017_Final.pdf). In general, fungicides coupled with herbicide is applied at 4 to 6 leaf stage in North Dakota to control tan spot (Friskop and Liu, 2016). Some fungicides are effectively used in seed treatments to control this disease. Additionally, decisions for fungicide application can be made based upon the results from the small grain disease forecasting model developed by NDSU extension services. The web-based computer model integrates weather data with that of plant growth stage to determine the risk of having diseases including tan spot (http://www.ag.ndsu.nodak.edu/cropdisease).

However, cultural practices and fungicide application are not always effective due to environmental issues and cost of production. Therefore, the most cost effective and environmentally sound way of controlling tan spot is to develop and plant genetically resistant cultivars. But most of the cultivars that are commonly grown in ND are susceptible for the disease (https://www.ag.ndsu.edu/publications/crops/north-dakota-hard-red-spring-wheat-variety-trial-results-for-2017-and-selection-guide/a574-17.pdf, 10/15/2018).

1.3. Host-parasite genetics

Plant pathogenic fungi represents diverse life styles and deploy different strategies to interact with the host including biotrophic, hemibiotrophic and necrotrophic strategies. Lifestyles of the fungi were defined based on the method of nutrient acquisition (Kabbage et al. 2015). Biotrophic plant pathogenic fungi such as *Puccinia graminis* f. sp. *tritici*, *Blumeria graminis* f. sp. *hordei*, obtained nutrients from living material whereas necrotrophic fungi such as *P. tritici-repentis*, *P. teres* f. *teres*, *P. nodorum* get nutrients from dead plant material (Presti et al. 2015). Hemibiotrophic fungi, such as *Magnaporthe oryzae*, *Z. tritici*, *F. graminearum*, *Venturia inaequalis and Colletotrichum* sp., combines both the biotrophic and necrotrophic lifestyles (Presti et al. 2015). Based on the lifestyle of the fungi, variations in infection process, infection

structure and how pathogen manipulate host mechanisms have been observed based on different pathosystems (Horbach et al. 2011; Presti et al. 2015).

Plant pathogenic fungi are known to produce and secrete a large number of molecules, known as effectors, during the infection process that manipulate the host mechanisms including suppression of basal immune system to promote a compatible interaction (Franceschetti et al. 2017; Presti et al. 2015). However, plants have evolved the ability to recognize these effector molecules, through an innate immune system to initiate a defense response known as effector-triggered immunity (ETI) (Jones and Dangl, 2006). Typical ETI response includes, the activation of pathogenicity related (PR) genes, activation of mitogen-activated protein kinases, reprogramming of gene expression, accumulation of reactive oxygen species (ROS), and deposition of callose on infection sites, which eventually leads to the intense reaction called hypersensitive response (HR) that cause localized plant cell death surrounding the infection site (Dodds and Rathjen, 2010). These effector molecules were recognized by resistance genes known as R-genes in gene-for-gene manner and a typical R-gene consists of a nucleotide binding (NB) domain and a leucine rich receptor (LRR) (Flor, 1971; Presti et al. 2015).

Even though, localized cell death prevents the growth of the biotrophic fungal pathogens, necrotrophic fungal pathogens thrive on dying tissue. Therefore, necrotrophic fungal pathogens often induce necrosis by releasing necrotrophic effectors (NE) that interact with host susceptibility gene products for a compatible interaction (Wolpert et al. 2002). Such NE-susceptibility gene interaction caused susceptibility is known as effector-triggered susceptibility (ETS). These NEs can be small secreted proteins such as Ptr ToxA, Ptr ToxB, SnTox1, and SnTox3 (Ciuffetti et al. 1997; Martinez et al. 2001; Liu et al. 2012, 2009) or they can be small

secondary metabolites such as HC-toxin, T-toxin, and Victorin (Reviewed in Stergiopoulos et al. 2013).

Similar to ETI, ETS also shows the characteristics of the programmed cell death (PCD) such as cell shrinkage, deposition of callose, DNA laddering and accumulation of ROS (Liu et al. 2012; Manning et al. 2009; Hammond-Kosack and Rudd, 2008). Interestingly, susceptibility genes such as *Tsn1*, *LOV1*, and *Pc* that interacts with these NEs have NB and LRR domains which are typical characteristics of a R-gene in ETI whereas recently cloned *Snn1* has the characteristics of a pattern recognition receptors (PRR) that are involved in pathogen associated molecular pattern (PAMP)-triggered immunity (PTI) (Faris et al. 2010; Lorang et al. 2007; Nagy and Bennetzen, 2008, Shi et al. 2016). Therefore, it is evident that necrotrophic fungi can hijack the plant immune system to cause disease.

P. tritici-repentis is a necrotrophic pathogen. The wheat-*Ptr* pathosystem follows an inverse gene-for-gene model where interaction between the necrotrophic effector (NE), also known as host selective toxins (HST), produced by the pathogen and the product of the host sensitivity gene leads to susceptibility (Wolpert et al. 2002). Such interaction triggers an apoptosis-like reaction that kills the plant tissue around the infection site, which allows necrotrophic pathogen to survive on the dead tissue. Three *P. tritici-repentis* NEs have been identified including Ptr ToxA, Ptr ToxB and Ptr ToxC, which interact with host sensitivity genes *Tsn1*, *Tsc2* and *Tsc1*, respectively. The three host genes have been mapped to the chromosome arms *Tsn1* on 5BL, *Tsc2* on 2BS, and *Tsc1* on 1AS (Faris et al. 1996; Friesen and Faris 2004; Abeysekara et al. 2009; Effertz et al. 2001). Among them, *Tsn1* has been isolated from wheat (Faris et al. 2010). Interaction between Ptr ToxA and Tsn1 results in necrosis whereas Ptr ToxB-Tsc2 and Ptr ToxC-Tsc1 result in restricted and extensive chlorosis, respectively.

1.3.1. Genetic variability in pathogen virulence

P. tritici-repentis was known to show variation in pathogenicity along the way from saprophyte to a wheat pathogen with a global impact. Therefore, assessing the virulence of *P. tritici-repentis* on various host genotypes has been important to devise management strategies to control the disease. At the beginning, virulence was assessed using lesion size (Misra and Singh 1972), percent leaf area infected (Luz and Hosford 1980), and necrotic leaf area (Schilder and Bergstorm 1990). However, these rating scales did not produce consistent results, where different studies classified the same isolates under different races (Ackermann et al. 1988). With some rating systems based on lesion length and percent necrotrophic area, even though variation in virulence was observed, it could not be proven statistically (Krupinsky 1992).

Introduction of lesion type-based rating scale by Lamari and Bernier (1989) had been considered a landmark in studying genetics of *P. tritici-repentis* wheat pathosystem. Lamari and Bernier (1989, 1991) showed that *P. tritici-repentis* produce either necrosis or chlorosis on host genotypes and they are genetically distinct. Lamari and Bernier (1989) screened 92 isolates and classified them in to 4 pathotypes based on the lesion type on two wheat differential lines, Glenlea and 6B365. Isolates that produced both necrosis and chlorosis were considered as pathotype 1 (nec+chl+). Isolates that produced only necrosis and isolates that only produced chlorosis were classified under pathotype 2 (nec+chl-) and pathotype 3 (nec-chl+). Isolates that did not produced either of the symptoms were classified under pathotype 4 (nec-chl-). Pathotypes 1 and 2 produced necrosis on the differential line Glenlea whereas pathotypes 1 and 3 produced chlorosis on 6B365.

Lamari et al. (1995) characterized a group of isolates from Algeria that produced chlorosis on wheat genotypes such as Katepwa that were resistance to the chlorosis production

by pathotype 3 isolates. These isolates also failed to induce chlorosis on 6B365. Therefore, these Algerian isolates were characterized as a new pathotype. With the discovery of these isolates, a race classification system based on the reaction produced on the differential lines were established, where pathotype 1 to 4 were considered as races 1 to 4. A new pathotype was classified as race 5 and it was differentiated based on the reaction produced on 6B662 (Ptr ToxB differential line). In addition, differential lines were amended with Salamouni as the universal resistance line.

1.3.2. Race classification and distribution

With the use of an established differential set, eight races of *P. tritici-repentis* have been characterized. Race 2 only produces necrosis on Glenlea, whereas races 3 and 5 only produces chlorosis on 6B365 and 6B662 respectively. Race 1 produces necrosis on Glenlea and chlorosis on 6B365 combining the virulence of races 2 and 3. Likewise, race 6 produces chlorosis on both 6B365 and 6B662 combining the virulence of races 3 and 5 whereas race 7 produces necrosis on Glenlea and chlorosis on 6B662 combining the virulence of races 2 and 5. Finally, race 8 produce necrosis on Glenlea and chlorosis on both 6B365 and 6B662 combining the virulence of all the three basic races (Faris et al. 2013; Strelkov and Lamari, 2003). Currently, it is known that the variation in virulence is due to the necrotrophic effectors (NEs) produced by each race and their interaction with host susceptibility genes carried in differential lines. Races 2, 3 and 5 produces single NE: Ptr ToxA, Ptr ToxC and Ptr ToxB respectively. Race 1 produces Ptr ToxA and Ptr ToxC, race produces Ptr ToxC and Ptr ToxB, and race 7 produces Ptr ToxA and Ptr ToxB. Race 8 produces all the three NEs currently identified in *P. tritici-repentis*. Race 4 lacks any of these known NEs (Ciuffetti et al. 2014).

Ali et al. (2010) characterized a set of isolates collected from Arkansas that the lacked *ToxA* gene but caused necrosis on the Ptr ToxA differential line Glenlea. Therefore, currently it was characterized as a new race that does not fit into the current race classification. Furthermore, another group of Algerian isolates were identified that failed to cause disease on Glenlea despite having the *ToxA* gene. However, those isolates were able to cause disease on tetraploid wheat lines suggesting a new virulent pattern (Benslimane et al. 2018). Therefore, all these isolates were not placed on the current race classification system.

Races 1 and 2, considered to be the most predominant race in the North America (Lamari et al. 1998), Australia and in the Southern Cone Region of South America (Gamba et al. 2012). Races 1 and 2 have also been found in the wheat center of diversity (Lamari et al. 2005). Recently, race 1 was also reported in Eastern Europe and North African countries including Morocco (Abdullah et al. 2017a, Gamba et al. 2017). Race 3 has been reported in North America, the Caucasus region, and Eastern Europe. However, in either region race 3 isolates have been less commonly found compared to other races (Lamari et al.1998; Ali and Francl 1998; Lamari et al. 2005; Abdullah et al. 2017a). Similarly race 4 was also found in low frequency and was identified in the Great Plains and North Africa (Ali and Francl 2003). Race 5 was first identified in Algeria, followed by the United States, Canada, Azerbaijan, Syria and Morocco (Lamari et al. 1995; Ali et al. 1999; Lamari and Strelkov, 2010; Gamba et al. 2017). Race 6 has been observed less frequently, and only found in North African countries such as Algeria and Morocco (Strelkov et al. 2002; Gamba et al. 2017). Race 7 and 8 have been reported in Middle East, Caucasus region, Algeria and Morocco (Lamari et al. 2005; Benslimane et al. 2011).

In last 20 years tan spot has spread in to wheat growing regions of Europe such as Latvia, Lithuania, and Romania (Abdullah et al. 2017a). It was also found in major wheat growing regions of South Asia including India and Pakistan (Misra and Singh, 1972; Ali et al. 2001) and has become a serious issue in Australia.

1.3.3. NEs of P. tritici-repentis

1.3.3.1. Ptr ToxA

Ptr ToxA is the most extensively studied NE in the *P. tritici-repentis* -wheat pathosystem. Tomas and Bockus (1987) revealed that NEs were present in the culture infiltrate of *P. tritici-repentis* isolates that produce necrosis on some genotypes of wheat. This study also showed the strong correlation between NE sensitivity and susceptibility to the fungal isolates that produces the NE. These results were further confirmed by Lamari and Bernier (1989). Balance et al. (1989) purified the NE by gel electrophoresis and ion exchange chromatography and later it was named Ptr ToxA (Ciuffetti et al. 1998).

Ptr ToxA contains both pre- and pro-protein domains. Pre- domain consists of 23 amino acids functioning as the signal peptide. Pro- domain is 4.3 kDa in size and it plays an important role in protein folding and its activity (Tuori et al. 2000). Both pre and pro domains are cleaved off prior to the secretion of the mature protein which has a size of 13.2 kDa (Balance et al. 1996; Ciuffetti et al. 1997). Further analysis of the protein structure revealed that Ptr ToxA consists of a conserved RGD (Arg-Gly-Asp) motif, which is known to be involved in protein-protein interactions. Ptr ToxA is hypothesized to bind to a putative extracellular receptor through the RGD motif (Sarma et al. 2005). Mutational analysis of the RGD motif and nearby amino acids suggested that the RGD is essential for the activity of the NE (Sarma et al. 2005). Multiple studies showed that the RGD motif is important for Ptr ToxA to transport to the cytoplasm of the

mesophyll cells of susceptible genotypes (Meinhardt et al. 2002; Manning et al, 2008). Furthermore, green florescence protein (GFP) tagged Ptr ToxA showed the sub-cellular localization to the chloroplast following the internalization in susceptible mesophyll cells (Manning and Ciuffetti, 2005). Therefore, these findings agree with the findings of Faris et al. (2010) that speculated the cytoplasmic localization of *Tsn1*. However, no direct interaction between Ptr ToxA and Tsn1 has been observed (Faris et al. 2010).

With the perception of Ptr ToxA, changes in gene expression was suggested by the work done by Rasmussen et al. (2004). Furthermore, with the perception of Ptr ToxA, also induced major transcriptional reprogramming including activation of defense related genes (such as WAKs, RLKs, MAPKs) and transcription factors required for the control of those genetic factors were upregulated whereas transcription of gene encoding reactive oxygen species (ROS) detoxification enzymes associated with the chloroplast being down-regulated (Pandelova et al. 2009; Adhikari et al. 2009). ROS accumulation is also supported by the ethylene production induced by the Ptr ToxA and it was shown that accumulation predominantly takes place in the chloroplast. Accumulation of ROS is known to disrupt the protein homeostasis in both photosystems (Manning et al. 2009; Pandelova et al. 2009) that eventually lead to cell death. Ptr ToxA can also disrupt the chloroplast activity by binding to ToxA binding protein 1 (ToxABP1) that leads to the degradation of photosystem II (Manning et al. 2007; Manning et al. 2010).

Ciuffetti et al. (1997) showed that Ptr ToxA is encoded by the single copy gene *ToxA* in the fungus. Ciuffetti et al. (1997) developed cDNA from the mRNA of the purified protein and used the labeled cDNA as a probe to identify the genomic clones of *ToxA.* Predicted *ToxA* locus has a promoter region of 278 bp, a predicted open reading frame of 534 bp and an extra 137 bp which is transcribed, but not translated, which included the intron 1. In addition, another intron

was also identified in the C-terminal domain of the ORF. Recent sequencing of the *P. tritici-repentis* genome showed that the *ToxA* gene resides on chromosome 6 that is 2.8 Mb in length (Manning et al. 2013). Furthermore, it was proposed that *P. tritici-repentis* acquired the *ToxA* gene from *P. nodorum* via horizontal gene transfer and the gene had the sequence identity of 98 to 100% to *P. nodorum ToxA* genes (Friesen et al. 2006). Very recently, a new study discovered a nearly identical *ToxA* gene in *Bipolaris sorokiniana*, the causal agent of leaf blight and common root rot in wheat, suggesting the importance of this interaction in three disease pathosystems of wheat (McDonald et al. 2018; Friesen et al. 2018).

1.3.3.2. Ptr ToxB

Ptr ToxB is the second proteinaceous NE identified and characterized in race 5 isolates of *Ptr* (Orolaza et al. 1995). Strelkov et al. (1999) showed that the mature Ptr ToxB is a heat stable protein with a molecular mass of 6.61 kDa. Using the partial protein sequence of Ptr ToxB, a 300 bp fragment of cDNA was developed from isolate DW7 with the help of reverse transcriptase (RT)-PCR (Martinez et al. 2001). However, developed cDNA fragment only represented part of the *ToxB* gene. Rest of the *ToxB* containing region was completed by using thermal asymmetric interlaced (TAIL)-PCR. With the use of TAIL-PCR products primers were designed to obtain a 646 bp fragment containing an open reading frame of 261 bp which was ultimately identified as the *ToxB* gene. Unlike *ToxA,* multiple copies of the *ToxB* gene ranging from 2-10 are present in Ptr ToxB-producing isolates and the amount of Ptr ToxB production is proportional to the copy number of the gene (Martinaz et al. 2004; Amaike et al. 2008). *ToxB* encodes for a pre-protein and consists of 87 amino acids, including a signal peptide of 23 amino acids. However, the gene does not encode for any known functional domains, even though it consisted of four cysteine residues with one each located close to the N- and C-terminus (Martinez et al. 2001; Strelkov

and Lamari, 2003). Furthermore, a non-pathogenic isolate that lacked *ToxB* was transformed with *ToxB* and transformants gained the virulence function on susceptible lines validating the function of the gene (Strelkov et al. 2002; Ciuffetti et al. 2010)

Figueroa et al. (2015) showed that Ptr ToxB localized in the apoplast of wheat leaves and acts extracellularly. Figueroa et al. (2015) showed that Ptr ToxB is stable conformationally with the presence of disulfide bridges between cysteine residues. Therefore, Ptr ToxB can counteract the hostilities present in the apoplastic fluid. These authors observed the presence of Ptr ToxB in apoplast in both sensitive and insensitive genotypes and they speculated that Ptr ToxB might be interacting with another host protein in the apoplast to induce symptoms.

Homologs of the *ToxB* gene was observed in *P. tritici-repentis* races 3 and 4 that do not produce Ptr ToxB. Strelkov et al. (2006) showed that *ToxB*-like genes in race 3 had modifications in the signal peptide which could result in improper folding or processing, hence the inactivity. A single copy of *toxb* present in race 4 isolates showed 86% similarity to the *ToxB* gene and it encodes for a protein with an extra amino acid than Ptr ToxB (Martinez et al. 2004). However, *toxb* is transcriptionally active but does not produce chlorosis (Amaike et al. 2008). In addition, homologs of *ToxB* were also found in related species such as *P. bromi* (causal agent of brown spot of bromegrass) with 89% similarity. Like *ToxB* these genes were also found in multiple copies in *P. bromi*. Even though infiltrations of Pb ToxB cause chlorosis on Ptr ToxB sensitive wheat genotype, it failed to induce symptoms of its own host (Andrie and Ciuffetti, 2011).

As mentioned above, similar to Ptr ToxA, Ptr ToxB induce transcriptional reprogramming of defense related genes and their regulators (Pandelova et al. 2012). Transcription of these genes were up-regulated, and ROS-detoxification genes were down-

regulated. Likewise, Ptr ToxB inactivates the function of the chloroplast. However, Ptr ToxB takes 24 hours while Ptr ToxA takes 9 hours to cause program cell death (Pandelova et al. 2012).

1.3.3.3. Ptr ToxC

As mentioned earlier, Ptr ToxC cause extensive chlorosis which is distinct from chlorosis produced by Ptr ToxB in susceptible wheat genotypes. Extensive chlorosis produced by Ptr ToxC was one of the first symptoms to be identified together with necrosis produced by Ptr ToxA (Lamari and Bernier 1989). Lamari and Bernier (1989) also used chlorosis on 6B365 as a part of the pathotype classification system that they proposed. Later, Lamari and Bernier (1991) also showed that extensive chlorosis and necrosis production is genetically distinct. Multiple QTL mapping studies also showed that Ptr ToxC-*Tsc1* interaction plays a major role in multiple wheat genetics backgrounds (Faris et al. 1997; Efferts et al. 2001, 2002; Sun et al. 2010; Kariyawasam et al. 2016).

Effertz et al. (2002) partially purified Ptr ToxC using gel filtration, ion exchange, and reverse-phase chromatography. The study also revealed that Ptr ToxC is a polar, nonionic, low-molecular weight molecule. Authors used the crude filtrate of race 1 isolate 78-62 to obtain partially purified Ptr ToxC. Infiltration of crude culture extract and partially purified Ptr ToxC was able to produce chlorosis on Ptr ToxC sensitive wheat genotypes whereas infiltrates failed to produce chlorosis on wheat genotypes that were insensitive to Ptr ToxC. This result also agreed with conidial inoculations on the same genotypes. Effertz et al. (2002) also infiltrated the crude extract and the partially purified Ptr ToxC on a recombinant inbred line (RIL) population derived from the cross W-7984× Opata 85 and found that the genetic region associated with resistance to conidial inoculation and insensitivity to infiltration are the same or very closely linked on the short arm of chromosome 1A. These results confirmed that the partially both the crude extract

and the partially purified infiltrates contained Ptr ToxC. However, unlike Ptr ToxA and Ptr ToxB, Ptr ToxC is not proteinaceous in nature (Strelkov and Lamari, 2003). Therefore, virulence gene(s) responsible for the production of Ptr ToxC cannot be identified using traditional biochemical methods as for the other two NEs.

1.3.3.4. Evidence of other NEs

In addition to, known NEs, multiple studies have reported evidence for the presence of other NEs in the wheat- *P. tritici-repentis* system. Friesen et al. (2003) used mutants of Kulm that were insensitive to Ptr ToxA and demonstrated that race 2 isolate 86-124 had the ability to cause disease and suggested that other necrosis inducing NEs were at play. Several studies also hinted about the existence of putative Ptr ToxD (Meinhardt et al. 2003; Ciuffetti et al. 2003). Manning and Ciuffetti (2015) showed that *ToxA* mutant isolates were still able to cause disease on susceptible wheat genotypes. Manning and Ciuffetti (2015) also showed that mutant isolate produced chlorosis which was not produced by the wild type isolate. Therefore, they concluded that NEs such as Ptr ToxA can be epistatic to the expression of other NEs. This notion is further bolstered by, acquiring QTL for the *ToxA* mutants of 86-124, that were absent for the wild type isolate in the winter wheat recombinant inbred line (RIL) population developed from Harry and Wesley, discussed in paper 3. Guo et al. (2018) recently published that two isolates that lacked both *ToxA* and *ToxB* genes showed the same QTL as the *ToxA* mutant isolate of 86-124, in Harry-Wesley population. Furthermore, isolates that were collected from Arkansas such as AR CrossB10 lack *ToxA* but had the ability to cause necrosis on the *ToxA* differential line Glenlea (Ali et al. 2010). All these evidences suggest that there are other NEs produced by *P. tritici-repentis* in addition to Ptr ToxA, Ptr ToxB and Ptr ToxC.

1.3.4. P. tritici-repentis genome

Since *P. tritici-repentis* has been an economically important pathogen, the genome of the pathogen has been studied. Karyotypic analysis of *P. tritici-repentis* was first done by Lichter et al. (2002) and showed that chromosome size was different among non-pathogenic and pathogenic isolates and within the pathogenic isolates. Aboukhaddour et al. (2009) using 47 isolates showed that isolates belonging to various races of *P. tritici-repentis* consisted of different number of chromosomes ranged from 8-11 and the size of the genome ranged from 25.5 to 48 Mb. In addition, Manning et al. (2013) nonpathogenic isolates tend to have a smaller genome compared to pathogenic isolates. Aboukhaddour et al. (2009) observed variation in chromosome length as well as in chromosome number among the isolates within the race as well as between the races. Aboukhaddour et al. (2009) observed 29 karyotype patterns among 47 isolates.

In addition to the karyotypic analysis, both studies focused on identifying the chromosomal location of the *ToxA* and *ToxB* genes. Lichter et al. (2002) located the *ToxA* gene to a chromosome with the size of 3.0 Mb and identified a homologous chromosome in non-pathogenic isolates with the size of 2.75 Mb. However, Aboukhaddour et al. (2009) showed that chromosomes with the *ToxA* gene varied in size in Ptr ToxA producing isolates regardless of the race. Out of 47, 36 isolates contained the *ToxA* gene in a chromosome with the size of 2.9 Mb. Similar results were obtained for the isolates carrying *ToxB* as well, where chromosomes with *ToxB* size ranged from 2.2 to 5.7 Mb. In addition, for one of the race 5 isolates they were able to observe *ToxB* in two chromosomes with different band intensities suggesting variation in copy number at each locus.

High quality reference genomes are useful tools to clone genes that contribute to a phenotype of interest. Therefore, availability of such genome assemblies for fungal pathogens such as *P. tritici-repentis* increases the possibility of identifying effectors. In addition, it can also provide a basis for comparative genomics studies among isolates belonging to the same race, different race or different species (Ciuffetti et al. 2014).

Manning et al. (2013) published the first genome sequence and optical map of *Ptr* using the race 1 isolate Pt-1C-BFP, which was collected from North Dakota. The Pt-1C-BFP genome was sequenced using whole genome shotgun and Sanger sequencing methods using fosmid libraries. Forty-seven scaffolds were assembled and it consisted of 37.8 Mb. Out of 47, 26 of the supercontigs were mapped to the 11 chromosomes of the optical map based on the restriction enzyme recognition sequence and length of the restriction fragments with the use of map aligner software. The assembly had an N50 scaffold length of 1.99 Mb. Gene annotations revealed 12,141 gene models.

In addition, a race 5 isolate DW-7-ToxB and a race 4 isolate SD20-NP were also sequenced using Illumina short-read method. Only 85% of the sequenced reads of SD20-NP and 93% of the DW-7-ToxB were mapped to the reference sequence of Pt-1c-BFP (Manning et al. 2013). SD20-NP also showed 73,190 SNPs with reference sequence whereas DW7-ToxB showed only 7,429 SNPs. Therefore, these facts show the non-pathogenic SD-20 (race 4) isolate is more diverse from pathogenic Pt-1c-BFP (race 1) and DW7-ToxB (race 5) isolates (Manning et al. 2013).

Very recently, Moolhuijzen et al. (2018), published a novel genome sequence and a de novo optical map of *P. tritici-repentis* using an Australian race 1 isolate M4. The genome of M4 was sequenced with the use of long read single molecule real time (SMRT) PacBio sequencing

technology with 75x coverage. Genome sequence consists of 50 contiguous sequences, which were corrected for the errors in base calls using Illumina high-quality paired-end reads. These 50 contiguous sequences had a total length of 40.9 Mb where 39.9 Mb (98%) was assigned to 10 chromosomes with the help of the optical map. Genome sequence of M4 had a L50 and N50 values of 6 and 2.9 Mb respectively. Gene annotations of the M4 genome revealed 13,797 gene models, which is approximately 2,000 gene models more than that of Pt-1C-BFP (Moolhuijzen et al. 2018). In addition, Moolhuijzen et al. (2018) also sequenced genomes of seven other isolates belonging to races 1, 2, 5 and a new race (AR CrossB10).

Moolhuijzen et al. (2018) compared the M4 whole genome assembly with the Pt-1c-BFP genome assembly and identified structural rearrangements among the two race 1 isolates. They showed that chromosome 10 of M4 was a fusion of chromosomes 10 and 11 of Pt-1c-BFP. They also observed 2 major inversions in chromosome 3 of two isolates which accounted for 60% of the length of the chromosomes. In addition, they also showed a translocation in the distal end of chromosome 2. Most of the variation among the two assemblies seems to be coincided with the break points of the Pt-1c-BFP scaffolds. The authors validated these rearrangements in M4 with the use of PCR. However, they did not validate the Pt-1c-BFP isolate due to its unavailability.

Genome sequence assemblies also give an account of repeat content of the genome, hence the plasticity (Manning et al. 2013). Manning et al. (2013) showed that 16% of the Pt-1c-BFP genome consists of repeat elements and 81% of it had 95 to 100% similarity. Similar results were identified for M4 with 15% of the genome comprised of repeat content (Moolhuijzen et al. 2018). Similarity in repeat content also showed that repeat induced polymorphism (RIP) mechanism is inactive in the Pt-1c-BFP genome. RIP plays an important role in fungal genome evolution which is a mechanism of mutagenesis that modifies cytosine bases to thymine,

favoring CpA dinucleotides (Hane et al. 2008). However, in the M4 genome, authors have identified few repetitive genomic regions that were subjected to the RIP mechanism. However, AT-rich regions associated with RIPs were not observed (Moolhuijzen et al. 2018). Therefore, *P. tritici-repentis* genome lacked the characteristics of 'two speed' genome as many plant fungal pathogens. In such genomes, genome is sectioned in to gene rich, repeat spares regions, and gene sparse, repeat rich region (Dong et al. 2015; Wyatt et al. 2017)

With the availability of genome sequence, putative effector genes can be identified by looking for gene models that encodes for small secreted proteins with cysteine residues and signal molecules. Using the prediction software such as EffectorP, 224 candidate effectors have been identified in M4. Moolhuijzen et al. (2018) also showed that the number of putative effectors also varied based on the isolate. For the eleven isolates used by Moolhuijzen et al. (2018) predicted effectors ranged from 179 to 260. In addition to proteins, secondary metabolites can also act as NEs. These secondary metabolites are synthesized in gene clusters that usually includes non-ribosomal peptide synthases (NPRS) and polyketide synthases (PKS). In the M4 genome 28 such clusters such as seven NPRS, four NPRS-like, thirteen PKS and four PKS like were identified (Moolhuijzen et al. 2018). Therefore, coupling the information provided by such a robust reference genome with the information obtained from the genetic analysis using the method published by Ameen et al. (2017), strong candidate genes for the effectors such as Ptr ToxC can be identified.

1.4. Sexual reproduction and genetics

Sexual reproduction is one of the most important aspects of the tree of life that has been conserved among all the eukaryotes (Heitman et al. 2013). Sexual reproduction is beneficial for organisms since it serves to generate progeny with diverse genotypes through recombination that

provide polymorphism to adapt to ever changing environment. In addition, sexual reproduction also removes the deleterious mutations of the genome such as transposable elements that could degrade the integrity of the genome. Goddard et al. (2005) with the use of yeast strains that mutant for meiotic recombination showed that wild type strains with sexual reproduction respond well and survive well to and in stressful environmental conditions compared to the mutants that reproduced asexually.

Ascomycetes such as *P. tritici-repentis* also undergo sexual reproduction. Ascogonia (female reproductive structure) are differentiated from vegetative hyphae. In many fungi ascogonia have apical receptive hyphal element known as trichogyne. This trichogyne fuses with the male element such as microconidia, macroconidia or hypha. Fertilization is completed with the transfer of nuclei from the opposite mating type element in to the primary ascogonium. The fertilized ascogonium produces the fruiting body known as pseudothecia. Each nucleus from different mating types multiply and pair of nuclei from two parental types will transfer into specialized cells called ascogenous hyphae. These ascogenous hyphae forms the crozier cells in where two nuclei from the two parental mating types undergoes mitosis. After the formation of septa lateral and basal cells with one nucleus and middle cell with two nuclei are formed. The binucleate cell differentiate in to an ascus mother cell and karyogamy takes place with in this ascus mother cell. Finally, the diploid nucleus resulting from karyogamy undergoes meiosis to form 4 haploid nuclei. These four nuclei undergo post meiotic mitosis to give rise to 8 nuclei which eventually form eight haploid ascospores per ascus (reviewed in Coppin et al. 1997)

Sexual reproduction of ascomycetes is regulated by two idiomorphs *MAT1-1* and *MAT1-2* at the *MAT* locus. *MAT1-1-1* has conserved *Saccharomyces cerevisiae* alpha box (α1) motif and *MAT1-2-1* consists of high mobility group (HMG) motif (Debuchy et al. 2010). Some

ascomycetes have one of the idiomorphs in an individual whereas some carry both idiomorphs in an individual. Therefore, ascomycetes are categorized as heterothallic (self-sterile) and homothallic (self-fertile) (Turgeon and Yoder, 2000).

Previous research showed that *MAT* plays a major role in sexual reproduction. Deletion of the *MAT1* gene from heterothallic fungi such as *Cochliobolus heterostrophus* made them unable to mate successfully with strains with the *MAT2* gene (Wirsel et al. 1996). It was also shown that homothallic fungi such as *Fusarium graminearum* can be made self-sterile by deleting one of the mating type genes (Lee et al. 2003). Therefore, deleting of mating type genes has been used to convert homothallic strains to heterothallic strains (Lee et al. 2003).

P. tritici-repentis is a homothallic ascomycete. *P. tritici-repentis* contains both *MAT1-1-1* and *MAT1-2-1* in tandem spanning 4.5 kb (Lepoint et al. 2010). Ameen et al. (2017) created heterothallic strains of race 2 isolates 86-124 and race 5 isolate DW5 by deleting one of the *MAT* genes using a split marker strategy coupled with homologous recombination. These heterothallic strains failed to produce functional pseudothecia and showed both *MAT1-1-1* and *MAT1-2-1* were required for sexual reproduction. However, a cross between 86-124ΔMAT1-1× 86-124ΔMAT1-2 restored the formation of functional pseudothecia which resulted in asci with 8 ascospores similar to its homothallic wild type. They also reported that the cross DW5ΔMAT1-1× 86-124ΔMAT1-2 yielded functional pseudothecia, even though the fertility was reduced compared to the wild type isolates because the majority of asci consisted of two ascospores per asci.

Genetic mapping has been commonly used to map virulence/avirulence genes of many plant pathogenic fungi. Lendenmann et al. (2014) developed two genetic maps for *Zymoseptoria tritici* from the crosses 3D1×3D7 and 1A5×1E4 which consisted of 9,745 and 7,333 markers that

spanned over 4,255.4 cM and 5191.3 cM respectively. In this study they used these maps to identify QTL that involved in synthesis of melanin. Recently, Zhong et al. (2017) did QTL mapping based on the genetic map developed from the cross 1A5×1E4 which combined with genome wide association mapping to clone *AvrStb6* which interacts with *Stb6* resistance in a gene-for-gene manner. Furthermore, several genetic maps have been developed for *P. teres* f. *teres*. Weiland et al. (1999) used a cross between isolates 0-1 and 15A to identify *AvrHar* that conferred low virulence on cultivar 'Harbin'. The same population was used by Lai et al. (2007) to identify two genes of *AvrPra1* and *AvrPra2* which conferred virulence to barley line 'Prato'. Likewise, many more studies have developed genetic maps for *P. teres* f. *teres* which resulted in mapping virulence/avirulence factors for barley (Shjerve et al. 2014; Koladia et al. 2017).

However, the above mentioned pathogens are naturally out crossing, unlike *P. tritici-repentis.* Therefore, the work done by Ameen et al. (2017) opens up the possibility of developing mapping populations for *P. tritici-repentis* where we can couple it with genome sequence sources to provide an effective way to identify and clone virulence genes in *P. tritici-repentis*, in particular for those that, cannot be done using conventional biochemical methods, for example Ptr ToxC.

1.5. Host resistance

Host resistance to tan spot has been extensively studied and resistant genotypes have been reported from many places of the world, including but not limited to the United States, Brazil, Mexico, Chile, China, Germany, Ecuador (Rees and Platz, 1992). For example, Lamari et al. (1992) identified 695 resistant wheat lines from 1200 wheat accessions including diploid, tetraploid, hexaploid and octaploid wheat lines. From a recent study, Liu et al. (2015) identified seven resistant lines out of 120 winter wheat cultivars and breeding lines from the United States.

Over the past 20 years, genetic resistance to tan spot has been shown to be a complex, which involves the lack of host sensitivity genes, major recessive resistance genes, race-nonspecific QTL and other resistance QTL other than three susceptibility genes (Reviewed in Faris et al. 2013; Kariyawasam et al. 2016; Virdi et al. 2016; Liu et al. 2017).

1.5.1. Host susceptibility genes

In many cases, tan spot resistance has been demonstrated to be the absence of three host sensitivity genes, which are *Tsn1* (for Ptr ToxA), *Tsc2* (for Ptr ToxB) and *Tsc1* (for Ptr ToxC). Faris et al. (1996) mapped the *Tsn1* gene Ptr ToxA to the long arm of chromosome 5B using restriction fragment length polymorphism (RFLP) analysis. Saturation mapping later delimited the *Tsn1* gene in a gene-rich region of the long arm of chromosome 5B (Faris et al. 2000). A map-based cloning strategy was used to clone the *Tsn1* gene which showed that it has a typical resistance gene structure consisting of serine/threonine protein kinase (S/TPK), nucleotide binding (NB), and leucine rich repeat (LRR) domains (Faris et al. 2010). However, direct interaction between Tsn1 and Ptr ToxA was not detected in that study suggesting the involvement of other factors in Ptr ToxA-Tsn1 interaction.

Since the discovery of the *Tsn1* gene, many disease susceptibility QTL were mapped to the *Tsn1* locus using various wheat populations indicating Ptr ToxA-Tsn1 interaction is important for disease development, thus disease susceptibility (Faris et al. 1996; Chong et al. 2004; Singh et al. 2008; Noriel et al. 2011; Faris et al. 2012; Liu et al. 2017). However, in many other wheat populations, there was no QTL identified at the *Tsn1* locus even though the Ptr ToxA-Tsn1 interaction was presented in these populations, particularly in tetraploid wheat populations (Faris and Friesen 2005; Chu et al. 2008; 2010; Kariyawasam et al. 2016). Some of this was due to the presence of race non-specific resistance QTL that may have epistatic effect on

the disease induced by the Ptr ToxA-*Tsn1* interaction (Faris and Friesen 2005; Kariyawasam et al. 2016).

Orolaza et al. (1995) used the partially purified Ptr ToxB to show that sensitivity to the NE was governed by a single dominant host susceptibility gene. Later, the Ptr ToxB host sensitivity gene was mapped to the short arm of chromosome 2B using the International Triticeae Mapping Initiative (ITMI) population and named as *Tsc2* (Friesen and Faris, 2004). In the study, it was shown that the *Tsc2* locus explained 69% of the phenotypic variation in disease caused by the race 5 isolate used. The *Tsc2* genomic region was further saturated with molecular markers developed from wheat ESTs together with the information of synteny to rice and *Brachypodium* genomes by using a hexaploid mapping population developed from a cross between Salamouni and Katepwa (Abeysekara et al. 2010). The research located the *Tsc2* gene to a 3.3 cM genetic region at the distal end of 2B and identified the STS marker *XBE444541* that co-segreagated with *Tsc2* and was recommended for marker assisted breeding against Ptr ToxB sensitivity. So far, the *Tsc2* gene has not been cloned and structure of the gene remains unknown.

Faris et al. (1997) was the first to find that the chromosomal region on the short arm of chromosome 1A, designated QTL *QTsc.ndsu-1A*, is associated with disease caused by *P. tritici-repentis* races 1 and 3 isolates using the ITMI population derived from Opata 85 and W-7984. Later, Effertz et al. (2002) showed that insensitivity to Ptr ToxC is controlled by a single recessive gene on the short arm of 1A and they named it *tsc1*. Therefore, *QTsc.ndsu-1A* was likely underlined by the *Tsc1* locus. Similarly, in several follow up studies, QTL associated with tan spot susceptibility were also identified at the *Tsc1* locus with high significance level (Sun et al. 2010; Kariyawasam et al. 2016; Liu et al. 2017). In addition, Liu et al. (2017) indicated that

the Ptr ToxC-*Tsc1* can act additively with the Ptr ToxA-*Tsn1* in some wheat genotypes. So far, the *Tsc1* gene is not cloned either despite its importance in wheat tan spot disease.

1.5.2. Other genetic factors

In addition to the three major sensitivity (susceptibility) genes, several studies have identified qualitative recessive resistance genes against specific races/isolates of *P. tritici-repentis*. Singh et al. (2006) located recessive resistance gene *tsr2* on chromosome 3B that conferred resistance to necrosis induced by race 3 isolate 331-9 with the use of LDN-DIC disomic chromosomal substitution lines and an RIL population derived from the cross Coulter × PI 352519 (*T. turgidum* ssp. *turgidum*).

In another study, synthetic hexaploid wheat lines XX41, XX45 and XX110 derived from the crosses between *Ae. tauschii* and a tetraploid parental lines LDN (XX41, XX45) and *T. turgidum* ssp. *dicoccum* A38 (XX110), showed resistance to race 1 isolate ASC1b (Tadesse et al. 2006a). Resistance was hypothesized to derive from *Ae. tauschii*, since both the tetraploid parents were susceptible to tan spot.

Tadesse et al. (2006a) crossed each of these synthetic accessions to Chinese Spring D-genome monosomic lines and identified a single gene on chromosome 3D that confers resistance to tan spot caused by ASC1b. Furthermore, F2 hybrids obtained from these crosses showed that, resistance gene in XX41 and XX110 inherited as recessive resistance whereas resistance in XX45 is inherited as dominant. A follow up study showed that three genes in each line could be alleles of the same gene or three tightly linked genes and these genes were considered as *tsr3* recessive resistance genes (Tadesse et al. 2007).

Another recessive resistance gene *tsr4* was identified through analyzing the F2 populations derived from crosses between Salamouni and Chinese Spring monosomic lines. A

gene was located to chromosome 3A and conferred resistance to tan spot caused by ASC1a (Tadesse et al. 2006b). Singh et al. (2008) used the same population as Singh et al. (2006) and mapped resistance against the necrosis produced on tetraploid wheat by race 5 isolate DW13. Asingle recessive resistance gene was mapped to the long arm of 3B and was designated as *tsr5* (Singh et al. 2006). Since resistance conferred by these genes are recessive, these loci might also be dominant susceptibility genes for the NEs that are yet identified.

Furthermore, many bi-parental and association mapping studies have been carried out on various wheat genetic backgrounds to identify QTL conferring resistance to tan spot. From QTL mapping studies resistance for different isolates of *P. tritici-repentis* segregated in bi-parental populations derived from the crosses W-7984 × Opata 85, Cranbook × Halberd, Brookton × Krichauff, BR34 × Grandin, TA4152-60 × ND495, WH542 × HD29, Lebsock × PI94749, Wangshuibai × Ning7840, Frina × Batavia, Salamouni × Katepwa, Louise × Penawawa, Altar 84 × Langdon, Harry × Wesley and LMPG-6 × PI626573 had been well characterized and a number of resistant QTL have been identified (Reviewed in Faris et al. 2013; Kariyawasam et al. Virdi et al. 2016; Liu et al. 2017). Some of the QTL provided resistance to one or a few isolates and they were designated as race-specific QTL whereas other QTL conferred resistance to all the races and were designated as race-nonspecific resistance. Faris and Friesen (2005) were the first to report and map race-nonspecific resistance in chromosome 1BS and 3BL in an RI population derived from BR34 and Grandin. Two QTL confirmed resistant isolates belongs to the races 1, 2, 3, and 5. Later several studies identified race-nonspecific QTL in the chromosomes 2A, 3B, 5A, and 7B which were segregated in other mapping populations (Chu et al. 2008; Kariyawasam et al. 2016). Kariyawasam et al. (2016) also showed that in the presence of race-nonspecific

resistance governed by *QTs.zhl-3B*, Ptr ToxA-Tsn1 interaction does not play an important role in disease.

In addition, several association mapping studies also conducted with different genetic backgrounds including winter wheat and spring wheat identified resistance to tan spot (Gurung et al. 2011; Patel et al. 2013; Kollers et al. 2014; Liu et al. 2015; Juliana et al. 2018). From both association and bi-parental QTL mapping studies, all the wheat chromosomes except 6D have been shown to carry QTL associated with tan spot resistance or susceptibility.

1.6. References

Abdullah, S., Sehgal, S., K., Ali, S., Liatukas, Z., Ittu, M., and Kaur, N. (2017a.) Characterization of *Pyrenophora tritici-repentis* (Tan Spot of Wheat) Races in Baltic States and Romania. Plant Pathol. J. 33: 133-139.

Abdullah, S., Sehgal, S.K., Glover, K.D. and Ali, S., (2017b.) Reaction of Global Collection of Rye (Secale cereale L.) to Tan Spot and *Pyrenophora tritici-repentis* Races in South Dakota. Plant Pathol. J 33:229.

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

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

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

Aboukhaddour, R., Turkington, T. K., and Strelkov, S. E. (2013). Race structure of *Pyrenophora tritici-repentis* (tan spot of wheat) in Alberta, Canada. Can. J. Plant Pathol. 35:256–268.

Aboukhaddour, R. and Strelkov, S.E., (2016). Exploring de novo specificity: the *Pyrenophora tritici-repentis*–barley interaction. Plant Pathol. 65:1347-1357.

Adhikari, T.B., Bai, J., Meinhardt, S.W., Gurung, S., Myrfield, M., Patel, J., Ali, S., Gudmestad, N.C. and Rasmussen, J.B., (2009). Tsn1-mediated host responses to ToxA from *Pyrenophora tritici-repentis*. MPMI 22:1056-1068.

Andrie, R.M. and Ciuffetti, L.M., (2011). *Pyrenophora bromi*, causal agent of brownspot of bromegrass, expresses a gene encoding a protein with homology and similar activity to Ptr ToxB, a host-selective toxin of wheat. MPMI. 24:359-367.

Ali, S. and Francl, L.J. (1998). Race structure of *Pyrenophora tritici-repentis* isolated from wheat and grasses in the US Great Plains. Phytopathology. 88:S114.

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

Ali, S., Francl, L., J., Iram, S., and Ahmad, I. (2001). First report of tan spot on wheat in Pakistan. Plant Dis. 85:1031-1031.

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

Ali, S., and Francl, L. J. (2003). Population race structure of *Pyrenophora tritici-repentis* prevalent on wheat and noncereal grasses in the Great Plains. Plant. Dis. 87:418–422

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

Amaike, S., Ozga, J. A., Basu, U., and Strelkov, S. E. (2008). Quantification of ToxB gene expression and formation of appressoria by isolates of *Pyrenophora tritici repentis* differing in pathogenicity. Plant Pathol. 57:623–633

Ameen, G., Kariyawasam, G., Shi, G., Friesen, T. L., Faris, J. D., Ali, S., Rasmussen, J. B. and Liu, Z. (2017). Molecular manipulation of the mating-type system and development of a new approach for characterizing pathogen virulence in *Pyrenophora tritici-repentis*. Fungal Genet. Biol.109:16-25

Balance, G., M., Lamari, L., and Bernier, C. C. (1989). Purification and characterization of a host-selective necrosis toxin from *Pyrenophora tritici-repentis*. Physiol. Mol. Plant. Pathol. 35:203–213

Balance, G., Lamari, L., Kowatsch, R., and Bernier, C. (1996). Cloning, expression and occurrence of the gene encoding the Ptr necrosis toxin from *Pyrenophora tritici-repentis*. Mol Plant Pathol.

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

Benslimane, H., Lamari, L., Benbelkacem, A., Sayoud, R., and Bouzand, Z. (2011). Distribution of races of *Pyrenophora tritici-repentis* in Algeria and identification of a new virulence type. Phytopathol. Mediterr. 50: 203–211

Benslimane, H., (2018). Virulence Phenotyping and Molecular Characterization of a New Virulence Type of Pyrenophora tritici-repentis the Causal Agent of Tan Spot. Plant Pathol. J. 34: 139-142.

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

Cheong, J., Wallwork, H., and Williams, K. J. (2004). Identification of a major QTL for yellow leaf spot resistance in the wheat varieties Brookton and Cranbook. Aust. J. Agric. Res. 55:315–319

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

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

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

Ciuffetti, L., Francl, L., Ballance, G., Bockus, W., Lamari, L., Meinhardt, S., Rasmussen, J. (1998). Standardization of toxin nomenclature in the *Pyrenophora tritici-repentis*/wheat interaction. Can. J. Plant Pathol. 20:421–424

Ciuffetti, L.M., Manning, V.A., Martinez, J.P., Pandelova, I. and Andrie, R.M., (2002), July. Proteinaceous toxins of *Pyrenophora tritici-repentis* and investigation of the site-of-action of Ptr ToxA. In Proceedings of the Fourth International Wheat Tan Spot and Spot Blotch Workshop, JB Rasmussen, TL Friesen, and S. Ali, eds (Fargo, ND: North Dakota Agricultural Experiment Station) 96-102.

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

Ciuffetti, L.M., Manning, V.A., Pandelova, I., Faris, J.D., Friesen, T.L., Strelkov, S.E., Weber, G.L., Goodwin, S.B., Wolpert, T.J. and Figueroa, M. (2014). *Pyrenophora tritici-repentis*: A Plant Pathogenic Fungus with Global Impact. In Genomics of Plant-Associated Fungi: Monocot Pathogens (pp. 1-39). Springer Berlin Heidelberg.

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

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

Coppin, E., Debuchy, R., Arnaise, S. and Picard, M., (1997). Mating types and sexual development in filamentous ascomycetes. Microbiol. Mol. Biol. Rev. 61:411-428.

Croll, D., Zala, M., and McDonald, B., A. (2013). Breakage-fusion-bridge cycles and large insertions contribute to the rapid evolution of accessory chromosomes in a fungal pathogen. PLoS genet, 9:e1003567. doi:10.1371/journal.pgen.1003567

Debuchy, R., Berteaux-Lecellier, V. and Silar, P., (2010). Mating systems and sexual morphogenesis in ascomycetes. In Cellular and molecular biology of filamentous fungi 501-535. American Society of Microbiology.

De Wolf, E., D., Effertz, R. J., Ali, S., and Francl, L., J. (1998). Vistas of tan spot research. Can J Plant Pathol. 20:349–370

Diaz de Ackermann, M., Hosford Jr., R., M., Cox, D., J. and Hammond, J., J. (1988). Resistance in winter wheats to geographically differing isolates of *Pyrenophora tritici-repentis* and observations on pseudoperithecia. Plant Dis. 72:1028-1031.

Diedicke, H. (1902). Uber den Zusammenhang zwischen *Pleosporaund Helminthosporium*-arten. Centrablatt fur Bakteriologie und Parasitenkunde Jena, Abt. 9:317-329.

Dinoor, A. (1974). Role of wild and cultivated plants in the epidemiology of plant diseases in Israel. Annu. Rev. Phytopathol. 12:413-436.

Dodds, P.N. and Rathjen, J.P. (2010). Plant immunity: towards an integrated view of plant–pathogen interactions. Nature Rev. Genet.11:539-548

Dong, S., Raffaele, S. and Kamoun, S., (2015). The two-speed genomes of filamentous pathogens: waltz with plants. Curr Opin *Genetics* Dev.57-65.

Drechsler, C. (1923). Some graminicolons species of *Helminthosporium*. I. J Agric. Res. 24:641-739.

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

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

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

Evans, C., Hunger, R., Siegerist, W. (1999). Comparison of greenhouse and field testing to identify wheat resistant to tan spot. Plant Dis. 83:269–273

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

Faris, J. D., Anderson, J. A., Francl, L., J., and Jordahl, J., G. (1997). RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici- repentis* in wheat. Theor Appl Genet. 94:98–103

Faris, J., D., Haen, K., M., and Gill, B., S. (2000). Saturation mapping of a gene-rich recombination hot spot region in wheat. Genet. 154:823–835

Faris, J., D., and Friesen, T., L. (2005). Identification of quantitative trait loci for race-nonspecific resistance to tan spot of wheat. Theor. Appl. Genet. 111:386–392

Faris, J., D., Zhang, Z., Lu, H., J., Lu, S., W., Reddy, L., Cloutier, S., Fellers, J., P., Meinhardt, S., W., Rasmussen, J., B., Xu, S., S., Oliver, R., P., Simons, K., J., and Friesen, T., L. (2010). A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proc. Natl. Acad. Sci. USA 107:13544–13549

Faris, J., D., Liu, Z., and Xu, S., S. (2013). Genetics of tan spot resistance in wheat. Theor. Appl. Genet. 29:1–21

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

Feldman, M., and Levy, A. A. (2012). Genome evolution due to allopolyploidization in wheat. Genetics 192:763-774.

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

Figueroa, M., Manning, V., A., Pandelova, I., and Ciuffetti, L., M. (2015). Persistence of the host-selective toxin Ptr ToxB in the apoplast. Mol. Plant-Microbe Int. 28:1082-1090.

Flor, H.H., (1971). Current status of the gene-for-gene concept. Annu. Rev. Phytopathol.9:275-296.

Franceschetti, M., Maqbool, A., Jiménez-Dalmaroni, M., J., Pennington, H., G., Kamoun, S. and Banfield, M., J. (2017). Effectors of filamentous plant pathogens: commonalities amid diversity. Microbiol Mol Biol Rev. 81: pp.e00066-16.

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

Friesen, T., L., Ali, S., Stack, R., W., Francl, L., J., and Rasmussen, J., B. (2003). Rapid and efficient production of the *Pyrenophora tritici-repentis* teleomorph. Can. J. Bot. 81:890-895.

Friesen, T., L., and Faris, J., D. (2004). Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. Theor. Appl. Genet. 109:464–471

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

Friesen, T., L., and Faris, J., D. (2012). Characterization of plant-fungal interactions involving necrotrophic effector-producing plant pathogens. In Plant Fungal Pathogens (pp. 191-207). Humana Press.

Friesen, T.L., Holmes, D.J., Bowden, R. and Faris, J.D. (2018). ToxA is present in the United States Bipolaris sorokiniana population and is a significant virulence factor on wheat harboring Tsn1. Plant Dis.

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

Friskop, A., and Acevedo, M. (2015). Rust Diseases of Wheat in North Dakota. PP-1361 (Revised). North Dakota State University Extension Service.

Gamba, F., M., Strelkov, S., E., and Lamari, L. (2012). Virulence of Pyrenophora tritici-repentis in the Southern Cone Region of South America. Can. J. Plant Pathol. 34:545–550

Gamba, F., M., Bassi, F., M., and Finckh, M., R. (2017). Race structure of Pyrenophora tritici-repentis in Morocco. Phytopathol. Mediterr. 56: 119-126.

Goddard, M.R., Godfray, H.C.J. and Burt, A., (2005). Sex increases the efficacy of natural selection in experimental yeast populations. Nature. 434:636.

Guo, J., Shi, G. and Liu, Z., (2018). Characterizing Virulence of the *Pyrenophora tritici-repentis* Isolates Lacking Both *ToxA* and *ToxB* Genes. Pathogens. 7:74.

Gurung, S., Mamidi, S., Bonman, J.M., Jackson, E.W., Del Rio, L.E., Acevedo, M., Mergoum, M. and Adhikari, T.B., (2011). Identification of novel genomic regions associated with resistance to Pyrenophora tritici-repentis races 1 and 5 in spring wheat landraces using association analysis. Theor. Appl. Genet. 123:1029-1041.

Hammond-Kosack, K.E. and Rudd, J.J., (2008). Plant resistance signalling hijacked by a necrotrophic fungal pathogen. Plant Signal. Behav. 3:993-995.

Hane, J. K., and Oliver, R., P. (2008). RIPCAL: a tool for alignment-based analysis of repeat-induced point mutations in fungal genomic sequences. BMC Bioinformatics 9: 478.

Heffelfinger, C., Fragoso, C.A. and Lorieux, M., (2017). Constructing linkage maps in the genomics era with MapDisto 2.0. Bioinformatics. 33:2224-2225.

Heitman, J., Sun, S. and James, T.Y., (2013). Evolution of fungal sexual reproduction. Mycologia, 105:1-27.

Horbach, R., Navarro-Quesada, A.R., Knogge, W. and Deising, H.B., (2011). When and how to kill a plant cell: infection strategies of plant pathogenic fungi. J Plant Physiol 168:51-62.

Hosford, R., M., Jr. (1971). A form of *Pyrenophora trichostoma* pathogenic to wheat and other grasses. Phytopathology 61:28–32

Hosford, R.M. Jr., and R.H. Busch. (1974). Losses in wheat caused by *Pyrenophora trichostoma* and *Leptosphaeria avenaria* f. sp. *triticea*. Phytopathology 64:184-187.

Hosford, R., M., Jr. (1982). Tan spot-developing knowledge 1902–1981, virulent races and wheat differentials, methodology, rating systems, other leaf diseases, and literature. In: Tan Spot of Wheat and Related Diseases Workshop, Fargo, North Dakota, Agricultural Experimental Station, North Dakota State University, (pp 1–24)

Hussain, W., Baenziger, P. S., Belamkar, V., Guttieri, M. J., Venegas, J. P., Easterly, A., Sallam, A., and Poland, J. (2017). Genotyping-by-Sequencing Derived High-Density Linkage Map and its Application to QTL Mapping of Flag Leaf Traits in Bread Wheat. Sci. Rep. 7: 16394. doi.org/10.1038/s41598-017-16006-z

Ito, S. (1930). On some new ascigerous stages of the species of *Helminthosporium parasitic* on cereals. Proc. Imp. Acad. Tokyo 6:352-355.

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

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

Jones, J.D. and Dangl, J.L. (2006). The plant immune system. Nature. 444:323-329

Juliana, P., Singh, R.P., Singh, P.K., Poland, J.A., Bergstrom, G.C., Huerta-Espino, J., Bhavani, S., Crossa, J. and Sorrells, M.E., (2018). Genome-wide association mapping for resistance to leaf rust, stripe rust and tan spot in wheat reveals potential candidate genes. Theor. Appl. Genet. 1-18.

Kabbage, M., Yarden, O. and Dickman, M.B., (2015). Pathogenic attributes of *Sclerotinia sclerotiorum*: switching from a biotrophic to necrotrophic lifestyle. Plant Science 233:53-60.

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

Konieczny, A., Ausubel, F., M. (1993). A procedure for mapping *Arabidopsis* mutations using co-dominant ecotype-specific PCR-based markers. Plant J. 4:403-410.

Koladia, V.M., Richards, J.K., Wyatt, N.A., Faris, J.D., Brueggeman, R.S. and Friesen, T.L., (2017). Genetic analysis of virulence in the Pyrenophora teres f. teres population BB25× FGOH04Ptt-21. Fungal Genet.and Biol. 107:12-19.

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

Krupinsky, J., M. (1992). Grass hosts of *Pyrenophora triticirepentis*. Plant Dis. 76:92-95.

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

Lamari, L., and Bernier, C., C. (1991). Genetics of tan necrosis and extensive chlorosis in tan spot of wheat caused by *Pyrenophora tritici-repentis*. Phytopathology. 81:1092-1095.

Lamari, L., Bernier, C., C., and Balance, G., M. 1992. The necrosis-chlorosis model in tan spot of wheat. Pages 10-15 in L.J. Francl, J.M. Kuprinsky, and McMullen, M., P. eds., Advances in Tan Spot Research. North Dakota Agric. Exp. Station. Fargo, ND

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

Lamari, L., Gilbert, J. and Tekauz, A., (1998). Race differentiation in *Pyrenophora tritici-repentis* and survey of physiologic variation in western Canada. Can. J. Plant Pathol. 20:396-400.

Lamari, L., Strelkov, S., E., Yahyaoui, A., Amedov, M., Saidov, M., Djunusova, M. and Koichibayev, M. (2005). Virulence of *Pyrenophora tritici-repentis* in the countries of the Silk Road. Can. J. Plant Pathol. 27:383-388.

Lamari, L., and Strelkov, S., E. (2010). Minireview/Minisynthèse The wheat/*Pyrenophora tritici-repentis* interaction: progress towards an understanding of tan spot disease. Can. J. Plant Pathol. 32: 4-10.

Lai, Z., Faris, J., D., Weiland, J., J., Steffenson, B., J., and Friesen, T. L. (2007). Genetic mapping of *Pyrenophora teres f. teres* genes conferring avirulence on barley. Fungal Genet. Biol. 44: 323-329.

Larez, C.R., Hosford, R., M., Jr., and Freeman, T., P. (1986). Infection of wheat and oats by *Pyrenophora tritici-repentis* and initial characterization of resistance. Phytopathol. 76: 931-938.

Lee, J., Lee, T., Lee, Y.W., Yun, S.H. and Turgeon, B.G., (2003). Shifting fungal reproductive mode by manipulation of mating type genes: obligatory heterothallism of *Gibberella zeae.* Mol Microbial 50:145-152.

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

Lendenmann, M., H., Croll, D., Stewart, E., L., and McDonald, B., A. (2014). Quantitative trait locus mapping of melanization in the plant pathogenic fungus *Zymoseptoria tritici*. G3 (Bethesda) 4:2519-33.

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

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

Liu, Z., Faris, J.D., Oliver, R.P., Tan, K.C., Solomon, P.S., McDonald, M.C., McDonald, B.A., Nunez, A., Lu, S., Rasmussen, J.B. and Friesen, T.L. (2009). SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the Snn3 gene. PLoS Pathogens. 5: p.e1000581.

Liu, Z., Zhang, Z., Faris, J.D., Oliver, R.P., Syme, R., McDonald, M.C., McDonald, B.A., Solomon, P.S., Lu, S., Shelver, W.L. and Xu, S., (2012). The cysteine rich necrotrophic effector SnTox1 produced by *Stagonospora nodorum* triggers susceptibility of wheat lines harboring Snn1. PLoS Pathogens 8: p.e1002467.

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

Liu, Z., Zurn, J., D., Kariyawasam, G., Faris, J., D., Shi, G., Hansen, J., Rasmussen, J., B., and Acevedo, M. (2017). Inverse gene-for-gene interactions contribute additively to tan spot susceptibility in wheat. Theor. Appl. Genet. 6:1267-76.

Long, Y., M., Chao, W., S., Ma, G., J., Xu, S., S., and Qi, L., L. (2017). An innovative SNP genotyping method adapting to multiple platforms and throughputs. Theor. Appl. Genet. 130:597-607.

Lo Presti, L., Lanver, D., Schweizer, G., Tanaka, S., Liang, L., Tollot, M., Zuccaro, A., Reissmann, S. and Kahmann, R., (2015). Fungal effectors and plant susceptibility. Annu. Rev. Plant Biol. 66:513-545.

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

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

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

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

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

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

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

Manning, V., A., Hamilton, S., M., Karplus, P., A., and Ciuffetti, L., M. (2008). The Arg-Gly-Asp-containing, solvent-exposed loop of Ptr ToxA is required for internalization. Mol. Plant Microbe In. 21:315–325

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

Manning, V.A., Chu, A.L., Scofield, S.R. and Ciuffetti, L.M., (2010). Intracellular expression of a host-selective toxin, ToxA, in diverse plants phenocopies silencing of a ToxA-interacting protein, ToxABP1. New Phytol 187:1034–1047

Manning, V., A., Pandelova, I., Dhillon, B., Wilhelm, L., J., Goodwin, S., B., Berlin, A., M., Figueroa, M., Freitag, M., Hane, J., K., Henrissat, B., Holman, W., H., Kodira, C., D., Martin, J., Oliver, R., P., Robbertse, B., Schackwitz, W., Schwartz, D., C., Spatafora, J., W., Turgeon, B., G., Yandava, C., Young, S., Zhou, S., Zeng, Q., Grigoriev, I., V., Ma, L., J., and Ciuffetti, L., M. (2013). Comparative genomics of a plant-pathogenic fungus, *Pyrenophora tritici-repentis*, reveals transduplication and the impact of repeat elements on pathogenicity and population divergence. G3 (Bethesda) 3:41–63.

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

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

Martinez, J., P., Oesch. N., W., and Ciuffetti, L., M. (2004). Characterization of the multiple-copy host-selective toxin gene, ToxB, in pathogenic and nonpathogenic isolates of *Pyrenophora tritici-repentis*. Mol. Plant Microbe In. 17:467–474.

McMullen, M., Zhong S., Neate, S. (2008). Fusarium head blight (Scab) of Small Grains. PP-804 (Revised). North Dakota State University Extension Service.

McMullen, M., and Adhikari, T. (2009). Fungal Leaf Spot Diseases of Wheat: Tan Spot, Stagonospora nodorum blotch and Septoria tritici blotch. PP-1249. North Dakota State University Extension Service.

McMullen, M., and Adhikari, T. (2011). Bacterial Leaf Streak and Black Chaff of Wheat. PP-1566. North Dakota State University Extension Service.

McDonald, M. C., Ahren, D., Simpfendorfer, S., Milgate, A., and Solomon, P., S. (2018). The discovery of the virulence gene ToxA in the wheat and barley pathogen *Bipolaris sorokiniana*. Mol. plant pathol. 19:432-439.

Meinhardt, S., Ali, S., Ling, H., Francl, L., Rasmussen, J. and Friesen, T. (2003). A new race of *Pyrenophora tritici-repentis* that produces a putative host-selective toxin. In Proceedings of the fourth international wheat tan spot and spot blotch workshop. North Dakota Agric. Exp. Station, Fargo 117-121.

Moolhuijzen, P., See, P., T., Hane, J., K., Shi, G., Liu, Z., Oliver, R., P., and Moffat, C., S. (2018). Comparative genomics of the wheat fungal pathogen Pyrenophora tritici-repentis reveals chromosomal variations and genome plasticity. BMC genomics. 19: 279.

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

Mitra, M. (1934). A leaf spot disease of wheat caused by *Helminthosporium tritici-repentis* Died. Indian J. Agric. Sci, 4:692.

Nagy, E.D. and Bennetzen, J.L., (2008). Pathogen corruption and site-directed recombination at a plant disease resistance gene cluster. *Genome research*.

Nisikado, Y. (1928). Preliminary notes on yellow leaf spot diseases of wheat caused by *Helminthosporium tritici-vulgaris* Nisikado. Inst. Agric. Biol. 4:103-109.

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

Ohm, R., A., Feau, N., Henrissat, B., Schoch, C., L., Horwitz, B., A., Barry, K., W., Condon, B., J., Copeland, A., C., Dhillon, B., Glaser, F., and Hesse, C., N. (2012). Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen Dothideomycetes fungi. PLoS Pathog. 6:8:e1003037.

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

Pandelova, I., Betts, M.F., Manning, V.A., Wilhelm, L.J., Mockler, T.C. and Ciuffetti, L.M., (2009). Analysis of transcriptome changes induced by Ptr ToxA in wheat provides insights into the mechanisms of plant susceptibility. Mol Plant 2:1067–1083

Pandelova, I., Figueroa, M., Wilhelm, L., J., Manning, V., A., Mankaney, A., N., Mockler, T., C., Ciuffetti, L. M. (2012) Host-selective toxins of *Pyrenophora tritici-repentis* induce common responses associated with host susceptibility. Plos One 7. Doi: ARTN e40240 DOI 10. 1371/journal.pone.0040240

Patel, J. S., Mamidi, S., Bonman, J. M., and Adhikari, T. B. (2013). Identification of QTL in Spring Wheat Associated with Resistance to a Novel Isolate of. Crop Sci. 53:842-852.

Peterson, C., J., Shelton, D., R., Baenziger, P., S., Baltensperger, D., D., Graybosch, R., A., Worrall, W., D., Nelson, L., A., McVey, D., V., Watkins, J., E., and Krall, J. (2001). Registration of 'Wesley' Wheat. Crop sci. 41:260-.260

Rasmussen, J.B., Kwon, C.Y. and Meinhardt, S.W., (2004). Requirement of host signaling mechanisms for the action of Ptr ToxA in wheat. Eur J Plant Pathol 110:333-335.

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

Rees, R., G., Platz, G., J., and Mayer, R., J. (1982). Yield losses in wheat from yellow spot: comparison of estimates derived from single tillers and plots. Aust. J. Agric. Res. 33:899–908

Rees, R. G., Platz, G. J. (1983). Effects of yellow spot on wheat: comparison of epidemics at different stages of crop development. Aust. J. Agric. Res. 34:39–46

Rees, R. G., and Platz, G., J. (1992). Tan spot and its control—some Australian experiences. In: Francl LJ, Krupinsky JM, McMullen MP (eds) Advances in tan spot research. North Dakota Agric. Exp. Station, Fargo, pp 1–9

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

Schilder, A.M.C. and Bergstrom, G.C., (1990). Variation in virulence within the population of *Pyrenophora tritici-repentis* in New York. Phytopathology 80:84–90

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

Shjerve, R., A., Faris, J., D., Brueggeman, R., S., Yan, C., Zhu, Y., Koladia, V., and Friesen, T., L. (2014). Evaluation of a Pyrenophora teres f. teres mapping population reveals multiple independent interactions with a region of barley chromosome 6H. Fungal genet. Biol. 70:104-112.

Shi, G., Zhang, Z., Friesen, T.L., Raats, D., Fahima, T., Brueggeman, R.S., Lu, S., Trick, H.N., Liu, Z., Chao, W. and Frenkel, Z., (2016). The hijacking of a receptor kinase–driven pathway by a wheat fungal pathogen leads to disease. Sci. Adv. 2: p.e1600822.

Shoemaker, R.A. 1962. Drechslera Ito. Can. J. Bot. 40:809-836.

Singh, P.K., Gonzalez-Hernandez, J.L., Mergoum, M., Ali, S., Adhikari, T.B., Kianian, S.F., Elias, E.M. and Hughes, G.R., (2006). Identification and molecular mapping of a gene conferring resistance to Pyrenophora tritici-repentis race 3 in tetraploid wheat. Phytopathology 96:885–889

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

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

Soreng, R.J., Peterson, P.M., Romaschenko, K., Davidse, G., Teisher, J.K., Clark, L.G., Barberá, P., Gillespie, L.J., and Zuloaga, F.O. (2017). A worldwide phylogenetic classification of the Poaceae (Gramineae) II: An update and a comparison of two 2015 classifications. J Syst Evol 55:259-290.

Strelkov, S., E., Lamari, L., and Balance, G., M. (1999). Characterization of a host-specific protein toxin (Ptr ToxB) from *Pyrenophora triticirepentis*. Mol. Plant-Microbe Interact. 12:728–732

Strelkov, S.E., Lamari, L., Sayoud, R. and Smith, R.B. (2002). Comparative virulence of chlorosis-inducing races of *Pyrenophora tritici-repentis*. Can. J. Plant Pathol. 24:29-35.

Strelkov, S., and Lamari, L. (2003). Host parasite interactions in tan spot [*Pyrenophora tritici-repentis*] of wheat. Can. J. Plant Pathol. 25:339–349

Strelkov S, Kowatsch R, Ballance G, Lamari L. (2006). Characterization of the ToxB gene from North African and Canadian isolates of *Pyrenophora tritici-repentis*. Physiol Mol Plant Pathol 67:164–170

Stergiopoulos, I., Collemare, J., Mehrabi, R. and De Wit, P.J. (2013). Phytotoxic secondary metabolites and peptides produced by plant pathogenic Dothideomycete fungi. FEMS Microbiol reviews, 37:67-93.

Sun, X., C., Bockus, W., and Bai, G. (2010). Quantitative trait loci for resistance to *Pyrenophora tritici-repentis* race 1 in a Chinese wheat. Phytopathology100: 468-473.

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

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

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

Talbot, N. J., Ebbole, D., J., and Hamer, J., E. (1993). Identification and characterization of MPG1, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. The Plant Cell. 5:1575-90.

Tomas, A., Bockus, W., W. (1987). Cultivar-specific toxicity of culture filtrate of *Pyrenophora tritici-repentis*. Phytopathology 77:1337–1340

Tuori, R.P., Wolpert, T.J. and Ciuffetti, L.M., (2000). Heterologous expression of functional Ptr ToxA. Mol Plant Microbe In 13:456–464

Turgeon, B., G., and Yoder, O.C. (2000). Proposed nomenclature for mating type genes of filamentous ascomycetes. Fungal Genet. Biol. 31:1-5

Virdi, S., K., Liu, Z., Overlander, M., E., Zhang, Z., Xu, S., S., Friesen, T., L., and Faris, J., D. (2017). New insights into the roles of host gene-necrotrophic effector interactions in governing susceptibility of durum wheat to tan spot and Septoria nodorum blotch. G3: (Bethesda) 6:4139-4150.

Windels, C.E., (2009). Economic and social impacts of Fusarium head blight: changing farms and rural communities in the Northern Great Plains. Phytopathology. 90:17-21.

Wirsel, S., Turgeon, B.G. and Yoder, O.C., (1996). Deletion of the *Cochliobolus heterostrophus* mating-type (MAT) locus promotes the function of MAT transgenes. Curr. Genet. 29: 241-249.

Wolpert, T.J., Dunkle, L.D. and Ciuffetti, L.M., (2002). Host-selective toxins and avirulence determinants: what's in a name? Annu. Rev. Phytopatho.40:.251-285.

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

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

Weiland, J.J., Steffenson, B.J., Cartwright, R.D. and Webster, R.K., (1999). Identification of molecular genetic markers in *Pyrenophora teres* f. *teres* associated with low virulence on 'Harbin'barley. Phytopathology 89:176-181.

Wen, A., Jayawardana, M., Fiedler, J., Sapkota, S., Shi, G., Peng, Z., Liu, S., White, F.F., Bogdanove, A.J., Li, X. and Liu, Z. (2018). Genetic mapping of a major gene in triticale conferring resistance to bacterial leaf streak. Theor. Appl. Genet. 131: 649-658.

Wolpert, T., J., Dunkle, L., D., and Ciuffetti, L., M. (2002) Host-selective toxins and avirulence determinants: what's in a name? Ann. Rev. Phytopathol. 40:251–285

Wyatt, N.A., Richards, J.K., Brueggeman, R.S. and Friesen, T.L., (2017). Reference Assembly and Annotation of the *Pyrenophora teres* f. *teres* Isolate 0-1. G3: (Bethesda). G3-300196.

Zhong, Z., Marcel, T.C., Hartmann, F.E., Ma, X., Plissonneau, C., Zala, M., Ducasse, A., Confais, J., Compain, J., Lapalu, N. and Amselem, J., (2017). A small secreted protein in Zymoseptoria tritici is responsible for avirulence on wheat cultivars carrying the Stb6 resistance gene. New Phytol. 214:.619-631.

CHAPTER 2. GENETIC MAPPING OF THE FUNGAL GENE (S) CONDITIONING PTR TOXC PRODUCTION IN *PYRENOPHORA TRITICI-REPENTIS*

2.1. Abstract

Pyrenophora tritici-repentis is an economically important fungal pathogen that causes tan spot of wheat in most wheat growing regions of the world. *P. tritici-repentis* is known to produce necrotrophic effectors (NEs), including Ptr ToxA, Ptr ToxB and Ptr ToxC, which interact with their corresponding host susceptibility genes *Tsn1*, *Tsc2* and *Tsc1*, respectively, to cause disease in an inverse gene-for-gene manner. Both Ptr ToxA and Ptr ToxB are proteins and their encoding genes *ToxA* and *ToxB*, respectively, have been cloned from the fungus. However, the fungal gene (s) conditioning the production of Ptr ToxC, which was characterized as a polar, nonionic, low-molecular weight secondary metabolite, remains elusive, and it has been difficult to use traditional biochemical approaches to characterize the gene due to its non-proteinaceous nature. In this work, a genetic approach recently developed in our lab was used to map the fungal genetic factors contributing to Ptr ToxC production. The heterothallic strains of 86-124 (Ptr ToxC-non-producer) and AR CrossB10 (Ptr ToxC-producer) were first created by the deletion of one of the mating type genes followed by the sexual crossing and the development of a genetically segregating fungal population. The progeny was phenotyped on Ptr ToxC sensitive wheat genotypes and the results showed a 1:1 segregation ratio for chlorosis and no-chlorosis development. Whole genome sequencing was performed for the two parental isolates and twenty progenies of which ten caused chlorosis and ten caused no chlorosis. Using the sequence data and reference genome, we identified a total of 26 SNP loci on four super_contigs of the reference genome that are close or co-segregated with the chlorosis phenotypes. These SNPs were developed into PCR-based markers and then tested on the entire population, leading to the

identification of two genomic regions, one on supercontig_1.16 and the other on supercontig_1.38, that both co-segregated with the phenotype. This work demonstrated that genetic mapping is an effective way to identify genomic regions that contains virulence genes in *P. tritici-repentis*.

2.2. Introduction

Pyrenophora tritici-repentis is a destructive necrotrophic fungal pathogen that causes tan/yellow spot on wheat. The disease occurs in almost all wheat growing regions of the world. Generally, yield loss due to tan spot ranges from 2%-15%, while under environmentally favorable conditions, tan spot can cause yield losses of up to 49% (Ciuffetti et al. 2014; Friskope and Liu 2016; Rees et al. 1982; Hosford 1982). The fungal infection leads to the formation of necrotic and/or chlorotic lesions or extensive chlorosis on the leaves of susceptible cultivars, which can reduce leaf photosynthesis, and thus grain yield. Although tan spot of wheat can be partially managed by using some cultural practices and timely fungicide application, the use of resistant cultivars is the preferred method for disease control.

Understanding pathogen virulence and how virulence factors interact with the host is important for breeding resistant wheat cultivars. *P. tritici-repentis* is known to produce multiple host selective toxins (HSTs), now called necrotrophic effectors (NEs), as important virulence factors. Three NEs have been identified and characterized, including Ptr ToxA, Ptr ToxB, and Ptr ToxC. These NEs specifically interact with their corresponding host sensitivity genes, which are *Tsn1* for Ptr ToxA, *Tsc2* for Ptr ToxB and *Tsc1* for Ptr ToxC (Ciuffetti et al. 2010) to cause disease. The Ptr ToxA-*Tsn1* interaction induces necrosis, whereas the Ptr ToxB-*Tsc1* and Ptr ToxC-*Tsc1* interactions induce chlorosis (Ciuffetti et al. 2010; Faris et al. 2013). Because the NE-Senssitivity gene interactions result in susceptibility, the wheat-*P. tritici-repentis*

pathosystem has been described as an inverse gene-for-gene model (Wolpert et al. 2002; Ciuffetti et al. 2010). Many disease systems involving NEs (or HST) have been shown to follow this model (Wolpert et al. 2002; Friesen et al. 2007; Liu et al. 2015).

Both Ptr ToxA and Ptr ToxB are small proteins, and they have been purified and their mode of action and structure have been well characterized (Ciuffetti et al. 1997; Balance et al. 1996; Zhang et al. 1997; Sarma et al. 2005). Ptr ToxA is encoded by a single copy gene designate *ToxA* (Ciuffetti et al. 1997). The *ToxA* gene has also been identified in other wheat fungal pathogens including *Parastagonospora nodorum* (Friesen et al. 2006) and *Cochliobolus sativus* (McDonald et al. 2018; Friesen et al. 2018), and horizontal gene transfer was suggested for the presence of this gene in multiple fungal pathogens. Ptr ToxA contains pre- and pro-domains, and its mature protein is 13.2 kDa after cleavage of the pre-and pro- domians. RGD (Arg-Gly-Asp) motif located in the C-terminus has been shown to be important for Ptr ToxA activity, internalization into plant cells and its mode of action (Meinhardt et al. 2003; Manning et al. 2005; Manning et al. 2008). The crystal structure of the protein also suggests the RGD motif may involved in protein-protein interacton (Sarma et al. 2005). Ptr ToxB is encoded by the *ToxB* gene which is present in multiple copies in the Ptr ToxB-producing isolates. The chlorosis inducing activity is correlated with the gene copy numbers for the fungus (Martinaz et al. 2004; Amaike et al. 2008). Figueroa et al. (2015) showed that Ptr ToxB was an apoplastic effector without the need to enter the plant cell. The cystal structure of Ptr ToxB has been revealed.

On the other hand, Ptr ToxC has not been well studied because it was not easy to purify. Ptr ToxC is produced by isolates belonging to races 1, 3, 6 and 8 as well as AR CrossB10 which is a new race (Strelkov and Lamari 2003; Kariyawasam et al. 2016). Effertz et al. (2002) partially purified Ptr ToxC using gel filtration, ion exchange, and reverse-phase chromatography

and identified Ptr ToxC as a polar, nonionic, low-molecular weight molecule. Many QTL mapping studies have revealed that the Ptr ToxC-Tsc1 interaction, when present, was an important component in disease (Faris et al. 1997; Effertz et al. 2001; Sun et al. 2010; Kariyawasam et al. 2016; Liu et al. 2017). Although Ptr ToxC and its interaction with host gene is important in the tan spot disease system, the gene(s) conditioning the Ptr ToxC production has not been identified or cloned.

Map-based cloning has been a common method to identify virulence genes in fungal pathogens such as *Magnaporthe grisea* (Talbolt et al. 1993), *C. sativus* (Zhong et al. 2002) and *Zymoseptoria tritici* (Zhong et al. 2017). The availability of good quality reference genome assemblies, gene annotation and effector gene prediction programs have also amended map-based cloning to be a more powerful tool for identifying virulence factors/gene in plant pathogenic fungi (Yoshida et al. 2009; Manning et al 2013; Sperschneider et al. 2016; Zhong et al. 2017; Moolhuijzen et al. 2018).

P. tritici-repentis is a homothallic fungus that carries both *MAT1-1-1* and *MAT1-2-1* genes in tandem spanning over 4.5 kb making it self-fertile (Lepoint et al. 2010). Therefore, development of a genetically powerful bi-parental population in natural conditions is difficult. Ameen et al. (2017) showed that heterothallic strains of *P. tritici-repentis* can be created by deleting one of the mating type genes, and genetic crosses among such heterothallic strains can used for the development of segregating bi-parental populations. The objectives of this study were to: 1) develop a bi-parental population that segregates for Ptr ToxC production; 2) phenotype and genotype the population; and 3) map the gene (s) conditioning Ptr ToxC production.

2.3. Materials and methods

2.3.1. The development of a fungal bi-parental population

A fungal bi-parental population was developed using 86-124 and AR CrossB10 as parental isolates. 86-124 is a race 2 isolate collected from Manitoba, Canada in the 1980s (Ameen et al. 2017) that is known to produce Ptr ToxA, and AR CrossB10 is an isolate that has not been categorized under the current race classification. It was collected from Arkansas, USA in 2010 and is known to produce Ptr ToxC (Ali et al. 2010; Kariyawasam et al. 2016). First, the heterothallic strains of 86-124ΔMAT1-1-1 and AR CrossB10ΔMAT1-2-1 were created by deleting the corresponding *MAT* genes. The deletion of the *MAT* genes followed the methods described in Ameen et al. (2017). In brief, two constructs were developed through fusion PCR with one containing the 5' flanking region of the targeted *MAT* gene and part of the hygromycin B phosphotransferase (*Hph*, referred as *HYG* hereafter), and the other containing a part of the *HYG* and the 3' flanking region of the targeted *MAT* gene. The resulting fusion fragments were transformed into *P. tritici-repentis* using the PEG-mediated method as described in Liu and Friesen (2012). The transformed protoplasts were selected in the regeneration media amended with hygromycin B. The plates were incubated at 30℃ for 4-6 days. Transformant cultures were purified through the standard single-sporing method (Choi et al. 1999). DNA was extracted from the single-spored cultures and regular PCR was performed using primers PtrPLP2 and PtrPLP4 for *MAT1-1-1* and primers PtrPLP7 and PtrPLP10 for *MAT1-2-1* to validate the true transformants (Table 2.1).

Heterothallic strains (86-124ΔMAT1-1-1 and AR CrossB10ΔMAT1-2-1) were crossed according to the protocol described in Ameen et al (2017). In brief, conidial suspension of each heterothallic strain were prepared, adjusted to a concentration 3000 spores/ml and mixed with an

equal volume. Then, a drop of mixed conidial suspension was inoculated onto a piece of dried and sterilized corn leaf that was laid on water agar. Agar plates were sealed with parafilm and incubated in a Percival incubator at 15 ± 1.5 ˚C with a 12h photoperiod. Pseudothecia formed on the corn leaves approximately 30 days post inoculation. The ascospores discharged from mature pseudothecia were collected using a petri dish lid filled with a thin layer of water agar. The widely separated ascospores picked from the agar lid were transferred to V8-PDA. The resulting colonies were purified using the standard single sporing method. Colonies developed from each ascospore were considered as an independent progeny of the fungal bi-parental population. A total of 142 single ascospore derived progeny were obtained for the cross to establish the bi-parental population, hereafter referred to as the AR population. Out of 142, four were removed due to the slow growth rate and 26 isolates were removed because they were found lately as redundant genotypes using 10 SSR markers, which left 112 progeny isolates for mapping.

To check the fertility of the cross, the asci produced in pseudothecia were examined under a dissecting microscope. Mature pseudothecia were picked from the crossing plate and placed on a glass slide and gently crushed prior to observation. The approximate number of ascospores in each asci were counted in pseudothecia formed by the wildtypes of 86-124, AR CrossB10, 86-124ΔMAT1-1-1×AR CrossB10ΔMAT1-2-1, and DW5ΔMAT1-1-1×86-124ΔMAT1-2-1.

2.3.2. Phenotyping of the fungal population for the production of Ptr ToxC

Fungal progenies were phenotyped by inoculating onto Ptr ToxC sensitive wheat lines including 6B365 (Ptr ToxC differential line), ‘Prosper’ (NDSU spring wheat cultivar), ‘Harry’ (Canadian winter wheat line) and Jerry (hard red winter wheat cultivar). In addition, the differential lines ‘Glenlea’ (Ptr ToxA differential line), ‘Salamouni’ (universal resistant line) and 6B662 (Ptr ToxB differential line) were also included for each inoculation. The tan spot

differential lines were arranged in half of the RL98 racks with the outside border planted to Jerry wheat. Each line was planted in two cone-tainers with three seeds/cone. At planting, Osmocote Plus 15-19-12 (Scotts Sierra Horticultural Product Company, Maysville, OH) was applied to each cone- tainers. All plants were grown in a greenhouse room with the temperature ranging from 20-25°C. The fungal inoculations were performed when the plants reached the two to three leaf stage. The experiment was repeated at least three times to obtain consistent data for each progeny.

Fungal cultures and inoculation preparation were done according to the standard procedure described in Lamari and Bernier (1989). Fungal spores (conidia) were harvested from the plates to make a spore suspension. The concentration of the spore suspension was adjusted to approximately 2000 spores per ml for inoculation. Tween 20 was added to the inoculum with two drops per 100 ml solution before inoculation. Plants at the two or three leaf stage were inoculated in a closed room by spraying with an air-pressured spray gun. After inoculation, the plants were kept in a mist chambers with 100% relative humidity in the light at 21°C for 24 hours. Plants were then transferred to a growth chamber with a 12-hour photoperiod at 21°C for disease to develop. Disease reactions were scored at 4^{th} - 7^{th} day post-inoculation based on the presence or absence of chlorosis. Due to limited space availability in the misting and growth chambers, inoculations for 6-8 progeny isolates with two parental isolates were performed for each inoculation. The production of chlorosis on the sensitive wheat lines was considered as the isolate having the gene conditioning Ptr ToxC production, and vice versa.

2.3.3. Genetic analysis of mating type genes and the *ToxA* gene in the population

Fungal cultures were grown in V8-PDA in the dark untill the mycelium covered the whole plate and from each culture aerial mycelium was collected by gentle scraping using a

sterilized scalpel. Collected tissue was freeze dried overnight using a lyophilizer. Dried tissue was ground with 700 µl of extraction buffer and a pinch of sand using a TissueLyzer. The mixture was vortexed, and all the tissue particles and sand were spun down. Then 200 µl of solution III was added and vortexed to mix them well. The mix was centrifuged at 13,000 rpm for 10 mins and the supernatant was transferred to a tube with an equal amount of chilled isopropanol and incubated on ice for 15 mins. The DNA was pelleted by centrifuging at 13,000 rpm for 4 mins. The supernatant was discarded, and the pellet was washed using 70% ethanol. The pellet was dried for 20 mins and finally dissolved in 50 µl of distilled water.

Genetic analysis of known *P. tritici-repentis* genes including *MAT1-1-1*, *MAT1-2-1* and *ToxA* were carried out using corresponding primer pairs (Table 2.1). The whole population was genotyped for these three genes. Primers for *ToxA* and *MAT1-1-1* were multiplexed whereas *MAT1-2-1* was run individually. PCR was conducted with a PCR reaction mix that contained 1× buffer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.5 µM of each forward and reverse primer, 10-20 ng of DNA and 2U of Bullseye Taq DNA polymerase (MIDSCI, St. Louis) in 20µl volume with the PCR program: 95 ºC for 5 mins, 30 cycles at 95 ºC for 30s, 60 ºC for 30s, 72 ºC for 1 min, followed by a 72 ºC final elongation step for 5 mins and 4 ºC holding step. Products were visualized using 1% agarose gel electrophoresis. PCR products were genotyped as '1' for the progenies with the 86-124ΔMAT1-1-1 allele and '0' for the progenies with the AR CrossB10ΔMAT1-2-1 allele. Finally, ratio of the *ToxA* and *MAT* gene presence and absence was calculated and a χ^2- test was performed to prove the expected ratio was significantly different from the observed ratio.

Table 2.1. List of primers used for the amplification of the *MAT* and *ToxA* genes

Primers	Sequences (from 5' to 3')	Purpose	Reference
PtrPLP2	CAGAACAAAGGCAGGACTGTGAGC	Amplify a region of *MAT1-1-1* gene	Lepoint et al. (2010)
PtrPLP4	ATGCGCTCAGCAAGGAAGGTCG		
PtrPLP7	GCTTTACTACAACTTTCCTCTACC	Amplify a region of *MAT1-2-1* gene	Lepoint et al. (2010)
PtrPLP10	GTACGGGCCAGCATGACGTGC		
TA51F	GCGTTCTATCCTCGTACTTC	Amplify the *ToxA* gene	Andrie et al. (2007)
TA52R	GCATTCTCCAATTTTCACG		

2.3.4. Bulked segregant analysis with SSR markers

After all the phenotyping work was done, I conducted bulked segregant analysis (BSA) with simple sequence repeat (SSR) markers with the hope to quickly identify genomic loci responsible for Ptr ToxC production. Ten progeny isolates that produce chlorosis symptoms and ten progeny isolates that do not produce chlorosis symptoms were selected, and equal amounts of DNA from these progeny isolates were mixed evenly to make chlorosis and no-chlorosis pool. The DNA concentrations of two pools as well as the parental isolates were adjusted to 5-10 ng/µl. The SSR of *P. tritici-repentis* were searched from the Pt-1C-BFP reference genome (Manning et al. 2013) and the corresponding primers for these SSR were designed using bioinformatics tools described in Zhong et al. (2009). The primer sequences for the SSR I used are listed in Appendix 2.2. The SSR PCR mix consisted of 1× buffer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.05 µM of SSR primer pair where the forward primer was tagged with M13 (5'-TGTAAAACGACGGCCAGT-3'), 0.1 µM DY682 fluorescently labeled M13 primer, 5-10 ng of DNA and 1U of Bullseye Taq DNA polymerase (MIDSCI, St. Louis) in a 10 µl volume (Wen et

al. 2017). The SSR products were amplified using the following program: 95°C for 5mins, 8 cycles at 95°C for 20s, 50°C for 20s, 72°C for 30s, 38 cycles at 95°C for 20s, 52°C for 20s, 72°C for 30s, followed by a 4°C holding step. The PCR products were visualized using a 4300 DNA analyzer (LI-COR Bioscience, Lincoln, NE, USA).

2.3.5. Genome sequencing and association analysis

Because no single SSR marker linked to the trait was identified, whole genome sequencing was done for the parental isolates and all the 20 progenies that I used to make pools. The fungal tissues prepared from each isolate were send to the commercial sequencing company NOVOGENE Corporation (Chula Vista, CA) where the DNA extraction, library preparation and genome sequencing were done. The sequencing was performed on the Illumina Hi-Seq 2500 platform with a target of obtaining at least 1 Gbp sequence for each isolate (approximately 25x coverage). Raw sequences for each isolate were cleaned using trimmomatic v.0.36. Cleaned sequenced reads were aligned to the reference sequence, Pt-1c-BFP (Manning et al. 2013) using the Burrows-Wheeler Aligner (BWA) with the 'bwa mem' function and a SAM file was developed for each isolate. Each SAM file was converted to a BAM file using SAMtools with the 'view' command. The resulting BAM files were converted to sorted BAM files with the 'sort' command, and those sorted BAM files were indexed using the 'index' command in SAMtools. Reference sequence was indexed using the 'faidx' command of SAMtools. These sorted indexed BAM files and indexed reference sequence was used for downstream single nucleotide polymorphism (SNP) calling.

SNP discovery was performed using the Unified Genotype program of genome analysis tool kit (GATK) with a minimum confidence threshold of 30.0. Both SNPs and INDELs were called using the '-glm' function and all the variants were output as a .vcf file. Output '.vcf' file

was used as the input file for the next step where a subset of polymorphic SNPs were selected using GATK's SelectVariants program. Finally, SNPs associated with the chlorosis production were identified using a BSRseq.R which was developed according the method explained in Liu et al. (2012).

2.3.6. Development of CAPS and STARP markers

These discovered associated SNPs were used to develop cleave amplified polymorphic sequence (CAPS) markers (Konieczny and Ausubel 1993) or semi-thermal asymmetric reverse PCR (STARP) markers (Long et al. 2017) and then mapped in the entire population. To develop CAPS markers, sequences spanning 150 bps upstream and downstream from SNPs were extracted and forward and reverse primers were designed using Primer3 v. 0.4.0 (Untergasser et al. 2012). Sequences were screened for restriction sites that include the SNP using NEBcutter v.2.0. The PCR reaction mix contained 1× buffer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.5 µM of each forward and reverse primer, 10-20 ng of DNA and 1U of Bullseye Taq DNA polymerase (MIDISCI, St. Louis) in a10 µl volume. PCR was performed with the following profile: 95 ºC for 5 mins, 30 cycles at 95 ºC for 30s, 58 ºC for 30s, 72 ºC for 1 min, followed by a 72 ºC final elongation step for 5 mins and 4 ºC holding step. Restriction digestions were carried out in a 10 µl reaction mix that contained 4.5 µl of PCR product, 2U of restriction enzyme, and 0.01 mg of BSA incubated at optimal temperature for 2 hours. Digestion products were visualized using 1-2% agarose gel electrophoresis.

When CAPS markers were impossible to develop, STARP strategy was then used to develop markers for the rest of SNPs as explained in Long et al. (2017). Two priming element-adjustable primers (PEA), two asymmetrically modified allele specific primers (AMAS), and a common reverse primer were designed as explained in Long et al. (2017). The STARP PCR mix

were consisted of 10 µl reactions contained 1 × NH_4^+ buffer (16 mM $(NH_4)_2$ and 67 mM Tris-HCl, pH 8.3 at 25 ºC) , 0.8 M betaine, 0.04% (W/V) bovine serum albumin (BSA), 1.5 mM $MgCl_2$, 50 µM of each dNTP, 200 nM each of PEA-primer 1 and PEA-primer 2, 40 nM each of AMAS-primer1 and AMAS-primer2, 200 nM of reverse primer, 1 U of Taq DNA polymerase (Homemade) and 10-100 ng of genomic DNA. The reactions were mixed well using a MixMate and spun down using a plate centrifuge prior to the PCR. The PCR for STARP markers was as follows: 94 ºC for 3 mins, 6 cycles at 94 ºC for 20s, 55 ºC for 30s with 1 ºC reduction per cycle, 37 cycles at, 94 ºC for 30s and 62 ºC for 30s, followed by 10 ºC holding step. The PCR products were visualized using 4300 DNA analyzer (LI-COR Bioscience, Lincoln, NE, USA).

2.3.7. Genetic mapping of gene conditioning Ptr ToxC production and identification of the candidate region

The marker data was scored as 1 for the 86-124 allele and 0 for AR CrossB10 allele. The phenotypic data was converted into 1 for the progeny isolates which did not induce chlorosis on sensitive lines as 86-124 did and 0 for the progeny that induce chlorosis as AR CrossB10 did. The genetic linkage mapping was performed with all the marker data using Mapdisto 2.0 (Heffelfinger et al. 2017). Linkage groups were formed using the 'find group' function with a LOD value of 5.0 and r max value of 0.35. The correct order of the markers was obtained using the 'order sequence', 'check inversions', 'ripple order' and 'drop locus' functions. The obtained SNP markers were aligned to the reference Pt-1c-BFP genome to identify the candidate region responsible for Ptr ToxC production.

2.4. Results

2.4.1. Generation, confirmation and virulence of the heterothallic strains

Heterothallic strains of 86-124 and AR CrossB10 were obtained by deleting *MAT1-1-1* and *MAT1-2-1*, respectively, and they were designated as 86-124ΔMAT1-1-1 and AR CrossB10ΔMAT1-2-1, respectively. These strains were confirmed using the corresponding *MAT* gene primer to be true knockouts before making the cross. 86-124ΔMAT1-1-1 did not produced the *MAT1-1-1* amplicon but produced the *MAT1-2-1* amplicon, whereas AR CrossB10ΔMAT1-2-1 produced a *MAT1-1-1* amplicon but lacked a *MAT1-2-1* (Fig. 2.1). The wild types of both isolates produced an amplicon of both *MAT* genes (Fig. 2.1). The two heterothallic strains showed similar growth rate and conidia morphology to that of the wild types (data not shown). The two heterothallic strains were also tested for virulence differential lines and other Ptr ToxC sensitive lines. It was shown that they had similar level of virulence as their wildtype strains. On the Ptr ToxC differential lines AR CrossB10ΔMAT1-2-1 was able to cause leaf spots coupled with extensive chlorosis along the infected leaf (Fig. 2.2). However, 86-124 ΔMAT1-1-1 was not able to produce extensive chlorosis on the infected leaves. 86-124 ΔMAT1-1-1 produced characteristic tan colored necrotic lesions on Harry and Glenlea, whereas it produced pinpoint black lesions on Prosper (Fig. 2.2).

2.4.2. Fertility of the cross between the heterothallic strains

The number of ascospores in each ascus from pseudothecia were examined to check fertility of the cross. Most of the mature asci from the wildtype 86-124 and AR CrossB10 had eight ascospores inside (Fig. 2.3). The heterothallic strains formed non-functional pseudothecia with no asci produced inside. The cross between two heterothallic strains produced a certain percentage of functional pseudothecia that contained asci. The mature asci from the cross

between 86-124ΔMAT1-1-1 and AR CrossB10ΔMAT1-2-1 had ascospores ranged from two to eight with the majority having four (Fig. 2.3). It was also observed that some asci in the cross contained no ascospores at all. This might be due to the growth stage (not the time to produce ascospores) or abnormal development of asci.

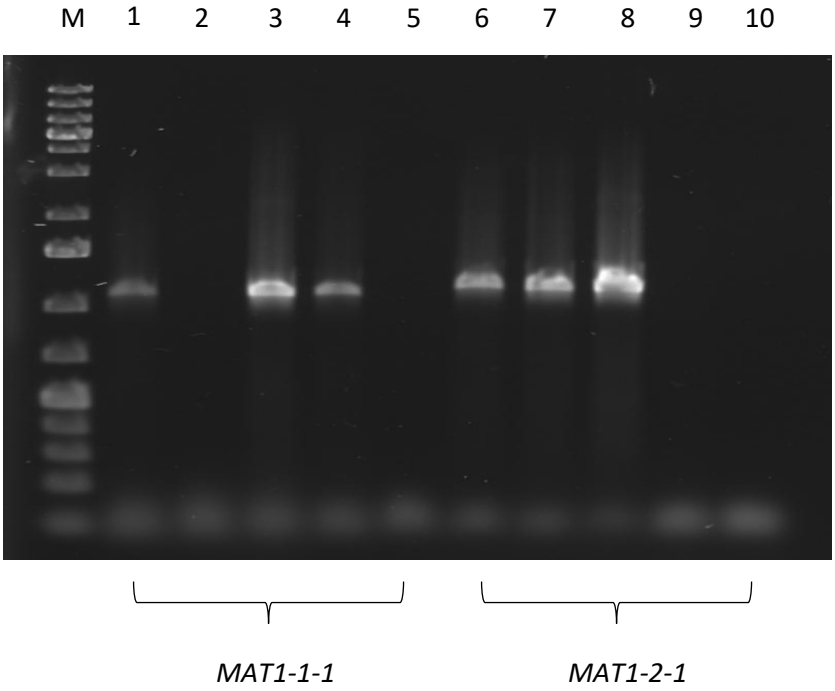


Figure 2.1. PCR confirmation of *MAT* gene deletion in the heterothallic 86-124 and AR CrossB10 strains. PCR amplification using primer pair for *MAT1-1-1* in lane 1-5 and *MAT1-2-1* in lane 6-10. M: Marker, 1:86-124, 2:86-124ΔMAT1-1-1, 3: AR CrossB10, 4: AR CrossB10ΔMAT1-2-1, 5: H_2O control, 6:86-124, 7:86-124ΔMAT1-1-1, 8: AR CrossB10, 9: AR CrossB10ΔMAT1-2-1, 10: H_2O control.

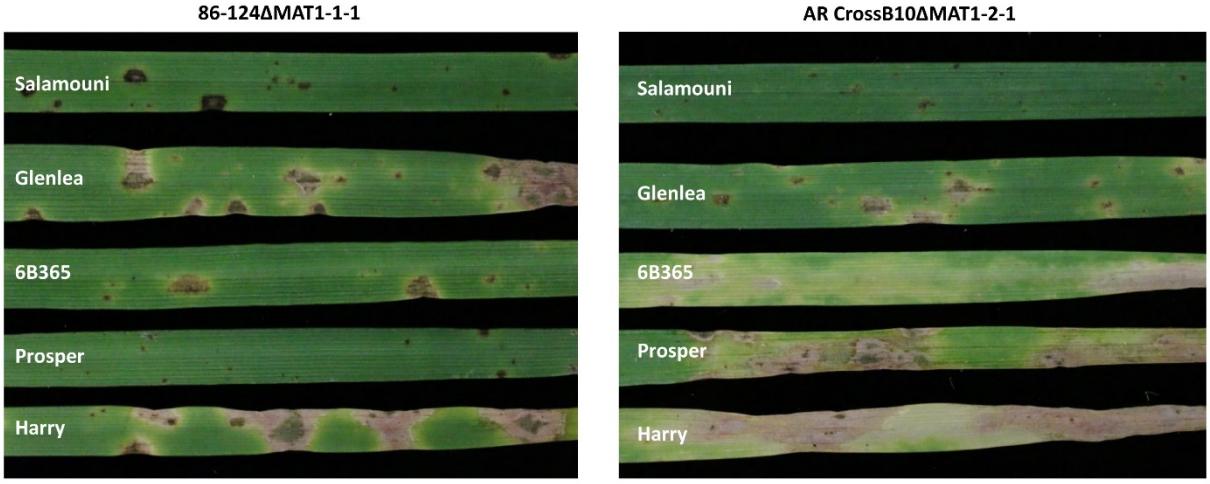


Figure 2.2. Reaction of tan spot differentials and other Ptr ToxC sensitive lines to the two heterothallic strains used for cross. These lines included Salamouni (universal resistant), Glenlea (Ptr ToxA differential), 6B365 (Ptr ToxC differential), Prosper (develop chlorosis) and Harry.

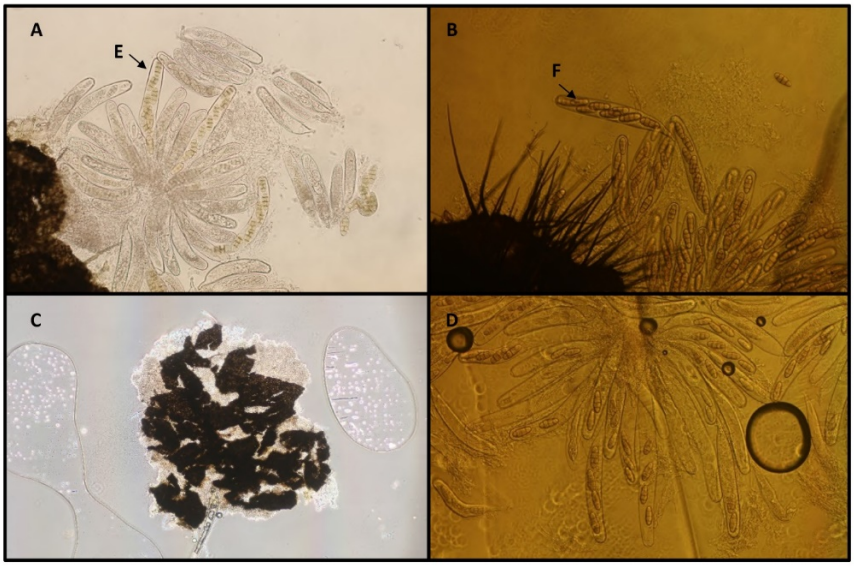


Figure 2.3. Fertility of the wildtype and cross between 86-124ΔMAT1-1-1 × AR CrossB10ΔMAT1-2-1. A: pesudothecium containing asci of wild type 86-124 with eight ascospore; B: pesudothecium containing asci of wild type AR CrossB10 with eight ascospores; C: A broken pesudothecium of 86-124ΔMAT1-1-1 that lacked asci; D: pesudothecium containing asci resulted from 86-124ΔMAT1-1-1 × AR CrossB10ΔMAT1-2-1 where most of asci contained four ascospores. Arrow in A and B indicates an ascus of wild type 86-124 and an ascospore of AR CrossB10, respectively.

2.4.3. Phenotyping of progeny isolates

All the 112 progeny isolates were phenotyped on the differential lines and the Ptr ToxC sensitive lines. Among them, 49 were able to produce extensive chlorosis as by AR CrossB10 ΔMAT1-2-1, whereas 63 produced no chlorosis and a reaction similar to 86-124 ΔMAT1-1-1 on three Ptr ToxC differential lines (Fig. 2.2). The reactions were consistent over three biological replicates. Based on the χ^2 test, the AR population segregated for the chlorosis production as a 1:1 ratio (P=0.19) (Table 2.2).

2.4.4. Genotyping for the *MAT* and *ToxA* genes

Out of 112, two progeny isolates contained both *MAT1-1-1* and *MAT1-2-1* genes, and one progeny isolate lacked both the genes. For the rest of the 109 isolates, 47 contained only *MAT1-1-1* and 62 isolates contained only *MAT1-2-1*. Therefore, as expected the two mating type genes segregated in 1:1 ratio (P=0.15). The *ToxA* gene was present in 47 progeny isolates and absent in 65. χ^2 test showed its segregation did not fit a 1:1 ratio (P=0.04) at the P= 0.05 level (Table 2.2).

2.4.5. Bulked segregant analysis

A total of 209 pairs of SSR primers developed across the reference genome were screened on the two parental isolates and two pools. Among them, 54 did not produce any specific bands, 60 produced specific bands but no polymorphism, and 95 produced polymorphic bands between two parental isolates. However, none of the SSR primers revealed polymorphisms between the between two pools.

Table 2.2. Genotyping of the fungal population for the presence of the *ToxA* and *MAT* genes.

Gene	Genotype	Number of Progeny	Segregation ration
ToxA	Gene present	47	1:1 (P=0.04)
	Gene absent	65	
MAT	*MAT1-1-1*	47	1:1 (P=0.15)
	MAT1-2-1	62	

2.4.6. Genome sequencing and association analysis

From the genome project, the total cleaned sequences obtained for each isolate ranged from 1,315.27 Mb to 2,064.68 Mb, which equals to ~33x to 54x coverage in each genome. Sequence alignments with the Pt-1c-BFP reference genome revealed a total of 5,095 SNPs between the two parental lines within the 38 Mb genome. Using the phenotypic data for the 20 isolates and the parental isolates, 26 SNPs were identified that were completely, or mostly segregated with the phenotype suggesting they linked to the gene (s) conditioning Ptr ToxC production. Of these SNPs, 18 were located to a physical region on supercontig_16, four to supercontig_38 and two each to supercontig_24 and 31. Supercontig_16 was placed on the distal end of chromosome 2 and supercontig_24 was placed on chromosome 8 on the optical map of the reference genome. However, the other two were not placed onto the optical map (Table 2.3). Seventeen of these SNPs co-segregated with chlorosis production, whereas the other nine had 1 or 2 recombination between genotype and phenotype (Table 2.3).

2.4.7. SNP development and genetic mapping

Out of 26, eight SNPs were developed as CAPS markers and eleven were developed as STARP markers (Table 2.4). Most of them (17) were able to produce polymorphic PCR product that could be clearly scored (Fig. 2.4). However, two performed poorly and were discarded. Among the CAPS or STARP markers that worked, one produced dominant amplicon and 16 produced co-dominant amplicons within the population (Fig. 2.4 and Table 2.4). Seven markers

including SC1.16.15k, SC1.16.17k, SC1.16.18k, SC1.16.23k, SC1.38.18k, SC1.38.20k and SC1.16.43k, co-segregated with the phenotype in the population while SC1.16.33k, SC1.16.36k, SC1.16.37k, SC1.16.40k, SC1.16.44k, SC1.16.45.2k, SC1.16.51k, SC1.16.69k, and SC1.16.78k still had recombination between phenotype and genotype (Table 2.4). The markers on supercontig 24 did not produce polymorphic bands, thus were not mapped. A genetic linkage map was generated, which consisted of 16 markers and spanned over 55.1 cM (Fig. 2.5). For the seven markers that co-segregated with the phenotype (Chlorosis), five of them aligned to the top of supercotig_1.16, which was ~ 693 kb long and located on the chromosome 2 (Fig. 2.5). It was also observed that there is an inversion involving 33K, 36K, 37K and 43 K markers. Therefore, the region on supercontig_1.16 from 1bp to 43K is likely one of the candidate regions that harbor the Ptr ToxC gene. The other two co-segregating markers were physically located on supercontig_1.38 which was about 21 kb long and could not be placed in optical map. Therefore, the region on supercontig_1.38 represents another candidate region for the Ptr ToxC production gene.

Candidate region of the supercontig_1.16 contained sixteen annotated genes whereas supercontig_1.38 did not contain any genes (Table 2.5). Of these candidate genes, twelve had predicted molecular functions. In addition, for eleven genes, biological process that they are involved in were predicted. These eleven candidate genes were involved in biological processes such as carbohydrate metabolism, transcription, proteolysis and peptidolysis, isoprenoid biosynthesis, carbohydrate transport and cell wall catabolism. However, for four genes molecular function and biological process that they involved in were not characterized (Table 2.5).

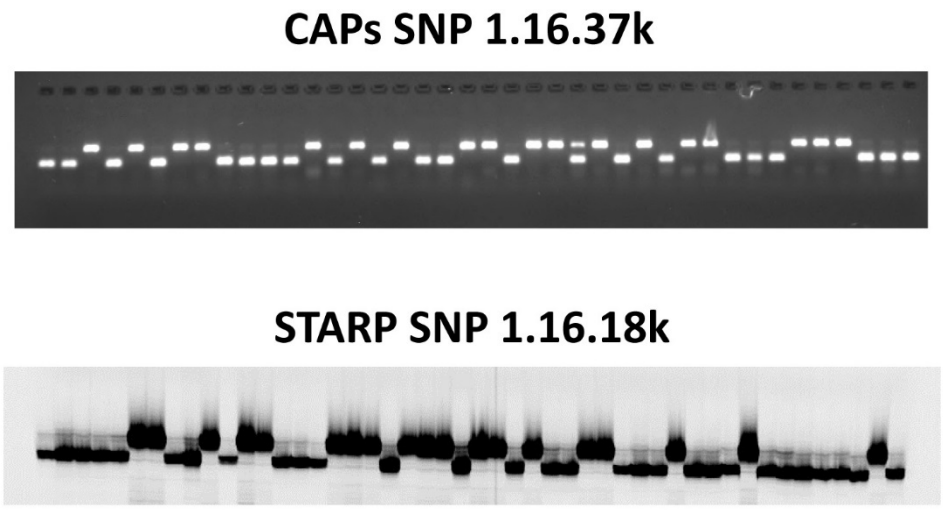


Figure 2.4. Examples of a CAPS marker (top) and a STARP marker (bottom) developed in the fungal population.

Table 2.3. SNPs identified from association analysis that associated completely with the phenotype.

Super_contig (SC) of the SNP[α]	Physical position of SNP[β]	Isolates with chlorosis production[γ]											Isolates lacked chlorosis production[γ]										
		ARCrossB10	AR86.9	AR86.10	AR86.26	AR86.30	AR86.44	AR86.68	AR86.87	AR86.106	AR86.128	AR86.136	86-124	AR86.12	AR86.29	AR86.35	AR86.48	AR86.63	AR86.82	AR86.91	AR86.101	AR86.109	AR86.121
SC1.16 (2)	9945	C	C	C	C	C	C	C	C	C	C	C	G	.	G	.	.	G	.	.	G	.	G
	15685	G	G	G	G	G	G	G	G	G	G	G	T	T	T	T	T	T	T	T	T	T	T
	17644	A	A	A	A	A	A	A	A	A	A	A	C	C	C	C	C	C	C	C	C	C	C
	18006	C	C	C	C	C	C	C	C	C	C	C	T	T	T	T	T	T	T	T	T	T	T
	23270	A	A	A	A	A	A	A	A	A	A	A	T	T	T	T	T	T	T	T	T	T	T
	33253	G	G	G	G	G	G	G	G	G	G	G	A	A	A	A	A	G	A	A	A	A	A
	36697	T	T	T	T	T	T	T	T	T	T	T	C	C	C	C	C	**T**	C	C	C	C	C
	37040	C	C	C	C	C	C	C	C	C	C	C	T	T	T	T	T	**C**	T	T	T	T	T
	40905	A	A	A	A	A	A	A	A	A	A	A	G	G	G	G	G	**G**	G	G	G	G	G
	43815	G	G	G	G	G	G	G	G	G	G	G	A	A	A	A	A	A	A	A	A	A	A
	44937	A	A	A	A	A	A	A	A	A	A	A	G	G	G	G	G	G	G	G	G	G	G
	46099	A	A	A	A	A	A	A	A	A	A	A	G	G	G	G	G	G	G	G	G	G	G
	51285	G	G	G	G	G	G	G	G	G	**A**	G	A	A	A	A	**G**	A	A	**G**	A	A	A
	69802	A	A	A	A	A	A	A	A	A	G	A	G	G	G	G	A	G	G	G	G	G	G
	74136	G	G	G	G	G	G	G	G	G	**A**	G	A	A	A	A	**G**	A	A	A	A	A	A
	78406	C	C	C	C	C	C	C	C	C	**A**	C	A	A	A	A	**C**	A	A	A	A	A	A
	78429	C	C	C	C	C	C	C	C	C	**G**	C	G	G	G	G	**C**	G	G	G	G	G	G
	116643	C	C	C	C	C	C	C	C	C	**A**	C	A	A	A	A	**C**	A	A	A	A	A	A
SC1.24(8)	158988	A	A	A	A	A	A	A	A	A	A	A	G	G	G	G	G	G	G	G	G	G	G
	159042	T	T	T	T	T	T	T	T	T	T	T	C	C	C	C	C	C	C	C	C	C	C
SC1.31	43707	T	T	T	T	T	T	T	T	T	T	T	C	C	C	C	C	C	C	C	C	C	.
	43747	A	A	A	A	A	A	A	A	C	A	A	C	C	C	C	C	C	C	C	C	C	C
SC1.38	17670	T	T	T	T	T	T	C	T	T	T	T	C	C	C	C	C	C	C	C	C	C	C
	18503	C	C	C	C	C	C	C	C	C	C	C	A	A	A	A	A	A	A	A	A	A	A
	18523	C	C	C	C	C	C	C	C	C	C	C	T	T	T	T	T	T	T	T	T	T	T
	20648	A	A	A	A	A	A	A	A	A	A	A	G	G	G	G	G	.	G	G	G	G	G

[α]Super contig_16 was mapped to chromosome 2 and super contig_24 was mapped to chromosome 8 of *P. tritici-repentis* whereas super contig_31 and 38 were not mapped to a chromosome of optical map (Manning et al. 2013) and chromosome number is given with in brackets.

[β]SNPs that have recombinants between phenotype and genotype were highlighted in bold font and period represent the missing data.

[γ]Genotypes of the isolates that does not co-segregate with phenotype were highlighted in bold font.

Table 2.4. Development of single nucleotide polymorphism markers in the fungal population

Marker name	Primer name	Primer sequence	Marker type[±]	Status[δ]
SC_1.16.9k	SC_1.16.9kAMAS1	GCAACAGGAACCAGCTATGACTCAAATTGGTAGACTCATAGAC	STARP	NP
	SC_1.16.9kAMAS2	GACGCAAGTGAGCAGTATGACTCAAATTGGTAGACTCATGAAG		
	SC_1.16.9kRev	GCTCAATTGGCTGAGCAAG		
SC_1.16.15k	SC_1.16.15AMAS1	GCAACAGGAACCAGCTATGACAAGAGGGGGATTGCCTTG	STARP	P
	SC_1.16.15AMAS2	GACGCAAGTGAGCAGTATGACAAGAGGGGGATTGCACTT		
	SC_1.16.15REV	GCCATGCCAAGACCTATTCT		
SC1.16.17k	SC1.16.17kF	GAAGGAGCGTAACTCGCATC	CAPS	P
	SC1.16.17kR	AACGCCCATGGGGATATAAT		
SC_1.16.18k	SC_1.16.18AMAS1	GCAACAGGAACCAGCTATGACGACTCTTGGACTTTGGATTTC	STARP	P
	SC_1.16.18AMAS2	GACGCAAGTGAGCAGTATGACGACTCTTGGACTTTGGACCTT		
	SC_1.16.18REV	AGACATGTTGTGCTCGTTGC		
SC_1.16.23k	SC_1.16.23AMAS1	GCAACAGGAACCAGCTATGACAATGGCGTACAGGGATCTTA	STARP	P
	SC_1.16.23AMAS2	GACGCAAGTGAGCAGTATGACAATGGCGTACAGGGATACTT		
	SC_1.16.23REV	GGGAAGGTCCAATGTGAAAA		
SC1.16.33k	SC1.16.33kF	GAGTCCATCAATTGGGCATT	CAPS	P
	SC1.16.33kR	GGAGATTGAGATTCTGCACCA		
SC1.16.36k	SC1.16.36kF	AAATCATAGGGCAAACTCAGGA	CAPS	P
	SC1.16.36kR	TTGCTCTGCCTTCTTCTTGG		
SC1.16.37k	SC1.16.37kF	TCTTCGGACGACATTGAACA	CAPS	P
	SC1.16.37kR	TTGCGGAGACTTCGGTAGTT		
SC_1.16.40k	SC_1.16.40AMAS1	GCAACAGGAACCAGCTATGACCCAAGAAGGACGGCAAGA	STARP	P
	SC_1.16.40AMAS2	GACGCAAGTGAGCAGTATGACCCAAGAAGGACGGCGCGG		
	SC_1.16.40Rev	CGATGGCGAAGAGATAGACC		
SC1.16.43k	SC1.16.43kF	GAGCCCTGATTCTGAATGGA	CAPS	P
	SC1.16.43kR	GCATGTCACTGCTGTCGTTT		
SC1.16.44k	SC1.16.44kF	GCGCTTGGAGAGGATGAAT	CAPS	P
	SC1.16.44kR	ATCTCTACTTGGCCCCGATT		
SC1.16.45.2k	SC_1.16.45.2AMAS1	GCAACAGGAACCAGCTATGACCGGTCTGGTAGGCGGA	STARP	P
	SC_1.16.45.2AMAS2	GACGCAAGTGAGCAGTATGACCGGTCTGGTAGGTAGG		
	SC_1.16.45Rev	ATCGAGTTGACACCCGAGAC		
SC1.16.51k	SC_1.16.51.AMAS1	GCAACAGGAACCAGCTATGACATGGTTTTTCCTGAACAGCG	STARP	P
	SC_1.16.51.AMAS2	GACGCAAGTGAGCAGTATGACATGGTTTTTCCTGAACGACA		
	SC_1.16.51.Rev	TACCACGGTATGCAGCAAAG		
SC1.16.69k	SC1.16.69kF	ACGTACCAAACAGCCACCAG	CAPS	P
	SC1.16.69kR	CTTCACCGATTGGAGTGGTT		
SC1.16.78k	SC1.16.78kF	TGGTAAGGGTGGATTGGTGT	CAPS	P
	SC1.16.78kR	GCAGGAACCTGGGTTCAATA		
SC1.24.158k	SC_1.24.158AMAS1	GCAACAGGAACCAGCTATGACAACTAAAAGTCATTAGAGAA	STARP	NP
	SC_1.24.158AMAS2	GACGCAAGTGAGCAGTATGACAACTAAAAGTCATTAGGAAG		
	SC_1.24.158REV	CCGTGATTTCAATGGAGGTT		
SC1.31.43k	SC_1.31.43AMAS1	GCAACAGGAACCAGCTATGACAATACAAAACAAGAGCCACT	STARP	P
	SC_1.31.43AMAS2	GACGCAAGTGAGCAGTATGACAATACAAAACAAGAGCACCC		
	SC_1.31.43REV	ATTGGTGCTGCGTGGTATTT		
SC1.38.18k	SC_1.38.18AMAS1	GCAACAGGAACCAGCTATGACCCCACCAGAATGGAGTTGCC	STARP	P
	SC_1.38.18AMAS2	GACGCAAGTGAGCAGTATGACCCCACCAGAATGGAGTCACT		
	SC_1.38.18REV	GGACGTCGATGGAGTATTGG		
SC1.38.20k	SC_1.38.20AMAS1	GCAACAGGAACCAGCTATGACTACCTACGCCGCCAAGAAGA	STARP	P
	SC_1.38.20AMAS2	GACGCAAGTGAGCAGTATGACTACCTACGCCGCCAAGGCGG		
	SC_1.38.20Rev	GCTTTGTACGCTCGTGATGA		

[±]SNPs were developed as cleave amplified polymorphic sequence (CAPS) markers or semi-thermal asymmetric reverse PCR (STARP) markers.

[δ]All the markers produced a PCR product and they were either P: polymorphic; or NP: monomorphic. All the markers except for SC1.16.40k were co-dominant.

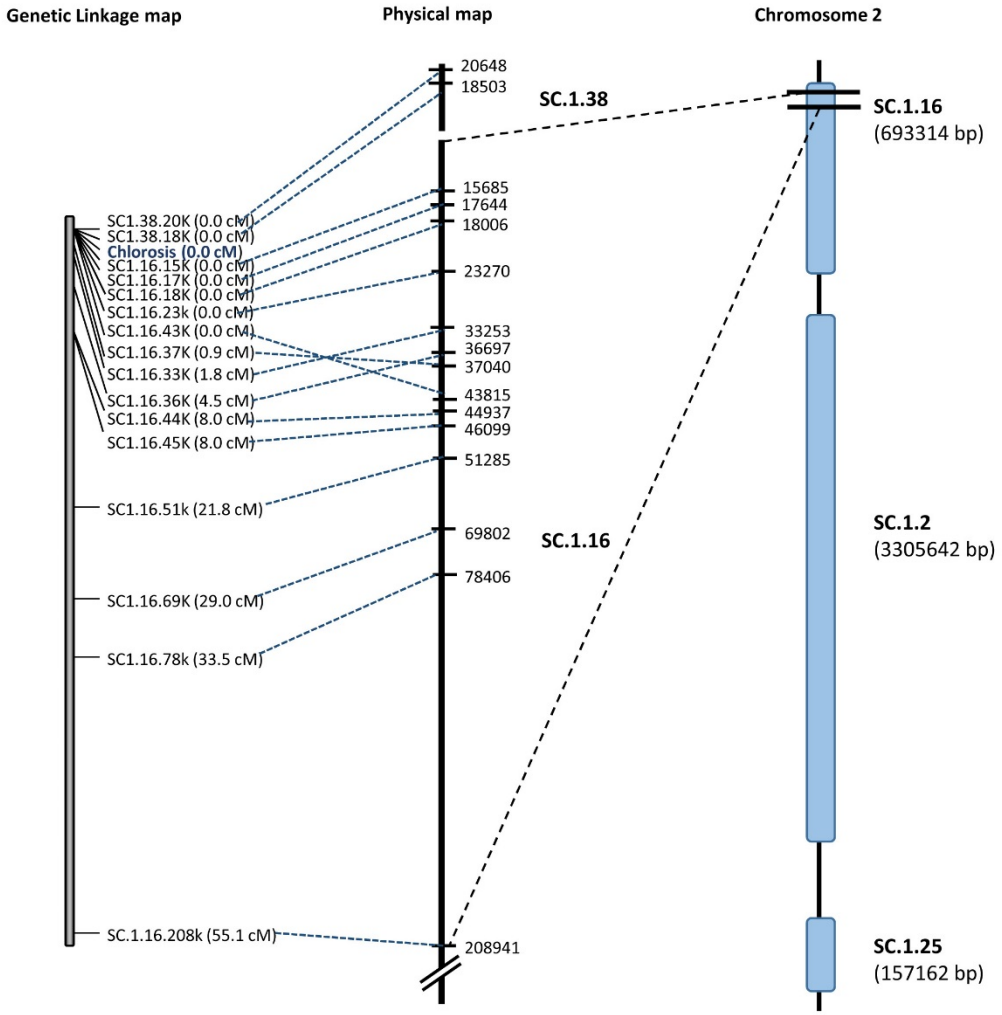


Figure 2.5. Genetic linkage map of the genomic regions that contain the gene conditioning Ptr ToxC production (chlorosis developed in sensitive wheat lines). A linkage map in cM is on the left, its corresponding physical map regions (bp) of Pt-1C-BFP is in the middle, and the chromosome 2 supercontigs are on the right.

Table 2.5. List of candidate genes annotated in reference genome assembly of Pt-1c-BFP

Gene ID[a]	Physical location of the gene in supercontig_1.16[a]	Biological process[a]	Molecular function[a]
PTRT_11087	1305-2999	-	-
PTRT_11088	7371-7682	-	-
PTRT_11089	9140-9750	-	-
PTRT_11090	12509-13260	-	-
PTRT_11091	13701-14846	Carbohydrate metabolism	Hydrolase activity Alpha-galactosidase activity
PTRT_11092	15808-17163	Transcription	DNA binding Zinc ion binding
PTRT_11093	18023-19934	Carbohydrate transport	Sugar porter activity Transporter activity
PTRT_11094	20703-22371	Proteolysis and peptidolysis	Pepsin A activity
PTRT_11095	25619-26134	Carbohydrate metabolism	Ribose/galactose isomerase
PTRT_11096	28348-30060	Regulation of transcription	Transcription regulator activity
PTRT_11097	31205-34356	Transcription	DNA binding Zinc ion binding
PTRT_11098	34684-35271	Isoprenoid biosynthesis	Isopentenyl-diphosphate delta-isomerase activity
PTRT_11099	35715-37277	-	-
PTRT_11100	37812-39813	Cell wall catabolism	Peptidoglycan-binding LysM
PTRT_11101	40155-42273	Proteolysis and peptidolysis	Membrane alanyl aminopeptidase activity Metallopeptidase activity Zinc ion binding
PTRT_11102	42939-43427	-	M-phase inducer phosphatase

[a]All the information was extracted from JGI genome portal (https://genome.jgi.doe.gov/) that contained the genome annotation of race 1 isolate Pt-1c-BFP published in Manning et al. (2013).

2.5. Discussion

P. tritici-repentis is a devastating fungal pathogen that causes significant yield and quality losses in wheat. The fungal pathogen is diverse in virulence by producing three known (Ptr ToxA, Ptr ToxB and Ptr ToxC) or other unknown NEs (Ciuffetti et al. 2010). Identification

and cloning of the fungal genes responsible for the production of these NEs is critical for understanding fungal virulence mechanisms and for developing resistant cultivars. Because Ptr ToxA and Ptr ToxB are proteins, they have been successfully purified and the encoding genes for them have been successfully cloned with the aid of protein sequence information (Ciuffetti et al. 1997; Martinez et al. 2001). However, Ptr ToxC was preliminarily characterized as a low molecular weight molecule that belongs to secondary metabolites (Effertz et al. 2002), thus it is difficult to use traditional approaches to identify its encoding genes or the genes involved in its biosynthesis. Since Ptr ToxC was discovered in 2002, the fungal gene (s) responsible for its production has not been identified even given that the predominant race 1 produces this NE and its interaction with host gene *Tsc1* is important. In this study, the genomic regions responsible for Ptr ToxC production were identified using a genetic mapping approach. This work demonstrates that the genetic approach which was first developed in our lab (Ameen et al. 2017) is an effective way to map and identify virulence factors in *P. tritici-repentis*.

Using the reference genome sequence and optical map for Pt-1c-BFP (Manning et al, 2013), two genomic regions were identified that possibly harbor the gene (s) for Ptr ToxC production with one on supercontig1.16 and the other on supercontig1.38. In the reference genome supercontig1.16 was placed on chromosome 2, but 1.38, which contains mainly repetitive sequence, was one of several unlinked small supercontigs (Manning et al. 2013). Therefore, it is unknown if the supercontig 1.38 is physically connected to supercontig1.16 in Pt-1c-BFP. In the very recently published reference genome M4 (race 1 from Australia producing Ptr ToxC), supercontig1.16 and 1.38 were linked and assembled in the single M4 supercontig 13 which was about 915 kb in length. However, the AR CrossB10 genome sequence we generated (see chapter 3), the two regions were in two different supercontig assemblies, but the genetic

linkage map suggests the two regions are connected in AR CrossB10. Molecular markers are being developed from the sequences that are extended by the M4 assemblies and will be tested in the population. The SNP marker developed in supercontig 1.24 lost polymorphism in the parental isolates and thus could not be mapped in the population. The physical relationships of these supercontigs with 1.16 and 1.38 remain unknown.

The segregation of progeny for the ability to induce chlorosis statistically fit a 1:1 ratio suggesting that a single Mendellian locus controlling Ptr ToxC production in the AR population. Many NEs belong to classes of polyketides and nonribosomal peptides and are biosynthesized by a series of polyketide synthetase (PKS) and nonribosome peptide synthetases (NRPS), respectively in fungi (reviewed in Wolpert et al. 2002). The PKS or NRPS required for a specific HST could distribute in different genomic regions, for example T-toxin produced by *Cochliobolus heterostrophus* (Kodama et al. 1999), or cluster in one locus, for example Hc-toxin produced by *C. carbonum* (Cheng et al. 2000). The characterization of Ptr ToxC structure was done only in one study (Effertz et al. 2002), thus more work is needed to confirm the chemical nature of Ptr ToxC. From the segregation ratio, it is possible that Ptr ToxC is a small protein and encoded by a single gene, but it is also possible that PKS or NRPS genes are required for Ptr ToxC production and are clustered and inherited as a single Mendellian locus. However, based on the genome annotation of M4, which contains the gene controlling Ptr ToxC production (Moolhuijzen et al. 2018), there are no obvious PKS and NRPS predicted in that region. Furthermore, sixteen genes were annotated in this region according to the annotation of the Pt-1c-BFP genome. Even according to the Pt-1c-BFP annotation PKS or NRPS genes were not predicted in the candidate regions. Of these only one gene *PTRT_11094* was predicted to have a

signal peptide. However, *PTRT_11094* was not predicted as an effector gene (Manning et al. 2013).

Eventhough genes encoding for effectors or PKS and NRPS were not predicted among these candidate genes, several have domains that can be involved in pathogenicity of the fungus. These includes genes that encodes for proteins with functional domains such as glycoside hydrolase domain (*PTRT_11091*), sugar transporter domain (*PTRT_11093*), peptidase A-pepsin activity domain (*PTRT_11094*), ribose/galactose isomerase activity domain (*PTRT_11095*), NOT2 activity domain (*PTRT_11096*), LysM domain (*PTRT_11100*) and fungal specific transcription factor activity domain (*PTRT_11092*) (JGI genome portal, Manning et al. 2013; Luo et al. 2016; Schuler et al. 2015: De Jonge and Thomma, 2009).

Bulked segregant analysis (BSA) is a method that can be used for rapid identification of genomic regions that are associated with a phenotype through closely linked genetic markers (Michelmore et al. 1991). The BSA method had been implemented to identify avirulence genes in *Magnaporthe grisea* (Dio et al. 2000). In this study, a total of 209 SSR markers across the genome were screened and tested on chlorosis producing and non-chlorosis producing pools together with parental isolates. However, none of the polymorphic SSR markers (95 total) were linked to the chlorosis producing trait. It is possible that the number of SSR markers is still limited. Now from the study of the Chapter 3, we know it is mainly due to the high levels of recombination rate during the sexual reproduction. In fact, the closest polymorphic SSR marker used in BSA was ~277 kb away from the candidate region on supercontig1.16, and it had a genetic distance of more than 43 cM from the trait (Chapter 3).

In the fertility testing, the genetically modified heterothallic strains, 86-124ΔMAT1-1-1 and AR CrossB10ΔMAT1-2-1 were completely sterile, which agrees with our previous finding

that deletion of one of the MAT genes can lead to complete sterility of the fungus (Ameen et al. 2017). Outcrossing of 86-124ΔMAT1-1-1 and AR CrossB10ΔMAT1-2-1 was partially fertile, but not completely fertile as the wild type strains (100%) in term of ascospore formation in each ascus. This is similar to the observation by Ameen et al. (2017) where the crossing of 86-124ΔMAT1-2-1 and DW5ΔMAT1-1-1 was tested. However, in the crossing of 86-124ΔMAT1-1-1 and AR CrossB10ΔMAT1-2-1, the majority of mature asci contained four ascospores compared to two ascospores in the majority of asci in the crossing of 86-124ΔMAT1-2-1 and DW5ΔMAT1-1-1. The difference is probably due to the genetic relatedness of the parental isolates. Both 86-124 and AR CrossB10 were collected from common wheat, whereas DW5 was collected from durum wheat (Friesen et al. 2004; Ali et al. 2010). It is likely that 86-124 is more related to AR CrossB10 thus having better fertility in the cross. If it is true, testing the fertility of a different cross could be a good way to quantify the genetic relatedness between different races or isolates from different geographic origins.

2.6. References

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

Ameen, G., Kariyawasam, G., Shi, G., Friesen, T.L., Faris, J.D., Ali, S., Rasmussen, J.B. and Liu, Z., (2017). Molecular manipulation of the mating-type system and development of a new approach for characterizing pathogen virulence in *Pyrenophora tritici-repentis*. Fungal Genet. Biol 109:16-25.

Amaike, S., Ozga, J.A., Basu, U. and Strelkov, S.E., (2008). Quantification of ToxB gene expression and formation of appressoria by isolates of *Pyrenophora tritici-repentis* differing in pathogenicity. Plant Pathol 57:.623-633.

Andrie, R.M. and Ciuffetti, L.M., (2011). *Pyrenophora bromi*, causal agent of brownspot of bromegrass, expresses a gene encoding a protein with homology and similar activity to Ptr ToxB, a host-selective toxin of wheat. MPMI. 24:359-367.

Azevedo-Martins, A. C., Machado, A.C., Klein, C.C., Ciapina, L., Gonzaga, L. Vasconcelos, A. T., Sagot, M. F., De Souza. W., Einicker-Lamas M., Galina, A., and Matta, M. C. (2015).

Mitochondrial respiration and genomic analysis provide insight into the influence of the symbiotic bacterium on host trypanosomatid oxygen consumption. Parasitol 142:352-362

Ballance, G.M., Lamari, L. and Bernier, C.C., (1989). Purification and characterization of a host-selective necrosis toxin from *Pyrenophora tritici-repentis*. Physiol Mol Plant Pathol 35:203-213.

Ballance, G.M., Lamari, L., Kowatsch, R. and Bernier, C.C., (1996). Cloning, expression and occurrence of the gene encoding the Ptr necrosis toxin from *Pyrenophora tritici-repentis*. Mol Plant Pathol 1209.

Choi, Y.W., Hyde, K.D. and Ho, W.H., (1999). Single spore isolation of fungi. Fungal diversity. 29-38

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

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

Ciuffetti, L.M., Manning, V.A., Pandelova, I., Faris, J.D., Friesen, T.L., Strelkov, S.E., Weber, G.L., Goodwin, S.B., Wolpert, T.J. and Figueroa, M., (2014). *Pyrenophora tritici-repentis*: a plant pathogenic fungus with global impact. In Genomics of Plant-Associated Fungi: Monocot Pathogens (pp. 1-39). Springer, Berlin, Heidelberg.

Condon, B.J., Leng, Y., Wu, D., Bushley, K.E., Ohm, R.A., Otillar, R., Martin, J., Schackwitz, W., Grimwood, J., MohdZainudin, N. and Xue, C., (2013). Comparative genome structure, secondary metabolite, and effector coding capacity across *Cochliobolus* pathogens. PLoS Genet 9 p.e1003233.

De Jonge, R. and Thomma, B.P., (2009). Fungal LysM effectors: extinguishers of host immunity? Trends Microbiol 17:151-157.

Dodds, P.N. and Rathjen, J.P., (2010). Plant immunity: towards an integrated view of plant–pathogen interactions. Nat Rev Genet 11:539-550

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

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

Faris, J.D., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J.P., Meinhardt, S.W., Rasmussen, J.B., Xu, S.S. and Oliver, R.P., (2010). A unique wheat disease resistance-

like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proc Natl Acad Sci107:13544-13549.

Figueroa, M., Manning, V., A., Pandelova, I., and Ciuffetti, L., M. (2015). Persistence of the host-selective toxin Ptr ToxB in the apoplast. Mol. Plant-Microbe Int. 28:1082-1090.

Flor, H.H., (1971). Current status of the gene-for-gene concept. Annu Rev Phytopathol 9:275-296.

Franceschetti, M., Maqbool, A., Jiménez-Dalmaroni, M.J., Pennington, H.G., Kamoun, S. and Banfield, M.J., (2017). Effectors of filamentous plant pathogens: commonalities amid diversity. Microbiol Mol Biol Rev 81:pp.e00066-16.

Friesen, T.L., Holmes, D.J., Bowden, R.L. and Faris, J.D., (2018). ToxA Is Present in the US *Bipolaris sorokiniana* Population and Is a Significant Virulence Factor on Wheat Harboring Tsn1. Plant dis. PDIS-03.

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

Hammond-Kosack, K.E. and Rudd, J.J., (2008). Plant resistance signalling hijacked by a necrotrophic fungal pathogen. Plant Signal Behav 3:993-995.

Heffelfinger, C., Fragoso, C.A. and Lorieux, M., (2017). Constructing linkage maps in the genomics era with MapDisto 2.0. Bioinformatics 33:2224-2225.

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

Howlett, B.J., (2006). Secondary metabolite toxins and nutrition of plant pathogenic fungi. Curr Opin Plant Biol 9:371-375.

Jones, J.D. and Dangl, J.L., (2006). The plant immune system. Nature 444: 323-329.

De Jonge, R., Ebert, M.K., Huitt-Roehl, C.R., Pal, P., Suttle, J.C., Spanner, R.E., Neubauer, J.D., Jurick, W.M., Stott, K.A., Secor, G.A. and Thomma, B.P., (2018). Gene cluster conservation provides insight into cercosporin biosynthesis and extends production to the genus *Colletotrichum*. Proc Natl Acad Sci 115:5459-5466.

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

Koladia, V.M., Richards, J.K., Wyatt, N.A., Faris, J.D., Brueggeman, R.S. and Friesen, T.L., (2017). Genetic analysis of virulence in the *Pyrenophora teres* f. *teres* population BB25× FGOH04Ptt-21. Fungal Genet Biol 107:12-19.

Lai, Z., Faris, J.D., Weiland, J.J., Steffenson, B.J. and Friesen, T.L., (2007). Genetic mapping of *Pyrenophora teres* f. *teres* genes conferring avirulence on barley. Fungal Genet Biol 44:323-329.

Lamari, L. and Bernier, C.C., (1989). Evaluation of wheat lines and cultivars to tan spot [*Pyrenophora tritici-repentis*] based on lesion type. Can J Plant Pathol 11:49-56.

Lee, B.N., Kroken, S., Chou, D.Y., Robbertse, B., Yoder, O.C. and Turgeon, B.G., (2005). Functional analysis of all nonribosomal peptide synthetases in *Cochliobolus heterostrophus* reveals a factor, NPS6, involved in virulence and resistance to oxidative stress. Eukaryot Cell 4:545-555.

Lendenmann, M.H., Croll, D., Stewart, E.L. and McDonald, B.A., 2014. Quantitative trait locus mapping of melanization in the plant pathogenic fungus *Zymoseptoria tritici*. G3: (Bethesda) 4:2519-2533

Lepoint, P., Renard, M.E., Legrève, A., Duveiller, E. and Maraite, H., (2010). Genetic diversity of the mating type and toxin production genes in *Pyrenophora tritici-repentis*. Phytopathol 100.474-483.

Liu, Z., Faris, J.D., Oliver, R.P., Tan, K.C., Solomon, P.S., McDonald, M.C., McDonald, B.A., Nunez, A., Lu, S., Rasmussen, J.B. and Friesen, T.L., (2009). SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the Snn3 gene. PLoS pathog 5.p.e1000581.

Liu, Z. and Friesen, T.L., (2012). Polyethylene glycol (PEG)-mediated transformation in filamentous fungal pathogens. In Plant Fungal Pathogens (pp. 365-375). Humana Press.

Liu, Z., Zhang, Z., Faris, J.D., Oliver, R.P., Syme, R., McDonald, M.C., McDonald, B.A., Solomon, P.S., Lu, S., Shelver, W.L. and Xu, S., (2012). The cysteine rich necrotrophic effector SnTox1 produced by *Stagonospora nodorum* triggers susceptibility of wheat lines harboring Snn1. PLoS Pathog 8 p.e1002467.

Liu, Z., Zurn, J., D., Kariyawasam, G., Faris, J., D., Shi, G., Hansen, J., Rasmussen, J., B., and Acevedo, M. (2017). Inverse gene-for-gene interactions contribute additively to tan spot susceptibility in wheat. Theor. Appl. Genet. 6:1267-76.

Long, Y.M., Chao, W.S., Ma, G.J., Xu, S.S. and Qi, L.L., (2017). An innovative SNP genotyping method adapting to multiple platforms and throughputs. Theor Appl Genet 130:597-607.

Lo Presti, L., Lanver, D., Schweizer, G., Tanaka, S., Liang, L., Tollot, M., Zuccaro, A., Reissmann, S. and Kahmann, R., (2015). Fungal effectors and plant susceptibility. Annu Rev Plant Biol 66:513-545.

Lorang, J.M., Sweat, T.A. and Wolpert, T.J., (2007). Plant disease susceptibility conferred by a "resistance" gene. Proc Natl Acad Sci 104:14861-14866.

Lorang, J., Kidarsa, T., Bradford, C.S., Gilbert, B., Curtis, M., Tzeng, S.C., Maier, C.S. and Wolpert, T.J., (2012). Tricking the guard: exploiting plant defense for disease susceptibility. Sci. 338:659-662.

Luo, X., Mao, H., Wei, Y., Cai, J., Xie, C., Sui, A., Yang, X. and Dong, J., (2016). The fungal-specific transcription factor Vdpf influences conidia production, melanized microsclerotia formation and pathogenicity in *Verticillium dahliae*. Mol Plant Pathol17:1364-1381.

McDonald, M. C., Ahren, D., Simpfendorfer, S., Milgate, A., and Solomon, P., S. (2018). The discovery of the virulence gene ToxA in the wheat and barley pathogen *Bipolaris sorokiniana*. Mol. plant pathol. 19:432-439.

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

Manning, V.A., Pandelova, I., Dhillon, B., Wilhelm, L.J., Goodwin, S.B., Berlin, A.M., Figueroa, M., Freitag, M., Hane, J.K., Henrissat, B. and Holman, W.H., (2013). Comparative genomics of a plant-pathogenic fungus, *Pyrenophora tritici-repentis*, reveals transduplication and the impact of repeat elements on pathogenicity and population divergence. G3: (Bethesda) 3:41-63.

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

Martinez, J.P., Oesch, N.W. and Ciuffetti, L.M., (2004). Characterization of the multiple-copy host-selective toxin gene, ToxB, in pathogenic and nonpathogenic isolates of *Pyrenophora tritici-repentis*. Mol Plant-Microbe Interact 17:467-474

Michelmore, R.W., Paran, I. and Kesseli, R.V., (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci 88:9828-9832.

Moolhuijzen, P., See, P.T., Hane, J.K., Shi, G., Liu, Z., Oliver, R.P. and Moffat, C.S., (2018). Correction to: Comparative genomics of the wheat fungal pathogen *Pyrenophora tritici-repentis* reveals chromosomal variations and genome plasticity. BMC genomics 19:279.

Nagy, E.D. and Bennetzen, J.L., (2008). Pathogen corruption and site-directed recombination at a plant disease resistance gene cluster. Genome Res.

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

Rees RG, Platz GJ, Mayer RJ .(1982). Yield losses in wheat from yellow spot: comparison of estimates derived from single tillers and plots. Aust J Agric Res 33:899–908

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

Schuler, D., Wahl, R., Wippel, K., Vranes, M., Münsterkötter, M., Sauer, N. and Kämper, J., 2015. Hxt1, a monosaccharide transporter and sensor required for virulence of the maize pathogen *Ustilago maydis*. New Phytol. 206:1086-1100.

Shi, G., Zhang, Z., Friesen, T.L., Raats, D., Fahima, T., Brueggeman, R.S., Lu, S., Trick, H.N., Liu, Z., Chao, W. and Frenkel, Z., (2016). The hijacking of a receptor kinase–driven pathway by a wheat fungal pathogen leads to disease. Sci. Adv. 2:p.e1600822.

Shjerve, R.A., Faris, J.D., Brueggeman, R.S., Yan, C., Zhu, Y., Koladia, V. and Friesen, T.L., (2014). Evaluation of a *Pyrenophora teres* f. *teres* mapping population reveals multiple independent interactions with a region of barley chromosome 6H. Fungal Genet Biol 70:.104-112.

Sperschneider, J., Dodds, P.N., Gardiner, D.M., Singh, K.B. and Taylor, J.M., (2018). Improved prediction of fungal effector proteins from secretomes with EffectorP 2.0. Mol Plant Pathol

Stergiopoulos, I., Collemare, J., Mehrabi, R. and De Wit, P.J., (2013). Phytotoxic secondary metabolites and peptides produced by plant pathogenic Dothideomycete fungi. FEMS Microbiol Rev 37:67-93.

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

Strelkov, S.E. and Lamari, L., (2003). Host–parasite interactions in tan spot [*Pyrenophora tritici-repentis*] of wheat. Can J Plant Pathol 25:339-349.

Sun, X., C., Bockus, W., and Bai, G. (2010). Quantitative trait loci for resistance to *Pyrenophora tritici-repentis* race 1 in a Chinese wheat. Phytopathology100: 468-473.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M. and Rozen, S.G., 2012. Primer3—new capabilities and interfaces. Nucleic Acids Res 40e115-e115.

Talbot, N. J., Ebbole, D. J., and Hamer, J. E. (1993). Identification and characterization of MPG1, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. Plant Cell 5: 1575-1590.

Tomas, A. and Bockus, W.W., (1987). Cultivar-specific toxicity of culture filtrates of *Pyrenophora tritici-repentis*. Phytopathol 77:1337-1340.

Trick, M., Adamski, N.M., Mugford, S.G., Jiang, C.C., Febrer, M. and Uauy, C., (2012). Combining SNP discovery from next-generation sequencing data with bulked segregant analysis (BSA) to fine-map genes in polyploid wheat. BMC Plant Biol 12:14.

Wen, A., Jayawardana, M., Fiedler, J., Sapkota, S., Shi, G., Peng, Z., Liu, S., White, F.F., Bogdanove, A.J., Li, X. and Liu, Z., (2018). Genetic mapping of a major gene in triticale conferring resistance to bacterial leaf streak. Theor Appl Genet 131:649-658.

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

Yoshida, K., Saitoh, H., Fujisawa, S., Kanzaki, H., Matsumura, H., Yoshida, K., Tosa, Y., Chuma, I., Takano, Y., Win, J. and Kamoun, S., (2009). Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen *Magnaporthe oryzae*. Plant Cell 21:1573-1591.

Zhang, H.F., Francl, L.J., Jordahl, J.G. and Meinhardt, S.W., (1997). Structural and physical properties of a necrosis-inducing toxin from *Pyrenophora tritici-repentis*. Phytopathol 87:154-160.

Zhong, S., Steffenson, B.J., Martinez, J.P. and Ciuffetti, L.M., (2002). A molecular genetic map and electrophoretic karyotype of the plant pathogenic fungus *Cochliobolus sativus*. Mol Plant-Microbe Interact 15:481-492.

Zhong, Z., Marcel, T.C., Hartmann, F.E., Ma, X., Plissonneau, C., Zala, M., Ducasse, A., Confais, J., Compain, J., Lapalu, N. and Amselem, J., (2017). A small secreted protein in *Zymoseptoria tritici* is responsible for avirulence on wheat cultivars carrying the *Stb6* resistance gene. New Phytol. 214:.619-631.

CHAPTER 3. DEVELOPMENT OF A GENOME-WIDE GENETIC LINKAGE MAP IN THE WHEAT PATHOGEN *PYRENOPHORA TRITICI-REPENTIS* AND ITS UTILIZATION IN COMPARATIVE GENOMICS AND DETECTION OF STRUCTURAL VARIATION RESULTING FROM SEXUAL RECOMBINATION

3.1. Abstract

Pyrenophora tritici-repentis is an ascomycete fungal pathogen that causes wheat tan spot. The disease has a worldwide distribution and can cause significant yield and quality losses in wheat production. The fungus usually undergoes sexual reproduction and produces pseudeothecia for overwintering. Due to the homothallic nature, no genetic linkage map has been developed in *P. tritici-repentis* and the assembling of *P. tritici-repentis* genome sequence data has been facilitated by optical mapping techniques. In this work, the first genetic linkage map in *P. tritici-repentis* was developed using a fungal population derived from the two genetically modified heterothallic strains: AR CrossB10 ΔMAT1-2-1 and 86-124 ΔMAT1-1-1. The two parental and twenty progeny isolates were first sequenced using Illumina pair-reads with at least 33 x coverage, and the data was mined for single nucleotide polymorphism (SNP) markers across the genome. In addition, SSR markers were employed in genetic linkage mapping. The resulting linkage map consisted of 16 linkage groups spanning 4922.8 cM in genetic distance with a strikingly high rate of genetic recombination at 7.57 kb/cM. A high-quality genome sequence was also obtained for AR CrossB10 using sequencing data from PAC-BIO and Nanopore technologies. The assembled contigs were aligned with the genetic linkage map and then compared to the recently published *P. tritici-repentis* genomes, which revealed a high level of similarity with only a few noticeable large structural variations between the isolates. Putative chromosomal structural variations were identified using whole genome sequences obtained for

progeny and parental isolates. The results showed that 42 new structural variations were detected in progeny but not in parental isolates, whereas 262 variations that were identified in parental isolates were lost in the progeny. This work indicates that linkage maps can provide an anchor for genome sequence assembly and comparison, and structural variation can take place during sexual reproduction in *P. tritici-repentis*.

3.2. Introduction

Pyrenophora tritici-repentis (anamorph: *Drechslera tritici-repentis*) is a destructive necrotrophic fungal pathogen that causes tan spot or yellow spot of wheat. The disease can occur in almost all wheat growing regions worldwide. Infection of the pathogen typically causes tan-colored necrotic lesions on wheat leaves with or without chlorotic halos that eventually coalesce leading to death of the entire leaf. The dead tissues on the leaves reduce the photosynthetic area and thus the yield (Friskop and Liu 2016; Moolhuijzen et al. 2018). Infection on wheat kernels can cause pink to red discoloration, downgrading the quality of the grain (Schilder and Bergstorm 1994). Epidemics of tan spot started in 1970s with the adoption of no or reduced tillage. It has been reported that under favorable environmental conditions the disease can cause yield losses up to 50% on susceptible lines (Hosfard 1982; Rees et al. 1982). A recent study revealed that tan spot is the most significant wheat disease in Australia where direct yield loss was valued at $212 million plus the cost of $463 million for disease control (Murry and Brennan 2009).

P. tritici-repentis is known to produce three necrotrophic effectors (NEs) including Ptr ToxA, Ptr ToxB, and Ptr ToxC, which interact with the host susceptibility genes *Tsn1*, *Tsc2* and *Tsc1*, respectively, to cause disease in an inverse gene-for-gene manner (Reviewed in Faris et al. 2013). Ptr ToxA produces a necrotic reaction and the other two produce chlorosis on the leaves

of sensitive wheat genotypes (Reviewed in Ciuffetti et al. 2010). Based on the production of NEs and/or their reactions on the four differential lines, Salamouni, Glenlea (*Tsn1*), 6B365 (*Tsc1*) and 6B662 (*Tsc2*), *P. tritici-repentis* isolates have been classified into eight races (Lamari and Strelkov 2010). However, recent studies have identified isolates that do not conform to the current race classification system (Ali et al. 2010; Benslimane et al. 2018). In addition, Aboukhaddour et al. (2011) characterized the genetic diversity of 80 isolates collected from Syria, Algeria, Azerbaijan and Canada using 31 SSR markers. Nei's analysis of genetic distance showed that these isolates grouped into four clusters where isolates from the same region clustered together suggesting genetic variability was significant between the *P. tritici-repentis* populations in different countries.

The genome of *P. tritici-repentis* was first studied using pulsed-field gel electrophoresis (PFGE). Lichter et al. (2002) found a great degree of variability in the karyotypes of pathogenic and non-pathogenic *P. tritici-repentis* isolates. Using more isolates representing eight races, Aboukhaddour et al. (2009) identified 29 karyotypes with chromosome number varying from 8 to 11 and genome size ranging from 25.5 to 48 Mb. Therefore, *P. tritici-repentis* genomes were highly variable which could be explained by chromosome length polymorphism (CLP) and chromosome number polymorphism (CNP).

In recent years, the genome sequences of several *P. tritici-repentis* isolates have been made available and used in comparative genomics to provide insights into genome variability in *P. tritici-repentis*. The first *P. tritici-repentis* genome sequence was reported by Manning et al. (2013) for the race 1 isolate Pt-1C-BFP which was collected from South Dakota, US. Sequencing for this isolate was done using a shot-gun fosmid library and the Sanger sequencing method. The assembled sequence was comprised of 47 scaffolds with a total length of 37.84 Mb, and 26 of

them were connected into eleven chromosomes using optical mapping. The genomes of an additional two isolates, one being race 5 and the other being race 4, were also sequenced in this study using Illumina short reads and compared to the race 1 isolate Pt-1C-BFP. A high-quality *P. tritici-repentis* genome sequence was recently published for the race 1 isolate M4, which was collected in Australia (Moolhuijzen et al. 2018). Because the long-read single –molecule real-time (SMRT) sequencing method was used in combination with high coverage short reads, this published reference sequence had fewer gaps and better genome assembly. The genome assembly contained 50 super contigs with a total size of 40.9 Mb arranged into ten chromosomes using optical mapping (Moolhuijzen et al. 2018). This genome sequence was highly comparable to that of Pt-1C-BFP, but several structural variations were detected.

A genetic linkage map is useful in many aspects, such as gene mapping and cloning, physical contig connection, and macro and micro synteny analysis in the genome (Zhong et al. 2017; Goodwin et al. 2011). Genetic linkage mapping requires the initial generation of segregating populations. For a heterothallic plant pathogenic fungus, which is self-sterile, natural isolates can be used directly to develop fungal populations for genetic mapping. Thus, genetic linkage maps have been developed for many heterothallic fungal pathogens, such as *Zymoseptoria tritici*, *Venturia inaequalis*, *Pyrenophora teres* f. *teres* and *Magnaporthe grisea* and have been successfully used in mapping and cloning of fungal effector genes (Wittenberg et al. 2009; Lendenmann et al. 2014; Xu et al. 2009; Lai et al. 2007; Koladia et al. 2017; Kaye et al. 2003, Zhong et al. 2017). In contrast, for the homothallic fungal pathogens, which are self-fertile by nature, genetic linkage mapping cannot be easily done due to the difficulty in the generation of segregating populations. However, there are two ways that segregating populations can be developed for homothallic fungi, including the utilization of nitrogen non-utilizing mutants,

example, *Fusarium graminearum* (Bowden and Leslie, 1999; Jurgenson et al. 2002; Gale et al. 2005), or conversion to heterothallism by deletion of one of the mating type genes (Lee et al. 2003). Recently, we showed that *P. tritici-repentis* isolates can be converted into heterothallic strains by deleting one of the mating type genes, and those heterothallic strains can be used to develop segregating fungal population (Ameen et al. 2017).

The *P. tritici-repentis* isolate AR CrossB10 represents a collection of isolates in Arkansas, US and was characterized as a new race because it did not contain the *ToxA* gene but produced necrosis on Glenlea (Ali et al. 2010). Those isolates likely carry new NEs to cause tan spot disease. The genome sequence for AR CrossB10 has been obtained using Illumina short reads (Moolhuijzen et al. 2018), but it remains unknown how different its genome is to the published reference genome sequences, which are based on race 1. Thus, in this study, I developed a high-quality genome sequence of AR CrossB10 from SMRT sequencing as well as a genetic linkage map on AR CrossB10 and used the genetic linkage map to anchor physical contigs into linkage groups for genome comparison to the published reference genomes. In addition, I also identified structural variations that could possibly occur during sexual reproduction.

3.3. Materials and methods

3.3.1. PAC-BIO sequencing and genome assembling of AR CrossB10

Genomic DNA of AR CrossB10 was extracted and sent to the Molecular Biology Core Facility at Mayo Clinic (Rochester, MN). Sequencing libraries were prepared using approximately 20 kb insertion and the sequencing was carried out on the PacBio RSII platform with three SMRT cells. AR CrossB10 genome sequence data was also produced from Nanopore sequencing using the Oxford Nanopore Ligation Sequencing kit v 1D R9 as per the user's

manual. FASTQ files that contained raw reads from both PacBio and Nanopore data were loaded to the Canu 1.0 assembler (Koren et al. 2017) and *de novo* genome assembly was performed by selecting the genome size estimate as 37.8 Mb and default parameters for correction and trimming. A second iteration of genome assembly was done by increasing the estimated genome size to 40.1 Mb. The resulting genome assembly of AR CrossB10 was finally polished using Pilon v1.21 (Walker et al. 2014) with the data from Illumina paired-end reads of AR crossB10 (Moolhuijzen et al. 2018), which was to improve base calling accuracy for the final genome assemblies. Quality of the genome assembly was also assessed by obtaining statistics such as N50, which defines the number of conigs that represents 50% of the genome and L50, which defines the length of the smallest contig that represents the ordered set of L50 contigs.

3.3.2. Genome annotation

The Maker2 pipeline (Holt et al. 2011) was used to develop gene models for AR CrossB10. *Ab initio* annotations were obtained via Augustus with the training set of the model fungi *Neurospora crassa* and Genemark-ES v.2 (Ter-Hovhannisyan et al. 2008) with unsupervised training. Transcript evidence from *P. tritici-repentis* isolate Pt-1C-BFP was input into the Maker2 pipeline along with protein evidence from *P. tritici-repentis* isolate Pt-1C-BFP (Manning et al. 2013), *P. teres* f. *teres* isolate 0-1 (Wyatt et al. 2018), and *Parastagonospora nodorum* isolates SN15 and Sn4 (Syme et al. 2016; Richards et al, 2018). For the first iteration of the annotation, “est2genome=1” and “protein2genome=1” commands were used to develop gene models based on the input evidence from other genomes and the option ‘split-hit=5000’ was set to avoid mis-annotation due to the overly large intron lengths. The *Ab initio* annotation program SNAP (Korf et al. 2004) was trained by the gene-models from the previous step and Maker2 pipeline was rerun with the addition of SNAP training file created specific to the AR CrossB10

genome. SNAP was retrained from gene set obtained from the second run of the Maker2 and ran again to further refine gene models. Finally, completeness of the annotated genes was measured using BUSCO. Once the genes were annotated, genes encoding for proteins with signal peptides were identified using SignalP v4.1 (Petersen et al. 2011). Then the mature proteins were used to predict genes encoding for effectors using EffectorP v2.0 (Sperschneider et al. 2015).

De novo annotation of the repeat regions was done using RepeatModeler v1.0.11 (Smit et al. 2015) to develop a custom repeat library for AR CrossB10. The resulting repeat library was input in to RepeatMasker (Smith et al. 2015) together with the latest release of fungal repeat sequences from Repbase (v22.10) (Bao et al. 2015) to obtain the final annotation of the repetitive elements identified for the AR CrossB10 genome. Summary of the statistics for different types of repeat content was obtained using the "buildSummary.pl" RepeatMasker script.

3.3.3. Fungal bi-parental population

The fungal population derived from the cross 86-124ΔMAT1-1-1 ×AR CrossB10ΔMAT1-2-1, which was descried in the second chapter of my dissertation, was used in this chapter to develop the genome-wide linkage map for *P. tritici-repentis*. The population is here after referred to as the AR population and consisted of 112 progeny, all of which were used for genotyping. Genomic DNA was extracted for each progeny as described in chapter 1 and used to develop genetic markers.

3.3.4. SNP calling and marker development

As mentioned in Chapter 1, the parental isolates and 20 progenies selected from the population were used for whole genome sequencing. Twenty progenies were sequenced by NOVOGENE corporations (Chula Vista, CA) using the Illumina HiSeq 2500 platform with a coverage ranging from 33 to 52 x. To identify single nucleotide polymorphisms (SNP) for

marker development, the cleaned sequencing data was aligned to the reference genome sequence of Pt-1c-BFP using BWA with the 'bwa-mem' function to create SAM files. SAM files were then converted to BAM files using the SAMtools 'view' function which is followed by the 'sort' function to create sorted BAM files of each assembly. The reference genome sequence and all the BAM files were indexed using 'faidx' and 'index' options of SAMtools followed by the calling of SNPs using 'mpileup' function. Finally, SNPs were obtained using BCFtools.

Based on the super contig and chromosomal arrangement of Pt-1c-BFP, SNPs were selected across the whole genome with an interval of approximately 200 kb in each scaffold, including the ones that are not mapped in chromosomes. Those SNPs were used to develop semi-thermal asymmetric reverse PCR (STARP) markers. Initially, primers were designed for 191 SNPs and later for 76 more SNPs at the regions where the first ones failed or there was a large genetic gap. Primers for all the SNPs were designed as described in Long et al. (2017) and listed in Appendix 2.1. Each STARP consisted of five primers, including two priming element-adjustable primers (PEA), two asymmetrically modified allele specific primers (AMAS), and a common reverse primer. The PEA-primer1 and PEA-primer2 are labeled with IRDye 700® and universal for all markers with a sequence of 5'-AGCTGGTT-Sp9-GCAACAGGAACCAGCTATGAC-3' and 5'-ACTGCTCAAGAG-Sp9-GACGCAAGTGAGCAGTATGAC-3', respectively (Long et al. 2017). Two AMAS primers were designed by selecting the 17-25 bps upstream of individual SNPs, and each contained a specific SNP nucleotide at the 3' end. The third nucleotide from the 3' end of the one AMAS sequence and the 4th nucleotide from the other AMAS sequence were substituted with a nucleotide according to the rule described in Long et al. (2017). Melting temperature (T_m) of the two AMAS primers were set between 54 to 58 ºC by adjusting the length of the primer.

Generally, the length of the two AMAS-primers were kept the same. Finally, universal Tail 1 (from Priming element 1) – 5'-GCAACAGGAACCAGCTATGAC-3' was added to the 5' end of all AMAS-primer1 and Tail 2 (from Priming element 2) – 5'-GACGCAAGTGAGCAGTATGAC-3' was added to the 5' end of all AMAS-primer2. The reverse primer was selected 70-170 bps downstream of the SNP where the T_m value falls between 58 and 62 ºC predicted by Primer3 v. 0.4.0 (Untergasser et al. 2012). Primer mix was prepared for each STARP SNP marker by mixing AMAS-primer 1: AMAS-primer 2: Reverse primer in 0.4:0.4:2 µM ratio.

The PCR mix consisted of a 10 µl reaction mix that contained 1 × NH_4^+ buffer (16 mM $(NH_4)_2$ and 67 mM Tris-HCl, pH8.3 at 25 ºC) , 0.8 M betaine, 0.04% (W/V) bovine serum albumin (BSA), 1.5 mM $MgCl_2$, 50 µM of each dNTP, 200 nM each of PEA-primer 1and PEA-primer 2, 40 nM each of AMAS-primer1 and AMAS-primer2, 200 nM of reverse primer, 1 U of Taq DNA polymerase (homemade) and 10-100 ng of genomic DNA. The PCR program was as following: 94 ºC for 3 m, 6 cycles at 94 ºC for 20s, 55 ºC for 30s with 1 ºC reduction per cycle, and 37 cycles of 94 ºC for 30s and 62 ºC for 30s. PCR products were visualized using a LI-COR 4300 DNA analyzer (LI-COR Bioscience, Lincoln, NE, USA). All the markers were screened for their activity and polymorphism prior to genotyping across the population.

Some of the SNP markers were developed as cleaved amplified polymorphic sequence (CAPS) markers (Konieczny and Ausubel, 1993). Sequence spanning 150 bps upstream and downstream from each SNP was extracted, and forward and reverse primers were designed using Primer3 v. 0.4.0 (Untergasser et al. 2012). The sequence was screened for restriction sites that included the SNP using NEBcutter v.2.0. PCR reaction mix containing 1× buffer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.5 µM of each forward and reverse primer, 10-20 ng of DNA and 1 U

of Bullseye Taq DNA polymerase (MIDISCI, St. Louis) in 10 µl volume. PCR was performed with the following profile that includes, 95 ºC for 5 mins, 30 cycles at 95 ºC for 30s, 58 ºC for 30s, 72 ºC for 1 min, followed by a 72 ºC final elongation step for 5 mins and a 4 ºC holding step.

Restriction digestions were carried out in a 10 µl reaction mix that contained 4.5 µl of PCR product, 2 U of restriction enzyme, and 0.01 mg BSA, and were incubated at optimal temperature for 2 hours. Digestion products were visualized using 1-2% agarose gel electrophoresis.

3.3.5. SSR marker development

A total of 313 SSRs were identified from the reference genome (assisted by Dr. Shaobin Zhong) as described in Zhong et al. (2009). All the SSR primers were designed using Primer3 v. 0.4.0 (Untergasser et al. 2012) and listed in Appendix 2.2. To visualize the PCR products in a 4300 DNA analyzer, all the forward primers were added with the sequence of the M13 primer (5'-TGTAAAACGACGGCCAGT-3'). All the primers were first screened between parental isolates and the polymorphic ones were used to genotype in the entire population. SSR products were amplified using the following PCR protocol: 95°C for 5mins, 8 cycles at 95°C for 20s, 50° C for 20s, 72°C for 30s, 38 cycles at 95°C for 20s, 52°C for 20s, 72°C for 30s. The PCR mix consisted of 1× buffer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.05 µM of SSR primer pair, 0.1 µM DY682 fluorescently labeled M13 primer, 5-10 ng of DNA and 1 U of Bullseye Taq DNA polymerase (MIDISCI, St. Louis) in a 10 µl volume (Wen et al. 2017). PCR product were separated based on size by performing polyacrylamide gel electrophoresis using a LI-COR 4300 DNA analyzer (LI-COR Bioscience, Lincoln, NE, USA).

3.3.6. Marker scoring and linkage mapping

For marker scoring, the progeny was recorded as '1' if the PCR produced the 86-124ΔMAT1-1-1 allele type and '0' if it produces the AR CrossB10ΔMAT1-2-1 allele type. Missing data was recorded as "3". If STARP produced two or more polymorphic bands, they were scored as separate markers as marker.1, marker.2, etc.

The genetic linkage map for the AR population was developed using Mapdisto v2.0 beta (Heffelfinger et al. 2017) where genotypic data for all the markers were entered in binary form. Linkage groups were developed using 'find linkage group' function with a LOD value of 3.3 and an r value of 0.35. Functions 'order sequence', 'check inversions', 'ripple order' and 'drop locus' were repeatedly performed to correct marker order and genetic distance.

3.3.7. Anchoring the assembled contigs of AR CrossB10 to the genetic linkage map

A local blast database was created for AR CrossB10 assemblies using the 'makeblastdb' command from BLAST+ 2.8.0 (Camacho et al. 2008). A FASTA file was created including all the DNA sequences that were used to develop SSR and SNP markers in FASTA format and it was used as the query to search against the AR CrossB10 local blast database using the 'blastn' option. Positions of the start and end nucleotides of the blast result for each DNA sequence, that was used to develop the markers were used as coordinates to anchor contigs of AR CrossB10 genome assembly to the genetic map.

3.3.8. Comparative genomics

The linkage map of AR CrossB10 was compared to the optical map of the reference genome assembly of M4 using the whole genome alignment pipeline 'NUCmer' that comes with MUMmer v3.23 package (Delcher et al. 2003). The '--mum' option was used to anchor the matches that are presented in both M4 and AR CrossB10 sequences. To obtain information about

the alignments, such as coordinates of the alignments in both M4 and AR CrossB10 assemblies, percent identity and alignment lengths, resulted delta alignment output from the NUCmer was analyzed using 'show-coords' command and parameters were set to obtain information of alignments at ≥ 95% identity and ≥ 10 kb in length. Coordinates of the contigs of M4 and AR CrossB10 assemblies were re-labeled based on the optical maps and linkage maps respectively to have one pseudomolecule for each *P. tritici-repentis* chromosome. Alignments were then visualized in RStudio using the genoPlotR (Guy et al. 2010) package.

In addition, information extracted from 'show-coords was used to identify the genomic regions that were not present in M4, but present in AR CrossB10 and vice-versa. Absence of regions of AR in M4 were further confirmed by reducing the alignment length to ≥ 1 kb.

3.3.9. Detection of chromosomal structural variations in progeny

The whole genome sequences of two parental isolates and 20 progeny isolates from Illumina paired-end reads were used to identify the possible structural variations (SV) in progeny during sexual reproduction, which was performed by using BreakDancer v1.3.6 as described in Fan et al. (2014). A Sequence Alignment Map (SAM) file with read group tag (RG) for each isolate was created using cleaned FASTQ file of each isolate by aligning the raw reads to the recently published reference genome of the M4 isolate (Moolhuijzen et al. 2018) using 'bwa-mem' command of Burrows- Wheeler Aligner (BWA) (Li and Durbin, 2009). SAM files were converted to a sorted binary alignment map (BAM) files using 'view' and 'sort' commands of the SAMtools. Multiple BAM files were generated for some of the genome sequences using the 'sort' command. Those BAM files were combined using the 'merge' command to obtain a single sorted BAM file for each isolate. The perl script 'bam2cfg.pl' was run on all the BAM files to generate a single configuration file that contained statistical information of read groups such as

location of the BAM file, mean insert size, standard deviation of insert size, average read length, unique tag for the library, and a command line for perl system to produce MAQ mapview alignment. In addition, a configuration file also provided the used thresholds for deletions and insertions

All the quality control checks were performed as suggested in Fan et al. (2014). In brief, commands "samtools view -H BAM_file| grep -c @RG" and "grep -c BAM_file config_file" were run for each BAM file. This test detects the missing RG or library (LB) tag information in the header of the input BAM file. If the value from the first command was less than the second value, RG and LB information of the BAM file were considered to be missing. A second quality check looked for the "NA" in read groups using the command "grep -cw readgroup:NA config_file" and any value greater than 0 showed RG and LB information was missing in at least one of the BAM files used in the study. A third quality check looked for the coefficient of variation of the insert size for each read group and it was calculated using the command "perl -ane ' ($mean)=($_=~/mean:(\S+)/);($std)=($_=~/std:(\S +)/);print $std/$mean ."\n" ' config_file". Values less than 0.2 were considered normal values. Finally, percentage of inter-chromosomal read pairs were calculated using "perl -ane '($CTX)=($_=~/32\((\S+?)\)/);print $CTX."\n" ' config_file". Typically, the percentage should be less than 3% and a higher value suggests problems in library preparation or sequencing.

Finally, the 'breakdancer-max' program was used to identify SVs from the statistical information in the configuration file. Out of these, SVs were reported if represented by more than ten reads and a confidence score of greater than 90. SVs were classified into deletions, insertions and inversions, which are defined as the following:, deletions are regions that are absent in the whole genome sequence (WGS) of test isolates but present in the reference isolate

(M4), an insertion was a DNA fragment inserted in the WGS of the test isolates that was absent in the reference genome assembly, and an inversion was defined as a segment of DNA of a progeny isolate that aligned to the reference sequence in the opposite direction. The parental lines and progeny isolates were compared for these SVs to identify those that are present in progeny, but not in parental isolates, and were therefore considered to have happened during sexual reproduction.

3.4. Results

3.4.1. Genome sequencing and genome assembly of AR CrossB10

PacBio SMRT sequencing and Nanopore sequencing of *P. tritici-repentis* isolate AR CrossB10 resulted in a total of 318,475 raw reads with an average read length of 8,035 bps. The total sequences from the two were 2,559,025,029 bps, which equals to approximately 65x coverage of the AR CrossB10 genome. De novo assembly with this data resulted in 149 contigs with a genome size of 40.1 Mb. The length of the contigs ranged from 1,161 bp to 1,995,139 bp with an L50 value of 17 and an N50 value of 687,436 bp (Table 3.1). The quality of the AR CrossB10 genome assembly was relatively lower than that of the published M4 reference genome, but they were comparable in genome size, GC content (50.71%) and gene content (Table 3.1).

Table 3.1. Statistics of AR CrossB10 compared to recently published reference genome

Feature	M4 Assembly	AR CrossB10 Assembly
Genome size	40.9 Mb	40.1 Mb
Total contigs	50	149
Largest contig	7,096,861	1,995,139
Smallest contig	3,304	1,161
L50	6	17
N50	2.930 Mb	687,436
GC%	50.73	50.71
Genes	13,797	13,768

Table 3.2. Genetic linkage map developed for the cross between *Pyrenophora tritici-repentis* isolates 86-124ΔMAT1-1-1 × AR CrossB10ΔMAT1-2-1

Linkage group	Chromosome of reference genome (M4)	Markers mapped	Total genetic distance (cM)	Physical distance (kb)	Average recombination rate (kb/cM)
LG1	1	66	1262.44	9731.386	7.71
LG2.1	2 and 9	61	677.30	6745.100	9.81
LG2.2	9	2	6.78		
LG3	3	29	503.80	3427.327	6.80
LG4	4	22	412.59	2951.382	7.15
LG5	5	28	482.06	2750.130	5.70
LG6	6	21	387.44	2704.255	6.98
LG7.1	7	2	16.82	2604.652	8.22
LG7.2	7	15	300.09		
LG8.1	8	14	189.37	1809.615	8.79
LG8.2	8	3	16.56		
LG8.3	8	8	107.57	677.450	6.30
LG10	10	16	348.07	2197.350	6.31
LG11.1	10	3	31.87	1669.066	7.87
LG.11.2	10	4	68.93		
LG11.3	10	9	111.15		
Total	All	303	4922.84	37267.713	7.57

Genome annotation of the AR CrossB10 assembly resulted in 13,768 gene models which was 29 genes less than the newly assembled M4 genome (Table 3.1). Out of those, 1,221 were predicted to encode a protein with a signal peptide, and only 312 were predicted to encode a secreted proteinaceous effector (Appendix 2.3).

Repeat analysis showed that 18.85 % of the AR CrossB10 whole genome sequence consisted of repetitive regions. These included DNA transposons (6.02%), long terminal repeats (LTR) (7.66%), and non-LTR elements such as long interspersed nuclear elements (LINEs) (1.74%). In addition, 4563 repeat elements were not shown to belong to any known type of repeat element and represented 3.34% of the genome sequence (Appendix 2.3).

3.4.2. Marker development and linkage mapping

In total, 256 STARP primer sets across the genome were designed and tested in the population (Appendix 2.1). Among them, 223 (87.1%) sets revealed polymorphisms and were mapped in the population. For the rest of them, 26 (10.1%) produced monomorphic amplicon between the parents and 7 (2.7%) sets produce no band at all, thus they were not run in the population. In addition, two SNPs were used to develop CAPS markers making it to 225 SNP markers. A total of 313 SSR primer sets (Appendix 2.2) were screened on the two parental isolates 86-124ΔMAT1-1-1 and AR CrossB10ΔMAT1-2-1. Out of them, 96 (30.6%) were polymorphic in the initial screening, but only 75 (23.9%) were mapped in the population and the remaining 21 were discarded because of weak and inconsistent products. The remaining 207 SSR primers sets either produced nonspecific weak bands (129 pairs) or produced no polymorphic bands between the two parental isolates (88). Therefore, a total of 303 markers including 225 SNPs, 75 SSRs, *MAT1-1-1*, *MAT 1-2-1*, and *ToxA* were obtained and used to construct the genetic linkage maps. The resulting genetic map consisted of 16 genetic linkage groups (LG) with a total map size of 4922.84 cM (Fig. 3.1, Table 3.2). LG1 was the largest, spanning 1262.44 cM and containing 66 markers while the LG2.2 was the smallest, having only two markers spanning 6.78 cM in genetic distance. Based on the physical distances in the reference genome of M4, the recombination rate for each linkage group ranged from 5.70 to 9.81 kb/cM with an average value of 7.57 kb/cM across the genome (Table 3.2). The largest genetic distance between the two markers was 40.2 cM. In addition, nine gaps greater than 35 cM were observed among the 16 linkage groups.

The *ToxA* gene was mapped to linkage group 6 that corresponds to the reference genome chromosome 6 as expected. However, segregation of the *ToxA* gene was distorted with 47

isolates with the gene and 65 lacking the gene (Chapter 2). The reason for distorted segregation is unknown. As expected *MAT1-1-1* and *MAT1-2-1* were mapped adjacent to each other separated by 3.6 cM in LG11.3, which one of the linkage group that corresponded to chromosome 10 of M4.

3.4.3. Anchoring of AR CrossB10 contigs to linkage map and genome comparison of AR CrossB10 and M4

Out of 149 contigs of the AR CrossB10 genome assembly, 76 contigs were assigned to the 16 linkage groups (Fig. 3.1. Table 3.4). These 76 contigs represented 37,267,661 bp, which was 92.8% of the total AR CrossB10 assembly. In addition, 26 contigs that did not have any developed markers were assigned to the linkage map based on synteny with the M4 reference genome sequence (Table 3.4). These contigs accounted for 1,875,470 bps making the total anchored sequence to be 39,143,131 which is about 97.5% of the total genome size of 40.1 Mb. The rest of the 47, which had sizes ranging from 1.3 kb to 237.1 kb and accounted for 1,065,086 bps in total could not be placed on the genetic maps either because there were no anchored markers or because there was no similarity (>95%) with the M4 genome.

The genetic linkage map of AR CrossB10 is highly comparable to the optical map of M4 except several noticeable chromosomal structural variations. These included inversions, translocations and fusions and they were identified in seven out of ten chromosomes (Fig. 3.1). Major inversions were also observed in alignments of chromosomes 2 (LG2.1 and LG2.2) and 5 (LG5), whereas minor inversions were observed in alignments of chromosome 1, 3, 5, 7 and 10 (Fig. 3.1).

Whole genome alignments also showed that chromosomes 2 and 9 of M4 were fused in chromosome 2 of AR CrossB10. In addition, chromosome 9 of M4 was split into two portions

where both were inverted in the AR CrossB10 genome and these inverted portions were represented by six AR CrossB10 contigs that were aligned in opposite directions. In addition, several minor intra chromosomal translocations were also observed in chromosomes 1 and 2 (Fig. 3.1). Overall, the majority of contigs of AR CrossB10 that were anchored to the linkage groups were in the same orientation as the M4 optical map.

Whole genome alignments enabled us to examine the genomic regions that were present in M4 but absent in AR CrossB10. Lack of DNA fragments greater than 10 kb had been observed in many chromosomes mostly located between adjacent contigs. The largest fragment that was absent in AR CrossB10 was observed in M4_PB_contig_00004 that spanned over 189.1 kb. In addition, the genomic region that accounted for the *ToxA* gene in M4 that was absent in AR CrossB10 was also identified at M4_PB_contig_00001:5685887-5832316 (*ToxA* gene spans over 5731867 - 5732482) that spanned over 116.1 kb (Table 3.3.). In addition, we were also able to find seven AR CrossB10 contigs that did not align to the M4 genome sequence. These contigs were small contigs that ranged from 1.3 kb to 7.0 kb. These regions were considered unique regions to AR CrossB10.

Table 3.3. List of genomic regions that are present in the M4 genome, but absent in the AR CrossB10 genome

Contig of M4	Start of the region	End of the region	Size of the region absent in AR CrossB10 (bp)
M4_PB_contig_00001	825316	900635	75319
M4_PB_contig_00001	3445740	3534312	88572
M4_PB_contig_00001	2386496	2400994	14498
M4_PB_contig_00001	5685887	5832316	116148
M4_PB_contig_00002	17323	60504	43181
M4_PB_contig_00003	2742357	2789596	47239
M4_PB_contig_00004	1355721	1544842	189121
M4_PB_contig_00004	1800881	1825164	24283
M4_PB_contig_00005	11276	106643	95367
M4_PB_contig_00005	218836	250914	32078
M4_PB_contig_00005	3085510	3156415	70905
M4_PB_contig_00007	307434	317313	9879
M4_PB_contig_00007	2039366	2069733	30367
M4_PB_contig_00009	1	92225	92224
M4_PB_contig_00009	2047363	2126057	78694
M4_PB_contig_00010	1	34979	34978
M4_PB_contig_00010	1759656	1826202	66546
M4_PB_contig_00012	37774	124804	87030
M4_PB_contig_00013	761912	813079	51167

3.4.4. Chromosomal structural variations of progeny and parental isolates of the AR population

SVs were identified for each of the 20 isolates and parental isolates by comparing its genome sequence to the reference genome M4. The configuration file that generated through the first step of the analysis contained information for only 21 out of 22 isolates where BAM information for “AR-48” was not included. However, all the 21 BAM files in the configuration passed the 4 quality tests suggested in Fan et al. (2014).

Table 3.4. AR CrossB10 contigs scaffolded to linkage groups and their alignment to the reference genome M4.

Linkage group (LG)	AR CrossB10 contigs represented by the LG[α]	M4 contigs represented by the LG
LG1	16890, **16889**, 85, 228, 82, 74, 76, 241, **242**, 243, **239**, 49, 244, 112,	2
	12 (1), 149, 126, **16895**, 16896, 247, 65, 112	6
	12 (2), 25, 206	11
LG2.1	38, **16883**, 16894, **16893**, 134, **263**, 213, 89, 146, 252, **230**, 227, **122**	1A
	58, 48, 223, **269**, 128	13
	118, 53, 97, 115, 57, 45, 212 (1)	8
LG2.2	212 (2)	8
LG3	**125**, **24**, 210, 204, **203**, 202,	3
LG4	108, 218, 16, 42	5
LG5	98, 221, **8**, 136, **16887**, 16888, 216, 63, 102, 215, **264**, 51, 111	4
LG6	22, 30, 249	1B
LG7.1	233, 72(1)	15
	72 (2), **54**, 32	14
LG7.2		
	29, **105**, 140, 32	12
LG8.1	235 (1), 94, 232, **231**	7
LG8.2	**69**, **277**, 235 (2)	
LG8.3	110	
LG10	7, 68	9
LG11.1	**259**, **200**, **254**, 238 (1), **47**	10
LG11.2	238 (2)	
LG11.3	77, **119**, 208, **262**	

[α] Contigs in bold font were assembled based on the synteny with M4. If two portions of one contig of AR CrossB10 anchored to two linkage groups or aligned to two adjacent contigs of M4 , in order represent two parts of such contig, contig name/number was followed by 1 or 2 with in the bracket.

A total of 711 deletions, 87 insertions, and 42 inversions were identified with 10 or more reads and confidence values greater than 90. The SVs shown only in progeny but not in parental isolates are listed in Table 3.5. These included 21 deletions, and 19 insertions. Deletions ranged from 235 bp to 5,946 bp in size, and the insertions were relatively constant ranging from 193 – 253 bps.

Table 3.5. List of genomic regions containing putative structural variation that were detected in progeny isolates.

Type of SV[a]	Contig of M4	Start of the SV	End of the SV	Size of the SV (bp)	Confidence Score	Number of_reads[b]	Number of progeny isolates[c]
DEL	M4_PB_contig_00001	6816478	6822361	5946	99	22	10
DEL	M4_PB_contig_00001	4702833	4702947	272	99	22	8
DEL	M4_PB_contig_00002	1034024	1034165	260	99	39	10
DEL	M4_PB_contig_00002	4185133	4185141	267	99	25	10
DEL	M4_PB_contig_00002	5366427	5366930	522	99	14	10
DEL	M4_PB_contig_00003	503848	504175	350	99	11	9
DEL	M4_PB_contig_00004	1249305	1249395	258	99	33	15
DEL	M4_PB_contig_00004	1716544	1716704	246	99	13	7
DEL	M4_PB_contig_00004	1249094	1249395	321	99	12	7
DEL	M4_PB_contig_00006	1314779	1314876	259	99	32	9
DEL	M4_PB_contig_00008	2008568	2011875	3353	99	10	10
DEL	M4_PB_contig_00011	470911	473909	3088	99	16	8
DEL	M4_PB_contig_00011	594042	594134	272	99	14	8
DEL	M4_PB_contig_00011	478931	478964	259	99	10	7
DEL	M4_PB_contig_00013	825266	825409	289	99	24	9
DEL	M4_PB_contig_00017	24068	24071	324	99	33	14
DEL	M4_PB_contig_00017	46106	46159	321	99	22	10
DEL	M4_PB_contig_00017	112147	112399	335	99	21	11
DEL	M4_PB_contig_00017	19578	19635	305	99	18	11
DEL	M4_PB_contig_00017	18952	19025	317	99	17	10
DEL	M4_PB_contig_00017	18170	18237	336	76	13	6
INS	M4_PB_contig_00001	5035379	5035562	248	99	14	7
INS	M4_PB_contig_00001	4458415	4458502	218	99	13	10
INS	M4_PB_contig_00001	321802	321837	244	99	10	3
INS	M4_PB_contig_00001	5697635	5697672	247	99	10	5
INS	M4_PB_contig_00002	388647	388864	218	99	32	16
INS	M4_PB_contig_00002	149794	22632	231	99	22	11
INS	M4_PB_contig_00002	2548946	2549030	213	99	18	9
INS	M4_PB_contig_00002	5598195	5598282	215	99	14	10
INS	M4_PB_contig_00002	4737662	4737694	249	99	11	4
INS	M4_PB_contig_00004	2357128	2357188	239	99	20	8
INS	M4_PB_contig_00004	1470730	1470776	207	99	19	7
INS	M4_PB_contig_00004	1452940	1452966	233	99	16	8
INS	M4_PB_contig_00005	2809180	2809489	230	99	14	11
INS	M4_PB_contig_00005	228712	228720	240	99	12	4
INS	M4_PB_contig_00007	1404160	1404172	243	99	10	5
INS	M4_PB_contig_00009	239092	239141	246	99	13	8
INS	M4_PB_contig_00010	371216	371245	212	99	10	6
INS	M4_PB_contig_00011	1740896	1741008	253	99	79	9
INS	M4_PB_contig_00015	143065	143152	232	99	15	8

[a]Explains the type of the structural variations (SVs), DEL-deletion and INS-insertion. Deletions is defined as a region absent in progeny but present in the reference genome assembly (M4) and parental isolates. Insertion is defined as a region presented in progeny isolate, but absent in reference genome and parental isolates.

[b]Cumilative number of reads that represented the specific SV across the progeny isolates where the SV was present.

[c]Number of progeny isolates that had the SV.

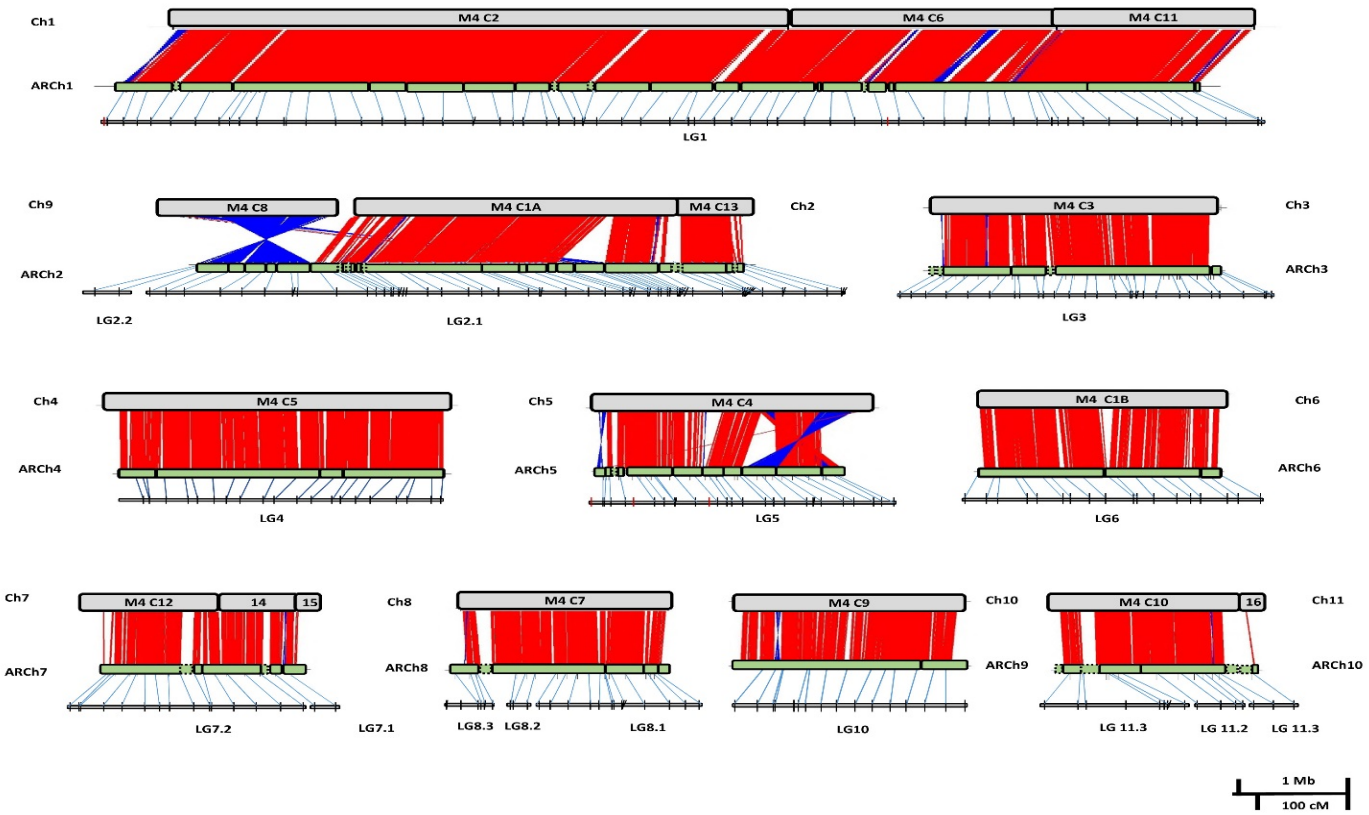


Figure 3.1. Whole genome alignment of M4 and AR CrossB10. The M4 chromosomes (Ch) were based on optical mapping from the genetic linkage group was used to link AR CrossB10 contigs into chromosomes (ARCh). Sequence alignments (connection lines between M4 contigs and AR CrossB10 contigs) were performed with an identity greater than 95% and the length greater than 10 kb. Red lines indicate alignment was in the same orientation and blue lines indicate opposite direction. M4 contigs are represented in grey color boxes and contigs of AR CrossB10 are represented in green color boxes. Green boxes with dotted frame represent the contigs positioned with the use of synteny to M4. The linkage map developed in this study were placed under the AR CrossB10 contigs with connection lines indicating the mapped markers that have blast hits to the corresponding contigs.

3.5. Discussion

Information on genome sequence and genome structure could provide important insights into systematic genetics, evolution and virulence of a fungal pathogen. *P. tritici-repentis* is a devastating fungal pathogen on wheat at a global level and it has a great deal of diversity in genetics and virulence (Lamari Strelkov, 2010). However, high-quality genome sequences were only available for two isolates so far, both of which belong to race 1 (Manning et al. 2013; Moolhuijzen et al. 2018). Here, we provided a high-quality genome sequence for AR CrossB10 which is a new race. Furthermore, the assembled contigs of the genome sequence were also arranged into chromosomes with the information from genetic linkage maps, which allowed us to conduct a thorough genome comparison and identify genome structural variations. The genome sequence and the linkage map for AR CrossB10 will not only help us to identify the virulence genes specific to this race, but also will provide another reference for further *P. tritici-repentis* genomic studies.

In this study, the first genetic linkage map was developed in *P. tritici-repentis*. Although the sexual reproduction of *P. tritici-repentis* can be induced in the lab, development of the genetic linkage map has not been done due to the homothallic nature of the fungus, which makes it difficult to produce segregating fungal populations. Our previous research demonstrated that *P. tritici-repentis* could be converted into heterothallic strains and could be used to develop segregating fungal populations (Ameen et al. 2017). In chapter 2 of my thesis research, I have demonstrated that the *P. tritici-repentis* segregating population can be developed and used in the genetic mapping of the factors involved in Ptr ToxC production. In this chapter, I demonstrated that the developed segregating population can be used in the development of genetic linkage maps for the whole genome. Using the genetic linkage map, we were able to order and connect

the physical contigs that were assembled from genome sequencing. In the previous two reference genomes, the ordering and connecting of physical contigs were done through optical mapping. This technique involves the use of sophisticated devices and computer algorithms; thus it cannot be easily performed in a regular lab (Manning et al. 2013). Saturated genetic linkage maps have been used in the construction of large genome sequence scaffolds in fungal pathogens such as *M. oryzae* and *Z. tritici* (Dean et al. 2005; Goodwin et al. 2011). Our genetic linkage allowed us to order 76 out of 149 AR CrossB10 physical contigs that accounted for 37.3 Mb (92.7% of the genome). In this research, a limited number of SNPs were chosen to develop markers due to the cost and the time involved. More SNP markers within the genome can be developed, which could allow us to anchor more AR CrossB10 physical contigs to the genetic map. I showed here that genetic linkage maps can provide an effective way for constructing genome sequences into large chromosomes in *P. tritici-repentis*.

The genetic map I developed for *P. tritici-repentis* spanned 4922.84 cM in genetic distance. Based on the AR CrossB10 genome, it was estimated that as an average every 7.6 kb could have a cross over (1 cM), which is a relatively high recombination rate. Most of the published fungal genetic linkage mainly accounted for 1,000 to 2,000 cM of total genetic distance with only few greater than 3,000 cM (Reviewed in Foulongne-Oriol, 2012; Lai et al. 2007; Koladia et al. 2017). The genetic map developed for the homothallic fungus *Fusarium graminearum* was also ~1,200 cM long in total genetic distance (Jurgenson et al. 2002; Gale et al. 2005). Nevertheless, two genetic linkage maps recently published for *Z. tritici* had a total genetic distance of 4255.4 cM and 5191.3 cM, respectively (Lendenmann et al. 2014). The recombination rates for these two maps were 7.97 and 7.06 kb/cM, respectively (Lendenmann et al. 2014). However, *Z. tritici* is a heterothallic fungus in nature. The high recombination rate in

Z. tritici and *P. tritici-repentis* might be due to the high level of sequence homology across the genomes, but the exact reason remains unknown. In addition, more bi-parental fungal populations with diverse origins or genetic variability in *P. tritici-repentis* need to be examined for the recombination rate to draw a conclusion.

The AR CrossB10 genome assembly consisted of 149 contigs that accounted for a total genome size of 40.1 Mb, which is only ~800 kb less than the newly published reference quality genome of M4. In addition, the AR CrossB10 genome had a coverage of 65x, which was error corrected with the use of Illumina paired-end reads. Therefore, we had a high quality PacBio genome assembly with statistics highly similar to the reference genome assembly of M4. Genome annotation of AR CrossB10 yielded 13,768 annotated genes, which is only 29 genes less than the M4 reference assembly and variation in number could be due to the presence/absence variation of the isolates and variation in training sets data used. Furthermore, our annotation was not supported by RNA-seq alignments unlike the M4 annotation. Therefore, RNA-seq evidence can increase the confidence of the annotation. However, BUSCO analysis using 1,315 conserved ascomycete orthologs showed a 97.4% completed annotation which is similar to the annotated genomes of *P. tritici-repentis* (Pt-1c-BFP), *P. nodorum*, *P. teres* f. *teres*, and *Leptosphaeria maculans* (Wyatt et al. 2017). Out of the total annotated genes 1,221 were predicted to encode secreted proteins, of which 312 were considered as genes encoding for effectors. This value is considerably high compared to the other *P. tritici-repentis* isolates annotated which ranged from 179 to 260 (Moolhuijzen et al. 2018). In AR CrossB10, the total repeat content was 18.85%, which was similar to that of Pt-1c-BFP (16%) and M4 (15%) (Manning et al. 2013; Moolhuijzen et al. 2018). Even though repeat content was similar to *Z. tritici* (21.2%), it is less compared to *P. tritici-repentis*, *P. teres* f. *teres* (32%) and considerably

higher compared to the close relatives of *P. nodorum* (4.5%) (Goodwin et al. 2011; Wyatt et al. 2016; Syme et al. 2016).

Whole genome alignment analysis carried out using NUCmer showed that 34.7 Mb (86.5%) of the AR CrossB10 genome showed 99.8% similarity to the M4 genome assembly (Moolhuijzen et al. 2018) which provides further evidence for lack of polymorphism among *P. tritici-repentis* isolates as suggested by Manning et al. (2013). In addition, some of the small contigs that were present in the AR CrossB10 were completely absent. These regions can represent genes that can be involved in virulence and make them different to other *P. tritici-repentis* races (Ali et al. 2010). In contrast, the AR CrossB10 genome lacked ~800 kb in sequence including regions within and between contigs compared to that of M4. However, all these regions should be confirmed by running PCR under laboratory conditions.

Major chromosomal variations were observed among the chromosomes of the two genomes. The largest structural variation was observed between chromosome 2 where ARCh2 consists of a fusion between Ch2 and Ch9 of the M4 genome. This could be due to the lack of a telomere because both chromosomes seem to lack telomeric tandem repeats of TTAGGG/CCCTAA in any of the contigs anchored to that chromosome (Murane 2006). A similar phenomenon was observed for the whole genome alignment between Pt-1c-BFP and M4 where M4 chromosome 10 was a result of a fusion between BFP chromosomes 9 and 10 (Moolhuijzen et al. 2018). Therefore, chromosome fusion could be one of the reasons for chromosome length and number polymorphism observed in previous studies (Aboukhaddour et al. 2009). In addition to chromosomal fusion, Ch9 fused as two large fragments with inversions. Between these two sections an insertion of large section of Ch2 can be seen. Structural variation analysis from BreakDancer showed several deletions of the terminal contigs of the two

fragments of Ch2. Therefore, the fragmentation could be due to chromosomal degradation through multiple breakage-fusion-bridge (BFB) cycles occurring during meiosis (Croll et al. 2013).

ARCh1, ARCh3, ARCh7, ARCh8, ARCh9 and ARCh10 showed structural variations such as inversions between two genomes. Inversions occurred at ARCh1, 3, 5 and 10 coincided with the LTR elements whereas others were recognized in regions with repeats that were not classified. Involvement of repetitive elements for the structural variations such as inversions were observed in the reference assembly of M4 as well as other fungal pathogens such as *Verticillium dahliae* (Faino et al. 2016). Some of the inversions such as the one in ARCh7 was observed on a break point between two contigs.

The SV analysis also showed 42 SVs that were novel and only found in progeny isolates. These putative variations occurred during sexual reproduction. Even though most of these SVs are small, they can actively contribute to CLPs. In addition, 262 SVs that were observed for two parental isolates were not observed in progeny isolates. However, the confidence level of these variations was low. Therefore, they could be detected due to sequencing errors. Therefore, more sequencing depth is required to increase the confidence level of such signals. In addition, use of pulse field gel electrophoresis can be performed on progeny isolates to identify if CLP and CNP occurs during sexual reproduction. However, these putative analyses show that sexual reproduction between isolates of *P. tritici-repentis* can highly increase the genetic diversity of the isolates.

3.6. References

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

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

Ameen, G., Kariyawasam, G., Shi, G., Friesen, T. L., Faris, J. D., Ali, S., Rasmussen, J. B. and Liu, Z. (2017). Molecular manipulation of the mating-type system and development of a new approach for characterizing pathogen virulence in *Pyrenophora tritici-repentis*. Fungal Genet. Biol.109:16-25

Bao, W., Kojima, K.K. and Kohany, O., (2015). Repbase Update, a database of repetitive elements in eukaryotic genomes. Mobile DNA. 6:11.

Bowden, R.L. and Leslie, J.F., (1999). Sexual recombination in *Gibberella zeae*. Phytopathol 89:182–188

Broggini, G.A., Bus, V.G., Parravicini, G., Kumar, S., Groenwold, R. and Gessler, C., (2011). Genetic mapping of 14 avirulence genes in an EU-B04 x 1639 progeny of *Venturia inaequalis*. Fungal Genet Biol 48:166–176

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

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

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

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

Croll, D., Zala, M. and McDonald, B.A., (2013). Breakage-fusion-bridge cycles and large insertions contribute to the rapid evolution of accessory chromosomes in a fungal pathogen. PLoS genetics, 9:p.e1003567.

Cumagun, C.J.R., Parzies, H.K. and Miedaner, T., (2007). Genetic variation and segregation of DNA polymorphisms in *Gibberella zeae* detected with AFLP and RAPD markers. Asian J Plant Sci 6:1174–1181

Delcher, A.L., Salzberg, S.L. and Phillippy, A.M., (2003). Using MUMmer to identify similar regions in large sequence sets. Curr Protoc Bioinformatics: 10-13.

Dean, R.A., Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., Orbach, M.J., Thon, M., Kulkarni, R., Xu, J.R., Pan, H. and Read, N.D., 2005. The genome sequence of the rice blast fungus Magnaporthe grisea. Nature 434: 980-986.

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

Faino, L., Seidl, M.F., Shi-Kunne, X., Pauper, M., van den Berg, G.C., Wittenberg, A.H. and Thomma, B.P., (2016). Transposons passively and actively contribute to evolution of the two-speed genome of a fungal pathogen. Genome research

Fan, X., Abbott, T.E., Larson, D. and Chen, K., (2014). BreakDancer: Identification of Genomic Structural Variation from Paired-End Read Mapping. Current protocols in bioinformatics. 45:15-1-11.

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

Faris, J., D., Liu, Z., and Xu, S., S. (2013). Genetics of tan spot resistance in wheat. Theor. Appl. Genet. 29:1–21

Foulongne-Oriol, M., 2012. Genetic linkage mapping in fungi: current state, applications, and future trends. Appl. Microbiol. Biotechnol. 95:891-904.

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

Gale, L.R., Bryant, J.D., Calvo, S., Giese, H., Katan, T., O'Donnell, K., Suga, H., Taga, M., Usgaard, T.R., Ward, T.J. and Kistler, H.C., (2005). Chromosome complement of the fungal plant pathogen *Fusarium graminearum* based on genetic and physical mapping and cytological observations. Genetics, 171: 985-1001.

Goodwin, S.B., M'barek, S.B., Dhillon, B., Wittenberg, A.H., Crane, C.F., Hane, J.K., Foster, A.J., Van der Lee, T.A., Grimwood, J., Aerts, A. and Antoniw, J., (2011). Finished genome of the fungal wheat pathogen Mycosphaerella graminicola reveals dispensome structure, chromosome plasticity, and stealth pathogenesis. PLoS Genetics. 7: p.e1002070.

Guy, L., Roat Kultima, J. and Andersson, S.G. (2010). GenoPlotR: comparative gene and genome visualization in R. Bioinformatics. 26:2334-2335.

Heffelfinger, C., Fragoso, C.A. and Lorieux, M. (2017). Constructing linkage maps in the genomics era with MapDisto 2.0. Bioinformatics. 33:2224-2225.

Holt, C. and Yandell, M. (2011). MAKER2: an annotation pipeline and genome-database management tool for second-generation genome projects. BMC bioinformatics. 12:491.

Hosford, R., M., Jr. (1982). Tan spot-developing knowledge 1902–1981, virulent races and wheat differentials, methodology, rating systems, other leaf diseases, and literature. In: Tan Spot of Wheat and Related Diseases Workshop, Fargo, North Dakota, Agricultural Experimental Station, North Dakota State University, (pp 1–24)

Hussain, W., Baenziger, P.S., Belamkar, V., Guttieri, M.J., Venegas, J.P., Easterly, A., Sallam, A. and Poland, J., (2017). Genotyping-by-sequencing derived high-density linkage map and its application to QTL mapping of flag leaf traits in bread wheat. Scientific reports, 7: p.16394.

Jurgenson, J.E., Bowden, R.L., Zeller, K.A., Leslie, J.F., Alexander, N.J. and Plattner, R.D., (2002). A genetic map of *Gibberella zeae* (*Fusarium graminearum*). Genetics 160:1451–1460

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

Kaye, C., Milazzo, J., Rozenfeld, S., Lebrun, M.H. and Tharreau, D., (2003). The development of simple sequence repeat markers for Magnaporthe grisea and their integration into an established genetic linkage map. Fungal Genet. Biol.40: 207-214.

Kema, G.H., Goodwin, S.B., Hamza, S., Verstappen, E.C., Cavaletto, J.R., Van der Lee, T.A., de Weerdt, M., Bonants, P.J. and Waalwijk, C., (2002). A combined amplified fragment length polymorphism and randomly amplified polymorphism DNA genetic kinkage map of *Mycosphaerella graminicola*, the septoria tritici leaf blotch pathogen of wheat. Genet. 161:1497–1505

Koladia, V.M., Richards, J.K., Wyatt, N.A., Faris, J.D., Brueggeman, R.S. and Friesen, T.L., (2017). Genetic analysis of virulence in the *Pyrenophora teres* f. *teres* population BB25× FGOH04Ptt-21. Fungal Genetics and Biology. 107:12-19.

Koren, S., Walenz, B.P., Berlin, K., Miller, J.R. and Phillippy, A.M., (2017). Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. BioRxiv, 071282.

Lai, Z., Faris, J.D., Weiland, J.J., Steffenson, B.J. and Friesen, T.L., (2007). Genetic mapping of *Pyrenophora teres* f. *teres* genes conferring avirulence on barley. Fungal Genet Biol 44:323–329

Lamari, L., and Strelkov, S., E. (2010). Minireview/Minisynthèse The wheat/*Pyrenophora tritici-repentis* interaction: progress towards an understanding of tan spot disease. Can. J. Plant Pathol. 32: 4-10.

Larraya, L.M., Idareta, E., Arana, D., Ritter, E., Pisabarro, A.G. and Ramírez, L., (2002). Quantitative trait loci controlling vegetative growth rate in the edible basidiomycete Pleurotus ostreatus. Appl Environ Microbiol 68:1109–1114

Lendenmann, M.H., Croll, D., Stewart, E.L. and McDonald, B.A., (2014). Quantitative trait locus mapping of melanization in the plant pathogenic fungus *Zymoseptoria tritici*. G3: 2519-2533.

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

Liu, Z.H., Anderson, J.A., Hu, J., Friesen, T.L., Rasmussen, J.B. and Faris, J.D., (2005). A wheat intervarietal genetic linkage map based on microsatellite and target region amplified polymorphism markers and its utility for detecting quantitative trait loci. Theor. Appl. Genet., 111:782-794.

Liu, Z. and Friesen, T.L., (2012). Polyethylene glycol (PEG)-mediated transformation in filamentous fungal pathogens. In *Plant Fungal Pathogens* (pp. 365-375). Humana Press.

Manning, V., A., Pandelova, I., Dhillon, B., Wilhelm, L., J., Goodwin, S., B., Berlin, A., M., Figueroa, M., Freitag, M., Hane, J., K., Henrissat, B., Holman, W., H., Kodira, C., D., Martin, J., Oliver, R., P., Robbertse, B., Schackwitz, W., Schwartz, D., C., Spatafora, J., W., Turgeon, B., G., Yandava, C., Young, S., Zhou, S., Zeng, Q., Grigoriev, I., V., Ma, L., J., and Ciuffetti, L., M. (2013). Comparative genomics of a plant-pathogenic fungus, *Pyrenophora tritici-repentis*, reveals transduplication and the impact of repeat elements on pathogenicity and population divergence. G3 (Bethesda) 3:41–63.

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

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

McClintock, B., (1941). The stability of broken ends of chromosomes in *Zea mays*. Genetics. 26:234-281

Moolhuijzen, P., See, P., T., Hane, J., K., Shi, G., Liu, Z., Oliver, R., P., and Moffat, C., S. (2018). Comparative genomics of the wheat fungal pathogen *Pyrenophora tritici-repentis* reveals chromosomal variations and genome plasticity. BMC genomics. 19: 279.

Murnane, J.P., (2006). Telomeres and chromosome instability. DNA repair. 5:1082-1092.

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

Petersen, T.N., Brunak, S., von Heijne, G. and Nielsen, H., (2011). SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat. methods 8:785.

Rees, R., G., Platz, G., J., and Mayer, R., J. (1982). Yield losses in wheat from yellow spot: comparison of estimates derived from single tillers and plots. Aust. J. Agric. Res. 33:899–908

Richards, J.K., Friesen, T.L. and Brueggeman, R.S., (2017). Association mapping utilizing diverse barley lines reveals net form net blotch seedling resistance/susceptibility loci. Theor. Appl. Genet. 130:915-927.

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

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

Sperschneider, J., Gardiner, D.M., Dodds, P.N., Tini, F., Covarelli, L., Singh, K.B., Manners, J.M. and Taylor, J.M., (2016). EffectorP: predicting fungal effector proteins from secretomes using machine learning. New Phytologist, 210:743-761.

Smit, A.F.A., Hubley, R. and Green, P., (2016). RepeatMasker Open-4.0. 2015. Google Scholar.

Syme, R.A., Tan, K.C., Hane, J.K., Dodhia, K., Stoll, T., Hastie, M., Furuki, E., Ellwood, S.R., Williams, A.H., Tan, Y.F. and Testa, A.C., (2016). Comprehensive annotation of the *Parastagonospora nodorum* reference genome using next-generation genomics, transcriptomics and proteogenomics. PloS one, 11: p.e0147221.

Ter-Hovhannisyan, V., Lomsadze, A., Chernoff, Y.O. and Borodovsky, M., (2008). Gene prediction in novel fungal genomes using an ab initio algorithm with unsupervised training. Genome research. pp-081612.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M. and Rozen, S.G., (2012). Primer3—new capabilities and interfaces. Nuc. Acid. Res. 40:115-115.

Völker, M., Backström, N., Skinner, B.M., Langley, E.J., Bunzey, S.K., Ellegren, H. and Griffin, D.K., (2010). Copy number variation, chromosome rearrangement, and their association with recombination during avian evolution. Genome research.

Wen, A., Jayawardana, M., Fiedler, J., Sapkota, S., Shi, G., Peng, Z., Liu, S., White, F.F., Bogdanove, A.J., Li, X. and Liu, Z., (2018). Genetic mapping of a major gene in triticale conferring resistance to bacterial leaf streak. Theor. Appl. Genet. 131:649-658.

Walker, B.J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C.A., Zeng, Q., Wortman, J., Young, S.K. and Earl, A.M., (2014). Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PloS one, 9: p.e112963.

Wu, Q.H., Chen, Y.X., Zhou, S.H., Fu, L., Chen, J.J., Xiao, Y., Zhang, D., Ouyang, S.H., Zhao, X.J., Cui, Y. and Zhang, D.Y., (2015). High-density genetic linkage map construction and QTL mapping of grain shape and size in the wheat population Yanda1817× Beinong6. PloS one, 10.p.e0118144.

Xu, J., Kerrigan, R.W., Horgen, P.A. and Anderson, J.B., (1993). Localization of the Mating type gene in Agaricus bisporus. Appl Environ Microbiol 59:3044–3049

Xu, X., Roberts, T., Barbara, D., Harvey, N.G., Gao, L. and Sargent, D.J., (2009). A genetic linkage map of *Venturia inaequalis*, the causal agent of apple scab. BMC Res Notes, 2:163.

Zhong, Z., Marcel, T.C., Hartmann, F.E., Ma, X., Plissonneau, C., Zala, M., Ducasse, A., Confais, J., Compain, J., Lapalu, N. and Amselem, J., (2017). A small secreted protein in *Zymoseptoria tritici* is responsible for avirulence on wheat cultivars carrying the Stb6 resistance gene. New Phytol. 214:.619-631.

CHAPTER 4. IDENTIFICATION OF QUANTITATIVE TRAIT LOCI CONFFERRING RESISTANCE TO TAN SPOT IN A BI-PARENTAL POPULATION DERIVED FROM TWO NEBRASKA HARD RED WINTER WHEAT CULTIVAR

4.1. Abstract

Tan spot, caused by *Pyrenophora tritici-repentis*, is a destructive foliar disease in all types of cultivated wheat worldwide. Genetics of tan spot resistance in wheat is complex, involving insensitivity to fungal-produced necrotrophic effectors (NEs), major resistance genes and quantitative trait loci (QTL) conferring race-nonspecific and race-specific resistance. The Nebraska hard red winter wheat (HRWW) cultivar 'Wesley' is insensitive to Ptr ToxA and highly resistant to multiple *P. tritici-repentis* races, but the genetics of resistance in this cultivar is unknown. In this study, we used a recombinant inbred line (RIL) population derived from a cross between Wesley and another Nebraska cultivar 'Harry' (Ptr ToxA sensitive and highly susceptible) to identify QTL associated with reaction to tan spot caused by multiple races/isolates. Sensitivity to Ptr ToxA conferred by the *Tsn1* gene was mapped to chromosome 5B as expected. The *Tsn1* locus was a major susceptibility QTL for the race 1 and race 2 isolates, but not for the race 2 isolate with the *ToxA* gene deleted. A second major susceptibility QTL was identified for all the Ptr ToxC-producing isolates and located to the distal end of the chromosome 1A, which likely corresponds to the *Tsc1* locus. Three additional QTL with minor effects were identified on chromosomes 7A, 7B and 7D. This work indicates that both Ptr ToxA-*Tsn1* and Ptr ToxC-*Tsc1* interactions are important for tan spot development in winter wheat, and Wesley is highly resistant largely due to the absence of the two tan spot sensitivity genes.

4.2. Introduction

Tan or yellow spot, caused by *Pyrenophora tritici-repentis*, can occur on all cultivated wheat crops including bread wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L.). The foliar symptom of the disease is a characteristic tan-colored and elliptical necrotic lesion, often with a yellow halo (Friskop and Liu 2016). The fungal pathogen overwinters on wheat residues, and thus it is believed that a wide adoption of no or reduced tillage production systems has increased disease incidence and made tan spot one of the most important diseases in most wheat-growing regions (Hosford 1982; Murry and Brenan 2009; Faris et al. 2013). Tan spot has been one of the most common diseases in North Dakota and surrounding areas where the majority of US hard red spring and durum wheat are produced (Friskop and Liu 2016).

Yield losses due to tan spot can reach up to 50% on highly susceptible cultivars when environmental conditions are favorable (Rees et al. 1982; Lamari and Bernier 1989). The disease can also diminish grain quality by causing pink to red discoloration of the grain, known as red smudge (Schilder and Bergstorm 1994). Disease management strategies for wheat tan spot include crop rotation, residue removal, and timely application of protective and systemic fungicides (Friskop and Liu 2016). Developing and deploying resistant cultivars is the most desirable way to control tan spot of wheat. However, the majority of wheat cultivars in North Dakota and surrounding areas are susceptible (Singh et al. 2006a; Liu et al. 2015; Friskop and Liu 2016). Breeding for tan spot resistance has been difficult due to the diverse and complex nature of pathogen virulence, host resistance and host-pathogen interactions.

P. tritici-repentis is known to produce three necrotrophic effectors (NE), namely Ptr ToxA, Ptr ToxB and Ptr ToxC, which interact with their wheat sensitivity genes to induce necrosis or chlorosis disease symptoms (Ciuffetti et al. 2010). The global *P. tritici-repentis*

isolates have been grouped into eight races according to their ability to produce combinations of the three NEs (Strelkov and Lamari 2003). However, new evidence has strongly suggested the existence of additional races (Ali et al. 2010; Mereno et al. 2015) as well as the presence of additional NEs in the current races (Friesen et al. 2002; Moffat et al. 2014). In addition, Ptr ToxA has been shown to have an epistatic effect on other unidentified NEs (Manning and Ciuffetti 2015; See et al. 2018).

Wheat sensitivity genes for the three *P. tritici-repentis* NEs have been identified and mapped to wheat chromosome arms, which are *Tsn1* on 5BL for Ptr ToxA (Faris et al. 1996), *Tsc1* on 1AS for Ptr ToxC (Effertz et al. 2002), and *Tsc2* on 2BS for Ptr ToxB (Friesen and Faris 2004; Abeysekara et al. 2009). Among them, *Tsn1* has been isolated from wheat and shown to be a NBS-LRR, resistance-like gene (Faris et al. 2010). Because each NE and host sensitivity gene interaction can lead to susceptibility/disease, and their effects can be additive, resistance is often seen as the lack of sensitivity genes, and removal of these sensitivity genes from wheat cultivars could reduce the levels of susceptibility (Liu et al. 2017). However, the effect of each pair of NE and host sensitivity gene interaction on disease can be highly variable, depending on the host genetic background and the isolate used (Faris et al. 2012; Virdi et al. 2016).

In addition to the three major sensitivity (susceptibility) genes, several studies have identified qualitative and recessive resistance genes against specific races/isolates of *P. tritici-repentis*, including *tsr2* on 3BL (Singh et al. 2006b), *tsr3* on 3DL (Tadesse et al. 2006a), *tsr4* on 3AL (Tadesse et al. 2006b) and *tsr5* on 3BL (Singh et al. 2008). Furthermore, many additional QTL conferring resistance/susceptibility to tan spot have also been identified using biparental and association mapping studies (Faris et al. 2013 review; Virdi et al. 2016; Kariyawasam et al. 2016; Liu et al. 2015, 2017). It is interesting that some of the identified QTL are race-

nonspecific, conferring resistance to multiple or all races (Faris and Friesen 2005; Chu et al. 2008; Kariyawasam et al. 2016).

Hard red winter wheat (HRWW) accounts for 3 to 10% of total wheat production in North Dakota (North Dakota Wheat Commission, www.ndwheat.com, accessed on July 5th 2018). Although growing HRWW in North Dakota is risky because of harsh winter conditions, HRWW has gained an increased interest due to its higher yield and the ability to spread seasonal workloads. 'Jerry', developed by North Dakota State University and the USDA-ARS and released in 2001 (Peel et al. 2004), has been the leading HRWW cultivar in the state. However, Jerry is highly susceptible to tan spot (Liu et al. 2015). The HRWW cultivar 'Wesley' from Nebraska has demonstrated resistance to multiple races of *P. tritici-repentis* (Liu et al. 2015). To better utilize Wesley in breeding programs, resistance in this cultivar needed to be characterized. The objectives of this study were to map Wesley's resistance to multiple *Ptr* races of tan spot using a recombinant inbred line (RIL) population derived from the cross between Harry and Wesley (Hussain et al. 2017), and to investigate the role of NE-wheat sensitivity gene interactions in the development of tan spot disease in winter wheat.

4.3. Materials and methods

4.3.1. Plant materials

The population derived from Harry/Wesley, hereafter referred to as HW population, consisted of one hundred and ninety-four recombinant inbred lines (RILs). Both Harry and Wesley are HRWW cultivars developed by Nebraska Agricultural Experiment Station in collaboration with the USDA-ARS. The HW population was originally developed for the mapping of drought tolerance (Hussain et al. 2017). In a previous study, we found that Wesley is insensitive to Ptr ToxA and highly resistant to major *P. tritici-repentis* races while Harry is

sensitive and highly susceptible (Liu et al. 2015). The two parental lines and all the RILs were evaluated for disease resistance using multiple races/isolates and NE infiltrations. In addition, four tan spot differential lines: Salamouni (insensitive to all three NEs), Glenlea (Ptr ToxA sensitive), 6B365 (Ptr ToxC sensitive) and 6B662 (Ptr ToxB sensitive) were also included making a total of 200 entries for each evaluation. Planting and growing the seedling plants followed the same protocols described in Liu et al. (2015). Briefly, seeds were sown in super-cell containers (Stuewe & Sons, Inc., Corvallis, OR) that were filled with Sunshine SB100 soil (Sun Grow Horticulture, Bellenvue, WA) and placed on RL98 trays (Stuewe & Sons, Inc., Corvallis, OR). The cultivar Jerry, highly susceptible to tan spot, was planted along the borders of the each RL98 rack to minimize the potential edge effect. The disease evaluations and NE infiltrations were conducted on the plants at the two to three leaf seedling stage, which required approximately two weeks of growth under temperatures ranging from 20 to 25 °C after seeds were sown. Three biological replications were performed with a randomized complete block design (RCBD) for each isolate and NE evaluation.

4.3.2. Fungal inoculations and NE infiltrations

Five *P. tritici-repentis* isolates were tested individually on the HW population, including Pti2, 86-124, 331-9, DW5 and AR CrossB10, which represented races 1, 2, 3, 5 and new race, respectively. These isolates were classified as different races based on the production of NEs or virulence on the differential lines (Table 1). The isolates 86-124 (race 2), 331-9 (race 3), and DW5 (race 5) each produce a single, known NE (Ptr ToxA, Ptr ToxC and Ptr ToxB, respectively). The isolate Pti2 (race 1) produces both Ptr ToxA and Ptr ToxC. AR CrossB10 was characterized as a new race because it produces no Ptr ToxA but is virulent on Glenlea (Ptr ToxA sensitive) (Ali et al. 2010). However, this isolate produces Ptr ToxC (Kariyawasam et al.

2016). The fungal strain 86-124ΔToxA was genetically modified from 86-124 (race 2) through deletion of the *ToxA* gene, thus producing no Ptr ToxA (Kariyawasam et al. 2016). Strain 86-124ΔToxA was used to test whether the effect of the 5B QTL was due to a Ptr ToxA-*Tsn1* interaction.

Fungal culturing and inoculum preparation followed the procedure described in Lamari and Bernier (1989). Briefly, the fungus was grown in dark for five days followed by the sporulation treatments. The conidiospores were harvested from the plates by adding sterilized distilled water to the plates and gently scrapping the surface of the fungal cultures. The concentration of the inoculum was defined by spore counting under microscope and adjusted to approximately 3,000 spores per mL and Tween-20 was added at a rate of two drops per 100 mL of the spore suspension before spraying inoculum. Plants were inoculated with the spore suspension using a paint sprayer (Husky; Home Depot) that was connected to an air pump with a pressure set at 1.0 bar. Inocula were applied till the leaves of all the plants were uniformly covered with water drops. Inoculated plants were transferred to a mist chamber with a 100% relative humidity and incubated for 24 h at 21 °C. Then, they were moved to and grown in a growth chamber with 12-hour photoperiod at 21 °C for 7 days. Disease severity was rated using a lesion type-based scale from 1 to 5 where 1 is highly resistant and 5 is highly susceptible (Lamari and Bernier 1989). An intermediate score was given if two types of reactions were observed. The disease score lower than 2.5 was considered to be resistant.

The HW population was also evaluated for reaction to Ptr ToxA and Ptr ToxB, which were produced from genetically modified *Pichia pastoris* yeast strain X33 expressing the individual NE gene (Liu et al. 2009; Abeysekara et al. 2010). The yeast *P. pastoris* strains were cultured for 48 h at 30 °C and the culture filtrates were harvested by centrifuging the yeast cells.

Approximately 20 µl of the culture filtrate was infiltrated into the fully expanded secondary leaf by using a 1 ml syringe without the needle. The infiltrated region was marked with a felt pen, and infiltrated plants were kept in a growth chamber at 21 °C with 12 h photoperiod. Reactions to NE were scored on the 5th day as 1 (sensitive, necrosis or chlorosis developed on the marked area) or 0 (insensitive, no reaction developed on the marked area). The scored data were transformed into marker data which were used for mapping the sensitivity locus.

4.3.3. Statistical analysis and QTL mapping

Normality of the disease data for each isolate was evaluated using the Shapiro-Wilk test in PROC UNIVARIATE in SAS 9.4 Software (SAS Institute, 2016). Disease data from different replicates were tested for homogeneity using Bartlett's chi-squared test (Snedecor and Cochran 1989) if the data fitted a normal distribution, or by Levene's test (Levene 1960) if the data did not fit a normal distribution. Analyses of variance were conducted using PROC GLM (SAS Institute, 2016). The data from homogeneous replications were combined to compute disease means for each RIL, which were then used in QTL analysis.

The genetic linkage map of the HW population contained 3,641 SNP markers from genotyping by sequencing (GBS) and covered all 21 wheat chromosomes with a total genetic distance of 1,959 cM and a marker density of 1.8 cM per marker (Hussain et al. 2017). For the QTL analysis, the linkage maps were reconstructed to remove most co-segregating markers and some tightly linked markers without affecting the quality of the maps using MapDisto 1.7.7 (Lorieux 2012). The resulting map consisted of 2,749 markers that spanned 1,911.84 cM with marker density at 1.43 cM. Because the population segregated for reaction to Ptr ToxA, sensitivity to Ptr ToxA was also mapped as a qualitative trait in the previous linkage map using MapDisto (Lorieux 2012). QTL mapping was conducted using QGene 4.4.0 (Joehanes and

Nelson 2008). Simple interval mapping (SIM) was used initially to identify the genomic region associated with tan spot reaction and to quantify the disease variations explained by the QTL (R^2). Composite interval mapping (CIM) was then performed to define the genomic locations. A permutation test with 1,000 iterations resulted in a LOD threshold of 4.2 for an experiment-wise significance level of 0.05.

4.4. Results

4.4.1. Reactions of the parental lines to fungal inoculations and NE infiltrations

Wesley exhibited black to brown colored, small size lesions on the secondary leaves for all the isolates tested, and it had average disease ratings ranging from 1.33 to 2.00 (Fig. 1, Table 1), indicating high levels of resistance. In contrast, Harry developed large necrotic lesions or extensive chlorosis on the secondary leaves and its disease rating ranged from 4.00 to 4.50, which was highly susceptible (Fig. 4.1, Table 4.1). For Ptr ToxA infiltration, Wesley was insensitive while Harry was sensitive (Fig. 4.1). Although the differential line 6B662 had a sensitive reaction to Ptr ToxB, neither Wesley nor Harry was sensitive to Ptr ToxB (Fig. 4.1). Extensive chlorosis developed on the leaves of Harry when inoculated with Pti2, 331-9 and AR CrossB10, but did not develop when inoculated with 86-124, 86-124ΔToxA and DW5 (Fig. 4.1). As Pti2, 331-9 and AR CrossB10 all produce Ptr ToxC, and extensive chlorosis is characteristic of the Ptr ToxC-*Tsc1* interaction, Harry must carry *Tsc1* conferring sensitivity to Ptr ToxC.

4.4.2. Reactions of the HW population to Ptr ToxA infiltration and mapping of sensitivity to Ptr ToxA

The HW population segregated for reaction to Ptr ToxA as 92 sensitive to 100 insensitive, which fits a 1:1 ratio (χ^2=0.33, P=0.56). Sensitivity to Ptr ToxA was mapped to

chromosome 5B as expected. The newly constructed chromosome 5B map was 135.2 cM in length and *Tsn1* was located at 47.1 cM, between markers HWGBS3693 and HWGBS3680.

4.4.3. Reaction of the HW population to fungal inoculations

The HW population segregated for reaction to tan spot caused by all the isolates tested. The mean disease severity for the whole population ranged from 2.74 (isolate DW5) to 3.20 (isolate 331-9) (Table 4.1, Fig. 4.2). No obvious transgressive segregation was observed. For all the isolates tested, the majority of RILs had intermediate reactions and only a few RILs had reactions similar to the resistant or susceptible parents (Fig. 4.2). The fungal strain 86-124ΔToxA produces no known NE, but still caused disease on Harry and susceptible RILs strongly indicating the presence of an unidentified NE(s) or another virulence factor(s) (Fig 4.2). Normality tests rejected a normal distribution of the disease reaction to all the isolates except the race1 isolate Pti2 (P=0.07) and race5 isolate DW5 (P=0.26).

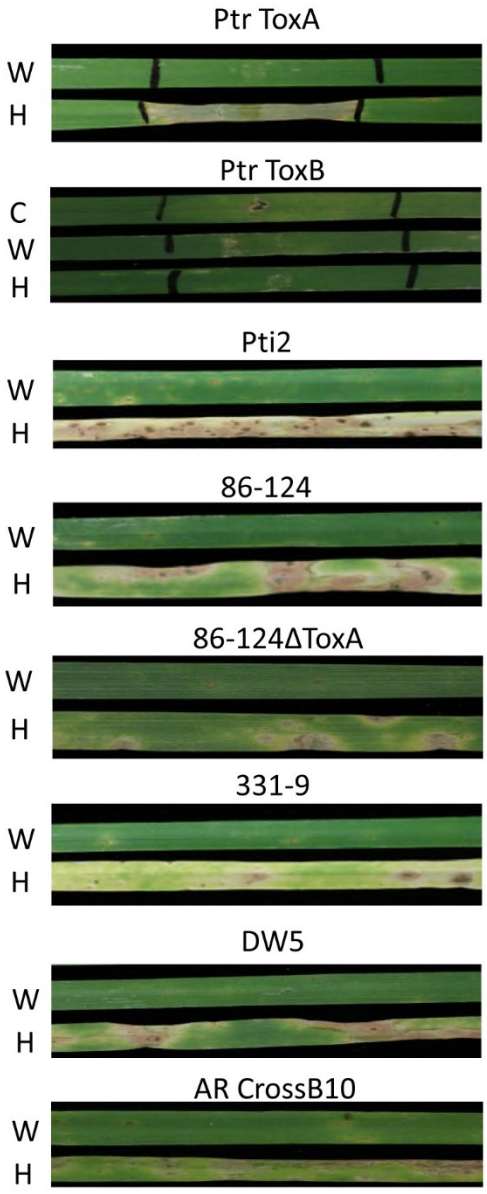


Figure 4.1. Reaction of the parental lines to different *Pyrenophora tritici-repentis* race/isolate inoculations and necrotrophic effector infiltrations. The *P. tritici-repentis* races/isolates included Pti2 (race 1), 86-124 (race 2), 86-124ΔToxA, 331-9 (race 3), DW5 (race 5) and AR CrossB10 (new race) and *P. tritici-repentis.* The NEs included Ptr ToxA and Ptr ToxB. W: Wesley, H: Harry. C: control 6B662 (for the Ptr ToxB infiltration only).

Table 4.1. Reaction of the parental lines and the HW population to *Pyrenphora tritici-repentis* races/isolates.

Isolate (race)[a]	NE produced[b]	Harry[c]	Wesley[c]	HW population mean	HW population range
Pti2 (race 1)	Ptr ToxA, Ptr ToxC	4.00	1.67	2.98	1.33-4.33
86-124 (race 2)	Ptr ToxA	4.00	1.33	3.00	1.33-4.17
86-124ΔToxA	-	4.00	2.00	2.76	1.17-4.17
331-9 (race 3)	Ptr ToxC	4.50	2.00	3.20	1.83-4.67
DW5 (race 5)	Ptr ToxB	4.00	1.33	2.74	1.17-4.17
AR CrossB10 (New)	Ptr ToxC	4.00	2.00	3.13	1.83-4.33

[a]Six isolates representing different *P. tritici-repentis* races were used to evaluate the HW population and parental lines. Fungal strain 86-124ΔToxA derives from 86-124 but lacks the *ToxA* gene (Kariyawasam et al. 2016).
[b]The Ptr races are known to produce different necrotrophic effectors (NEs): Ptr ToxA, Ptr ToxB and Ptr ToxC. 86-124ΔToxA does not produce Ptr ToxA, but it might produce other unknown effectors.
[c]Disease was scored using a 1 to 5 scale with 1 being resistant and 5 being susceptible. Means of three replicates are given.

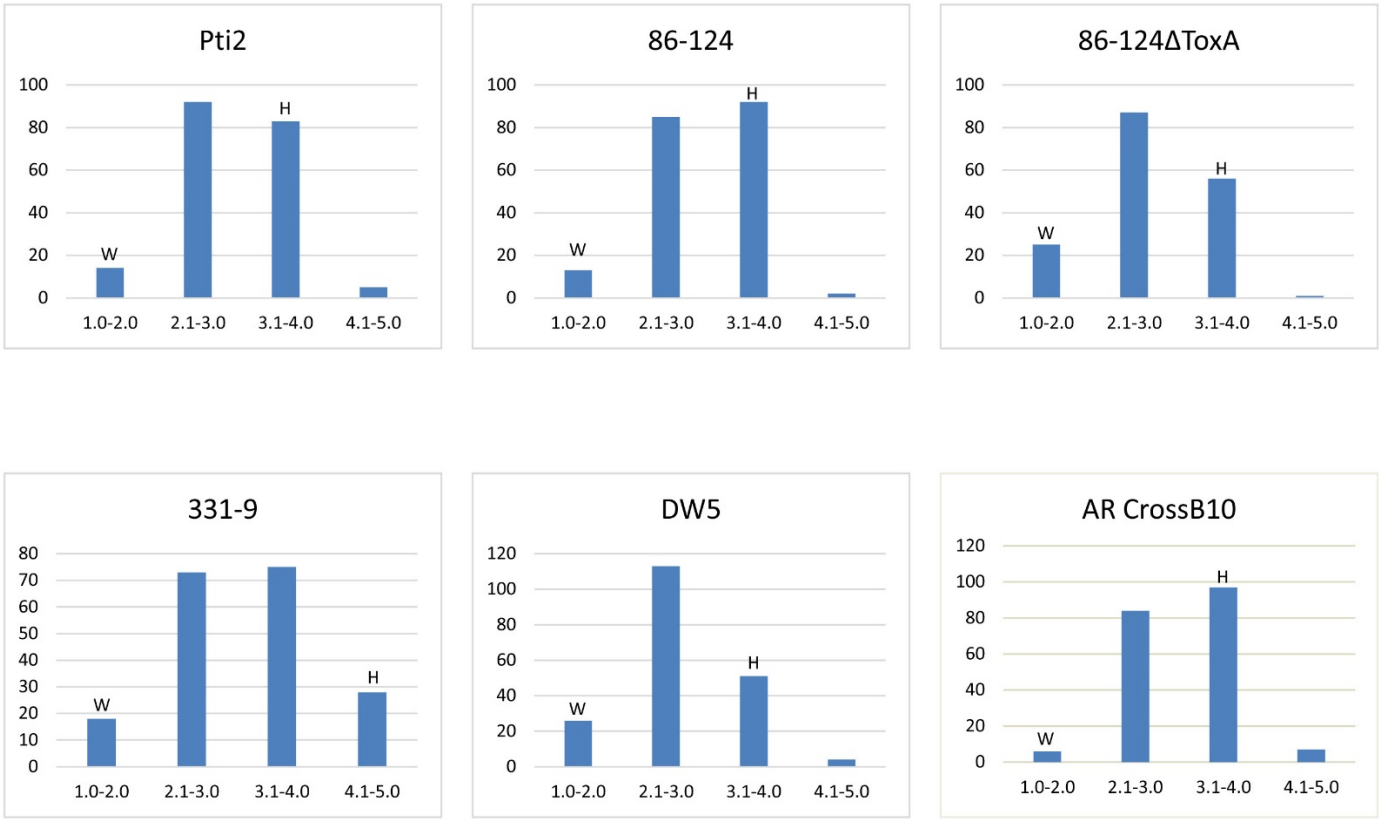


Figure 4.2. Histograms showing the disease reaction of the Harry × Wesley population to individual *Pyrenophora tritici-repentis* races/isolates. The races/isolates used for the evaluations included Pti2 (race1), 86-124 (race 2), 86124ΔToxA, 331-9 (race 3), DW5 (race 5) and AR CrossB10 (new race). The disease phenotype was rated using a 1-5 scale with 1 being highly resistant and 5 being highly susceptible. The x-axis is the disease scale, and the y-axis is the number of recombinant inbred lines.

4.4.4. QTL identification

Homogeneity analysis with Barlett's chi-squared test (for Pti2 and DW5) and Levene's test (for the remaining isolates) indicated that the variance among the replicates for each isolate was not significant (P=0.06-0.17). Therefore, the means of the three replicates for each isolate were used in QTL identification. A total of five QTL associated with reaction to tan spot were identified in the HW population. These QTL were located on chromosomes 1A, 5B, 7A, 7B, and 7D, and were designated as *QTs.zhl-1A.1*, *QTs.zhl-5B.1*, *QTs.zhl-7A.1*, *QTs.zhl-7B.1* and *QTs.zhl-7D.1*, respectively (Table 4.2). The resistance alleles for these QTL are all from Wesley, the resistant parent (Table 4.2).

QTs.zhl-1A.1 was located on the distal end of 1AS between markers *HWGBS60* and *HWGBS5150* and was significant for Pti2, 331-9 and AR CrossB10, all of which produce Ptr ToxC (Fig. 3, Table 2). The other markers *HWGBS58* and *HWGBS59* co-segregated with HWGBS60. This QTL is likely due to the Ptr ToxC-*Tsc1* interaction, had LOD values ranging from 9.9 to 46.9 and accounted for 10 to 64% of the variation in disease. *QTs.zhl-5B.1* was identified for Pti2 and 86-124 which produce Ptr ToxA and mapped at the *Tsn1* locus which confers sensitivity to Ptr ToxA (Fig. 3, Table 4.1). This QTL was not associated with reactions to strain 86-124ΔToxA, which does not produce Ptr ToxA (Fig. 4.3). These results indicate that *QTs.zhl-5B.1* is due to the Ptr ToxA-*Tsn1* interaction. The QTL had similar LOD and R^2 values for Pti2 and 86-124. *QTs.zhl-7B.1* is a minor QTL located on the short arm of chromosome 7B, and it was the only QTL associated with reaction to DW5 (Fig. 4.3). The QTL explained 8% of the disease variation with a LOD value of 6.6 (Table 4.2).

Table 4.2. QTL associated with reaction to tan spot caused by different *Pyrenophora tritici-repentis* races/ isolates in the HW population.

QTL	Interval (cM)	Flanking markers	LOD (R^2) [a]						Source[b]
			Pti2	86-124	86124ΔToxA	331-9	DW5	AR crossB10	
QTs.zhl-1A	0.0-2.0	*HWGBS60-HWGBS150*	9.9 (0.10)	-	-	46.9 (0.64)	-	18.6 (0.31)	W
QTs.zhl-5B	20.0-52.0	*HWGBS3693-HWGBS3672*	16.9 (0.28)	14.9 (0.22)	-	-	-	-	W
QTs.zhl-7A	124.0-130.0	*HWGBS5420-HWGBS5422*	-	-	8.8 (0.12)	-	-	-	W
QTs.zhl-7B	18.0-26.0	*HWGBS5696-HWGBS5992*	-	-	-	-	6.6 (0.08)	-	W
QTs.zhl-7D	176.0-180.0	*HWGBS6047-HWGBS6066*	-	-	8.2(0.13)	-	-	-	W

[a]A permutation test with 1,000 iterations yielded a LOD value of 4.2 and it was used as the cut-off to identify significant QTL. R^2 values are given in parenthesis for each QTL, indicating the amount of phenotypic variation explained by the QTL.
[b]The parental line that contributed the resistant allele where 'H' = Harry and 'W' = Wesley.

This QTL was flanked by *HWGBS5696* and *HWGBS5992* and two co-segregating markers: *HWGBS5678* and *HWGBS5672* mapped very closely to *HWGBS5696* (Table 4.2 and Fig.4.3). The QTL on 7A and 7D: *QTs.zhl-7A.1* and *QTs.zhl-7D.1* were identified for 86-124ΔToxA, the isolate producing no known NE. *QTs.zhl-7A.1* was flanked by *HWGBS5420* and *HWGBS5422* explaining 12% of the disease variation and *QTs.zhl-7D.1* was located between *HWGBS6047* and *HWGBS6066* explaining 13% of the disease variation (Fig. 4.3, Table 4.2). Three other markers *HWGBS6029*, *HWGBS6031* and *HWGBS6046* co-segregated with *HWGBS6047* on 7D. Interestingly, the two QTL were not identified using its wild type isolate 86-124 (Fig. 4.3).

4.4.5. The additive effect of the identified QTL

Because *QTs.zhl-1A.1* and *QTs.zhl-5B.1* are the two major QTL identified and they are due to the NE-wheat sensitivity gene interactions, we also investigated the genetic relationships between these two interactions by categorizing the RILs based on the genotype at the two loci and comparing the disease means in the reaction to Pti2 which produces both Ptr ToxA and Ptr ToxC. There are four genotypic groups based on the combination of the parental alleles at two QTL including the Harry allele at both loci (H,H), the Wesley allele at both loci (W,W) and the Harry allele at one locus and Wesley at the other locus (W,H and H,W) (Table 4.3). Significant differences were obtained for the disease means among all four groups with the genotypic group with Harry the allele at both loci having the highest disease mean (3.51) and that with the Wesley allele at both loci having the lowest disease mean (2.54) (Table 4.3). It is very interesting to notice that Wesley has a significant low disease mean than the group carrying Wesley's allele at both loci. This might be due to Wesley does not have the susceptibility QTL on 7A, 7B and 7D

.

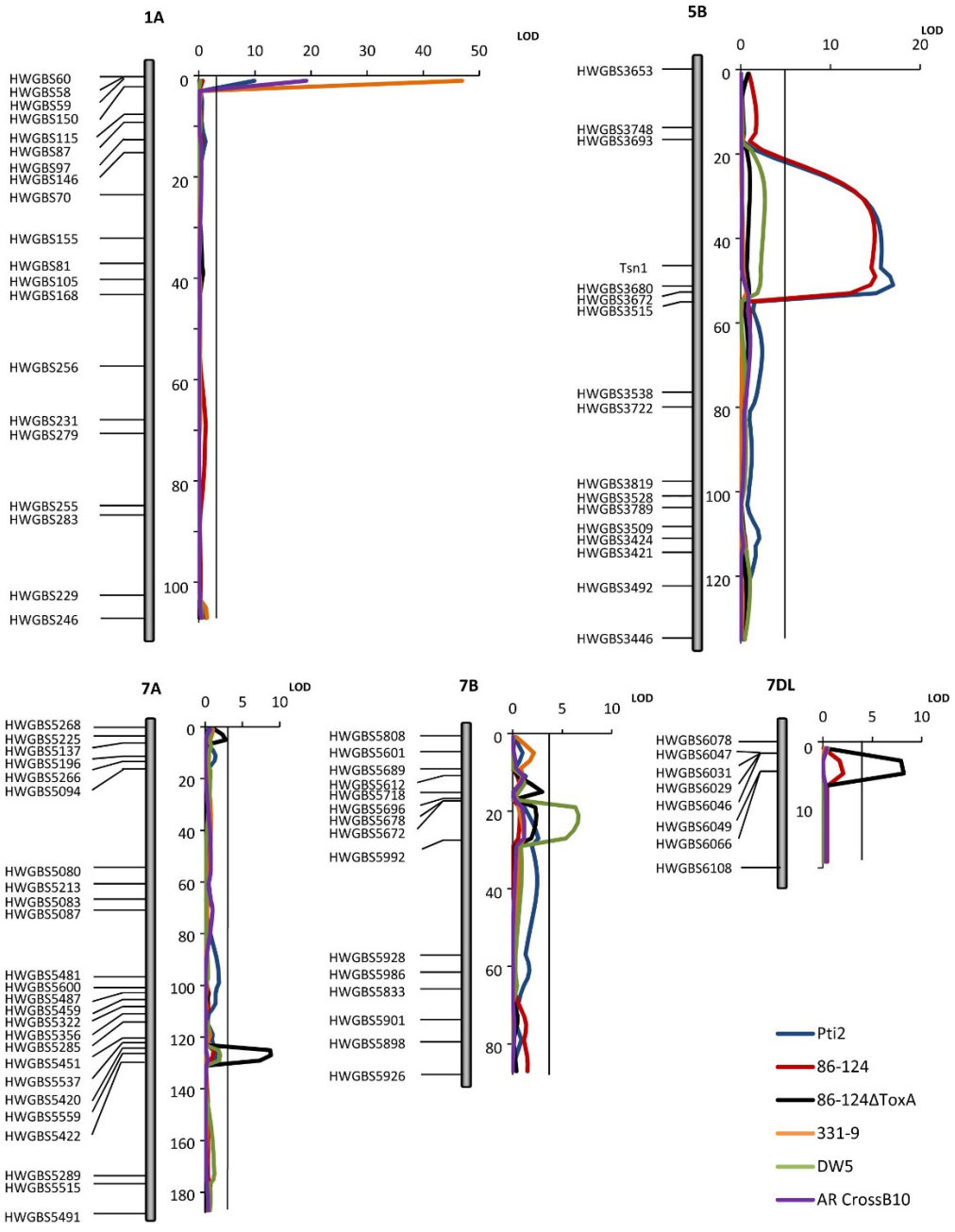


Figure 4.3. Composite interval regression maps of chromosomes 1A, 5B, 7A, 7B, and 7D containing QTLs significantly associated with reaction to tan spot in the HW population. QTL mapping was conducted on the HW population for different *Pyrenophora tritici-repentis* races/isolates, which are represented by different colors, including Pti2 (race 1), 86-124 (race 2), 86124ΔToxA, 331-9 (race 3), DW5 (race 5) and AR crossB10 (new race). The positions of marker loci are shown to the left of the linkage groups and genetic scales in centiMorgan (cM) are shown to the right of each chromosome. A solid line represents the logarithm of the odds (LOD) significance threshold of 4.2. The LOD and R^2 values for each QTL are presented in Table 2.

Table 4.3. Disease means of four categories of RILs based on alleles at *QTs.zhl-1A.1* and *QTs.zl-5B.1* for the reaction caused by race 1 isolate Pti2.

Allele at *QTs.zhl-1A.1*, *QTs.zhl-5B.1*[a]	No. of RILs	Pti2 (Race 1) [b]
H,H	49	3.51a
W,H	43	3.07b
H,W	49	2.83c
W,W	53	2.54d
Wesley	-	1.67e
Harry	-	4.00a

[a] Indicates the source of the allele at each QTL where H and W are the alleles from Harry and Wesley, respectively. The parental lines were included as controls.
[b] Means with different letters were significantly different.

4.5. Discussion

Genetic resistance to tan spot has been shown to involve multiple factors, including the lack of NE sensitivity genes, the presence of race-nonspecific resistance QTL, the presence of qualitative recessive resistance genes and other less well characterized QTL (Faris et al. 2013 for review; Liu et al. 2017). Wesley, which was highly resistant to multiple races of tan spot pathogen, could be a good source of tan spot resistance in breeding programs for winter wheat. Using QTL analysis in a segregating winter wheat population derived from Harry and Wesley, we characterized genetic resistance in Wesley. Reaction to tan spot in this population was primarily due to the two NE-wheat sensitivity gene interactions: Ptr ToxA-*Tsn1* and Ptr ToxC-*Tsc1*, which indicates that resistance in Wesley is largely due to the lack of NE sensitivity genes *Tsn1* and *Tsc1*, rather than the presence of any active resistance genes. Therefore, breeders should place strong emphasis on selection for the absence of the two NE sensitivity genes in segregating populations. *Tsn1* has been cloned and a perfect marker, *Xfcp623,* has been developed from the gene itself, which can be used in marker assisted selection (Faris et al. 2010).

However, *Tsc1* has not yet been cloned and the closest marker developed so far is 4.7 cM away from it (Faris et al. 2013). Three co-segregating GBS markers: *HWGBS58*, *HWGBS59*, and *HWGBS60* mapped in the HW population were found to underlie the peak of the 1AS QTL (Fig.4.3), which might be very close to *Tsc1*. These GBS markers can be converted into PCR-based KASP or STARP (Semi-Thermal Asymmetric Reverse PCR, Long et al. 2017) markers for marker-aided selection against *Tsc1*.

The significance of the two major QTL, *QTs.zhl-1A.1* and *QTs.zhl-5B.1,* in the respective Ptr ToxC-*Tsc1* and Ptr ToxA-*Tsn1* interactions of this study, confirms their important role in tan spot development in winter wheat genetic backgrounds. Many studies have been conducted to investigate the role of the Ptr ToxA-*Tsn1* interaction in spring wheat germplasm and populations (Faris et al. 2013; Dinglasan et al 2018). Although the Ptr ToxA-*Tsn1* interaction usually plays a significant role in hexaploid wheat backgrounds, it has never been shown to be important in tetraploid wheat backgrounds (Faris et al. 2013 for review; Virdi et al. 2016). Very interestingly, SnToxA-*Tsn1* interactions in the wheat-*Parastagonospora nodorum* system have always been shown to be important regardless of wheat polyploid levels and host genotypes (Friesen et al. 2006; Virdi et al. 2016). Sensitivity to Ptr ToxA has been found to significantly correlate with susceptibility to Ptr ToxA-producing races in winter wheat germplasm indicating the importance of the Ptr ToxA-*Tsn1* interaction in disease in winter wheat backgrounds (Noriel et al. 2011; Kollers et al. 2014; Liu et al. 2015). In this study, we used QTL mapping in a biparental population to further confirm that Ptr ToxA-*Tsn1* interaction is important for tan spot development in winter wheat genetic backgrounds.

Because Ptr ToxC cannot be easily obtained and purified, the role of the Ptr ToxC-*Tsc1* interaction in disease has not been extensively investigated except for a few QTL mapping

studies, which suggested its important role (Faris et al. 1997; Effertz et al. 2001, 2002; Sun et al. 2010; Kariyawasam et al. 2016; Liu et al. 2017). Here, we demonstrated that the Ptr ToxC-*Tsc1* interaction is also important for disease in winter wheat backgrounds. However, the effect of the interaction on disease, which was measured by R^2, was variable depending on the race/isolate used, i.e. 10% for Pti2, 31% for AR CrossB10 and 64% for 331-9 (Table 4.2, Fig. 4.3). A similar result was obtained in a study performed by Kariyawasam et al. (2016) using a spring wheat population. Liu et al. (2017) demonstrated that the Ptr ToxA-*Tsn1* interaction and the Ptr ToxC-*Tsc1* interaction made additive contributions to the level of disease in a spring wheat population when both interactions were present. Here, we showed that the two interactions can also have an additive effect on disease development in winter wheat backgrounds (Table 4.3). This observation has been commonly found in the wheat-*P. nodorum* system where multiple NE-sensitivity gene interactions have been identified (Oliver et al. 2012 for review). Therefore, for these necrotrophic pathogens, the part of the disease system that is based on inverse gene-for-gene interactions involving multiple NE-host sensitivity gene combinations, these interactions often have an additive effect and produce quantitative differences in disease development and resistance responses (Friesen and Faris 2010).Thus, in breeding programs, the sensitivity loci should be removed systematically in order to obtain higher levels of tan spot resistance.

The wheat-*P. tritici-repentis* system has also been shown to involve QTL conferring resistance to multiple or all *Ptr* races, which was referred to as race-nonspecific resistance QTL (Faris and Friesen 2005). Race-nonspecific resistance QTL has been identified in hexaploid spring wheat lines which showed resistance to multiple races (Faris and Friesen 2005; Chu et al. 2010; Faris et al. 2012; Kariyawasam et al. 2016). Some race-nonspecific resistance QTL can have complete epistasis on the effect of the Ptr ToxA-*Tsn1* interaction, but partial epistasis on the

Ptr ToxC-*Tsc1* interaction (Kariyawasam et al. 2016). This type of resistance should be very useful in breeding programs to develop wheat cultivars with resistance to multiple races. Wesley is highly resistant to multiple races, but we did not identify any QTL conferring resistance to all the races tested in the population (Table 4.3), indicating that Wesley does not carry race-nonspecific resistance. The high levels of resistance to multiple races in Wesley is most likely due to its insensitivity to the three known NEs: Ptr ToxA, Ptr ToxB and Ptr ToxC, as well as other possibly unidentified NEs. It remains unknown whether or not race-nonspecific resistance is present in winter wheat germplasm.

The race 5 isolate DW5 produces Ptr ToxB, which interacts with the sensitivity gene *Tsc2* on 2BS to induce chlorosis (Strelkov et al. 1999; Martinez et al. 2004; Friesen and Faris 2004; Abeysekara et al. 2010). For this isolate, we only identified a minor QTL (*Qts.zhl-7B.1*) on 7B (Table 4.3). The fact that no QTL were identified at the *Tsc2* locus (2BS) is due to the lack of Ptr ToxB sensitivity in both Wesley and Harry (Fig.4.1). There are two possible reasons that can explain why no major QTL was identified for DW5. First, it is possible that DW5 produced multiple unidentified NEs, but effects of which are too small to detect in this population. Second, the genetic linkage map developed in the HW population has a poor coverage in most D genome chromosomes (Hussain et al. 2017) and it is possible that some major or minor QTL could be missed or not identified. Liu et al. (2015) conducted an association mapping in a collection of winter wheat germplasm which included Wesley and Harry, revealing a QTL on 7B for DW5. This QTL might be the same as *Qts.zhl-7B.1* identified in the HW population. Tan spot resistance/susceptibility QTL on 7B have been reported before, but the previous studies used different races (Faris et al. 2012; Kollers et al. 2014).

For AR CrossB10**,** *QTs.zhl-1A.1*, which is involved in the Ptr ToxC-*Tsn1* interaction, is the only QTL identified in the HW population. AR CrossB10 was defined as a new race because it does not produce Ptr ToxA, but caused necrosis symptoms on the Ptr ToxA differential line Glenlea (Ali et al. 2010). This suggests that AR CrossB10 produces a different NE(s) which interacts with an unidentified wheat sensitivity gene(s). Previous studies using biparental mapping or association mapping have revealed QTL on a number of other wheat chromosomes (Patel et al. 2013; Liu et al. 2015, 2017; Kariyawasam et al. 2016). However, none of those QTL was identified in the HW population, which might be due to no segregation for these loci or the low coverage in some areas of the genetic linkage maps in the HW population.

The two fungal strains 86-124 and 86-124ΔToxA are nearly identical except that 86-124ΔToxA is deficient in the production of Ptr ToxA compared to the wild type 86-124 (Kariyawasam et al. 2016). The *Tsn1* locus was associated with a major QTL for 86-124, but not for 86-124ΔToxA which strongly indicates that this QTL involves the Ptr ToxA-*Tsn1* interaction. On the contrary, two QTL, *QTs.zhl-7A.1* and *QTs.zhl-7D.1* were identified for 86-124ΔToxA, but not for 86-124 in this population (Table 4.2). This suggests that the effect of these QTL is masked by that of the Ptr ToxA-*Tsn1* interaction. Epitasis of the Ptr ToxA-*Tsn1* interaction over other interactions has been reported in the wheat- *P. tritici-repentis* system (Manning and Ciuffetti 2015; See et al. 2018). As mentioned above, the effect of Ptr ToxA-*Tsn1* interaction can be completely masked by the action of race-nonspecific resistance (Kariyawasam et al. 2016). These epistasis mechanisms remain unknown, which hinders breeding of tan spot resistant cultivars.

4.6. References

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

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

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

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

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

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

Dinglasan, E. G., Godwin, I. D., Phan, H. T., Tan, K. C., Platz, G. J., and Hickey, L.T. (2018) Vavilov wheat accessions provide useful sources of resistance to tan spot (syn. yellow spot) of wheat. Plant Pathol 67:1076-1087

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

Effertz RJ, Meinhardt SW, Anderson JA, Jordahl JG, and Francl LJ (2002) Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. Phytopathology 92:527–533

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

Faris, J. D., Anderson, J. A., Francl, L. J., and Jordahl, J. G. (1997) RFLP mapping of resistance to chlorosis induction by *Pyrenophora tritici-repentis* in wheat. Theor Appl Genet 94:98–103

Faris, J. D., and Friesen, T. L. (2005) Identification of quantitative trait loci for race-nonspecific resistance to tan spot of wheat. Theor Appl Genet 111:386–392

Faris, J. D., Zhang, Z., Lu, H. J., Lu, S. W., Reddy, L., Cloutier, S., Fellers, J. P., Meinhardt, S. W., Rasmussen, J. B., Xu, S. S., Oliver, R. P., Simons, K. J., and Friesen, T. L. (2010) A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. Proc Natl Acad Sci USA 107:13544–13549

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

Faris, J. D., Liu, Z., and Xu, S. S. (2013) Genetics of tan spot resistance in wheat. Theor Appl Genet 126:2197–2217

Friesen, T. L., Rasmussen, J. B., Kwon, C. Y., Francl, L. J., and Meinhardt, S. W. (2002) Reaction of Ptr ToxA-insensitive wheat mutants to *Pyrenophora tritici-repentis* race. Phytopathol 92:38-42.

Friesen, T. L., and Faris, J. D. (2004) Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity to Ptr ToxB in wheat. Theor Appl Genet 109:464–471

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

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

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

Hosford RM Jr (1982) Tan spot-developing knowledge 1902–1981, virulent races and differentials, methodology, rating systems, other leaf diseases, literature. In: Hosford RM Jr (ed) Tan spot of wheat and related diseases workshop. ND Agric Exp Stn, Fargo, pp 1–24

Hussain, W., Baenziger, P. S., Belamkar, V., Guttieri, M. J., Venegas, J. P., Easterly, A., Sallam, A., and Poland, J. (2017) Genotyping-by-sequencing derived high-density linkage map and its application to QTL mapping of flag leaf traits in bread wheat. Sci Rep 7:16394 DOI:10.1038/s41598-017-16006-z

Joehanes, R., and Nelson, J. C. (2008) QGene 4.0, an extensible Java QTLanalysis platform. Bioinformatics 24:2788–2789

Kariyawasam, G. K., Carter, A. H., Rasmussen, J. B., Faris, J. D., Xu, S. S., Mergoum, M., and Liu, Z. H. (2016) Genetic relationships between race-nonspecific and race-specific

interactions in the wheat– *Pyrenophora tritici-repentis* pathosystem. Theor Appl Genet 129:897–908

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

Lamari, L., and Bernier, C. C. (1989) Evaluations of wheat lines and cultivars to tan spot [*Pyrenophora tritici-repentis*] based on lesion type. Can J Plant Pathol 11:49–56

Levene, H. (1960) robust tests for equality of variances. In: Olkin, I, Ghurye, S. H, Hoeffding, W., Madow, W. G., and Maan, H. B (eds) Contributions to probability and statistics: essays in honor of Harold Hotelling. Stanford University Press, Stanford, pp 278–292

Liu, Z. H., Faris, J. D., Oliver, R. P., Tan, K., Solomon, P. S., Mcdonald, M. C., Mcdonald, B. A., Nunez, A., Lu, S., Rasmussen, J. B., and Friesen, T. L. (2009) SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the Snn3 gene. PLoS Pathog 5:1–15

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

Liu, Z., Zurn, J. D., Kariyawasam, G., Faris, J. D., Shi, G., Hansen, J., Rasmussen, J. B., and Acevedo, M. (2017) Inverse gene-for-gene interactions contribute additively to tan spot susceptibility in wheat. Theor Appl Genet 6:1267-1276

Long, Y., Chao, W. S., Ma, G., Xu, S. S., and Qi, L. (2017) An innovative SNP genotyping method adapting to multiple platforms and throughputs. Theor Appl Genet 130:597–607

Lorieux, M. (2012) MapDisto: fast and efficient computation of genetic linkage maps. Mol Breed 30:1231-1235

Manning, V. A., and Ciuffetti, L. M. (2015) Necrotrophic effector epistasis in the *Pyrenophora tritici-repentis*-wheat interaction. PLoS One https://doi.org/10.1371/journal.pone.0123548

Martinez, J. P., Oesch, N. W., and Ciuffetti, L. M. (2004) Characterization of the multiple-copy host-selective toxin gene, ToxB, in pathogenic and nonpathogenic isolates of *Pyrenophora tritici-repentis*. Mol Plant Microbe Interact 17:467-474

Mereno, M. V., Stenglein, S., and Perello, A. E. (2015) Distribution of races and Tox genes in *Pyrenophora tritici-repentis* isolates from wheat in Argentina. Trop Plant Pathol 40:141–146

Murray, G. M., and Brennan, J. P. (2009) Estimating disease losses to the Australian wheat industry. Australas Plant Pathol 38:558-70

Moffat, C. S., See, P. T., and Oliver, R. P. (2014) Generation of a *ToxA* knockout strain of the wheat tan spot pathogen *Pyrenophora tritici-repentis*. Mol Plant Pathol 15:918-26

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

Oliver, R. P., Friesen, T. L., Faris, J. D., and Solomon, P. S. (2012) *Stagonospora nodorum*: from pathology to genomics and host resistance. Annu Rev Phytopathol 50:23-43

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

Peel, M. D., Anderson, J. A., Rasmussen, J. B., Miller, J. D., Olsen, T. C., and Johnson, G. W. (2004) Registration of 'Jerry' wheat. Crop Sci 44:1026-1027

Rees, R. G., Platz, G. J., and Mayer, R.J. (1982) Yield losses in wheat from yellow spot: comparison of estimates derived from single tillers and plots. Aust J Agric Res 33:899–908

SAS Institute, (2016) SAS/IML 9.4 User's Guide. SAS Institute, Cary, NC

Schilder, A. M. C, and Bergstrom, G. C. (1994) Infection of wheat seed by *Pyrenophora tritici-repentis*. Can J Bot 72:510–519

See, P. T., Marathamuthu, K. A., Iagallo, E. M., Oliver, R. P., and Moffat, C, S (2018) Evaluating the importance of the tan spot ToxA–*Tsn1* interaction in Australian wheat varieties. Plant Pathol 67:1066-1075

Singh, P.K, Mergoum, M., Ali, S., Adhikari, T. B., Elias, E. M., Anderson, J. A., Glover, K. D., and Berzonsky, W. A. (2006a) Evaluation of elite wheat germ plasm for resistance to tan spot. Plant Dis 90:1320-1325

Singh, P. K., Gonzalez-Hernandez, J. L., Mergoum, M., Ali, S., Adhikari, T. B., Kianian SF, Elias, E, and Hughes G. R. (2006b) Identification and molecular mapping of a gene conferring resistance to *Pyrenophora tritici-repentis* race 3 in tetraploid wheat. Phytopathology 96:885–889

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

Snedecor GW, and Cochran WG (1989) Statistical methods, 8th edn. Iowa State University Press, Ames, Iowa

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

Strelkov SE, and Lamari L (2003) Host-parasite interactions in tan spot (*Pyrenophora tritici-repentis*) of wheat. Can J Plant Pathol 25:339–349

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

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

Tadesse, W., Hsam, SLK. , and Zeller, F. J. (2006b) Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'. Plant Breed 125:318–322

Virdi, S. K., Liu, Z. H., Overlander, M. E., Zhang, Z. C., Xu, S. S., Friesen, T. L., and Faris, J. D. (2016) New insights into the roles of host gene-necrotrophic effector interactions in governing susceptibility of durum wheat to tan spot and Septoria nodorum blotch. G3 doi: 10.1534/ g3.116.03

APPENDIX A. PHENOTYPIC DATA OF PYRENOPHORA TIRITI-RENPTIS ISOLATES FOR CHLOROSIS/NO CHLOROSIS PRODUCTIION ON PTR TOXC DIFFERENTIAL LINES

Progeny ID	Chlorosis/No-chlorosis	Prosper	6B365	Harry	Jerry
86-124	NO CHLOROSIS				
ARCrossB10	CHLOROSIS				
AR2 - 2	NO CHLOROSIS	1.5	2	2.5	3
		1	3.5	3.5	3
		2	3	3.5	4
AR2 - 3	NO CHLOROSIS	1	2.5	2.5	.
		1	4	4.5	4.5
		1*	4.5	4.5	4
AR2 - 6	NO CHLOROSIS	2	4wc	4	4.5
		1	4.5	4.5	4.5
		1	4.5	4.5	4
AR2 - 7	CHLOROSIS	4c	5c	5c	4
		3.5c	4.5c	4.5c	4.5c
		1.5	4c	4.5c	4c
AR2 - 8	CHLOROSIS	3.5c	4c	5c	5c
		4c	3.5c	4.5c	4.5c
		4c	4.5c	4c	3.5c
AR2 - 9	CHLOROSIS	3.5c	4c	3.5c	4c
		2.5wc	4c	4.5c	5c
		4c	4.5c	4c	4.5
		4.5c	3.5c	4.5c	4.5c
AR2 - 10	CHLOROSIS	5c	5c	4c	5c
		4.5c	5c	5c	4.5c
		4	4c	4.5c	4.5c
		4c	3.5c	4c	4.5c
AR2 - 12	NO CHLOROSIS	2	3.5	3.5	3.5
		1.5	3	4.5	4
		2	3.5	3.5	3
AR2 - 13	NO CHLOROSIS	1	3	4	3.5
		1.5	3.5	3	3.5
AR2 - 15	CHLOROSIS	4c	5c	5c	4c
		3.5c	4c	4c	4.5c
		3.5c	4c	4.5c	4
AR2 - 16	NO CHLOROSIS	1	3.5	4	4.5
		1.5	3.5	3.5	4
		1	3.5	4.5	4
AR2 - 17	NO CHLOROSIS	2	4	3.5	3.5
		1	4	4	4.5
		2.5	4.5	4	4
AR2 - 18	NO CHLOROSIS	1.5	4.5	4.5	4
		2.5	4	4	4.5
		1.5	4	4.5	4.5
AR2 - 19	CHLOROSIS	4c	4.5c	4.5c	5c
		3.5c	4.5c	4.5c	4.5c
		4c	3.5c	4.5c	4c
AR2 - 20	CHLOROSIS	4c	4c	5c	4wc
		3.5c	3wc	3.5c	3.5wc
		2.5wc	3.5c	4.5c	4c
		3.5c	3.5c	4.5c	4.5c

Progeny ID	Chlorosis/No-chlorosis	Prosper	6B365	Harry	Jerry
AR2 - 21	NO CHLOROSIS	2	3.5wc	3.5	4
		1	4	4	4.5
		1.5	4	4.5	4
		1.5	4	4.5	3.5
AR2 - 22	CHLOROSIS	4c	5c	5c	5c
		3.5c	4.5c	5c	4.5c
		4c	5c	4.5c	4c
AR2 - 23	NO CHLOROSIS	1.5	3	3.5	3
		1.5	4wc	4	4.5wc
		3.5	4	4.5	4.5
AR2 - 24	NO CHLOROSIS	2	4	4	4.5
		1.5	3.5	3.5	3.5
		2.5	4.5	4.5	4.5
AR2 - 25	CHLOROSIS	4c	4.5c	5c	5c
		3c	4.5c	4.5c	3.5wc
		3wc	4.5c	4.5c	4wc
AR2 - 26	CHLOROSIS	5c	4c	5c	4.5c
		3.5c	5c	4.5c	4.5c
		4.5c	5c	5c	5
		4.5c	5c	4.5c	4.5c
AR2 - 27	NO CHLOROSIS	2.5	4	4	4.5
		1.5	3.5	4.5	4.5
		3	4.5	4	4.5
AR2 - 28	NO CHLOROSIS	1.5	3	4	4
		2	3.5	4.5	4
		4	3	4	3.5
AR2 - 29	NO CHLOROSIS	1	3.5	4	4
		1	3.5	4.5	4
		2	3	4	3.5
		1.5	3.5	3.5	3
AR2 - 30	CHLOROSIS	5c	4c	5c	5c
		4c	5c	4.5c	4.5c
		4.5c	5c	5c	4c
AR2 - 31	CHLOROSIS	5c	4c	5	5c
		3.5c	4.5c	4c	4c
		3.5c	4.5c	4c	4c
AR2 - 32	NO CHLOROSIS	2.5	3.5	4	4
		2	4.5	4	4.5
		3	4	4	4.5
		1	3.5	3.5	4
AR2 - 33	NO CHLOROSIS	1	3	3.5	3.5
		1	4	3.5	4
		1.5	4	3.5	3
AR2 - 35	NO CHLOROSIS	1	3	3.5	4
		1	3.5	3.5	3
		2	3.5	4	3
		2.5	3.5	4	3.5
AR2 - 36	NO CHLOROSIS	1.5	2.5	4	3.5
		1	3.5	3	3.5
		3.5	3.5	4	3
AR2 - 42	NO CHLOROSIS	2	3	4	4
		1.5	4	3.5	3.5
		2.5	4	4.5	4
AR2 - 43	CHLOROSIS	4.5c	5c	5c	5c
		4c	5c	4c	4c
		3.5c	4c	4.5c	4wc

Progeny ID	Chlorosis/No-chlorosis	Prosper	6B365	Harry	Jerry
AR2 - 44	CHLOROSIS	4c	4c	4.5	4c
		3wc	3.5c	4c	3wc
		3.5c	4.5c	4.5c	5
AR2 - 45	NO CHLOROSIS	1.5	4	3.5	3.5
		1	3.5	3	3
		2	4.5	4.5	4
AR2 - 46	CHLOROSIS	4c	5c	5c	4.5c
		2.5	4.5c	4c	4.5c
		3c	4.5c	3.5c	4c
AR2 - 47	CHLOROSIS	4c	5c	5c	4c
		4c	5c	5c	4.5c
		4c	3.5c	5c	4c
AR2 - 48	NO CHLOROSIS	2	3.5	3	4
		1	3.5	3.5	4
		2.5	3.5	4.5wc	4
AR2 - 49	NO CHLOROSIS	1	3	3	4
		1	4	4.5	4.5
		2.5	3.5	4.5	4.5
AR2 - 50	CHLOROSIS	3.5c	4c	5c	4wc
		3.5c	4.5c	3.5c	4c
		4.5c	4.5c	4.5c	5c
AR2 - 51	NO CHLOROSIS	1.5	3.5	3	4
		1.5	4.5	4	4.5
		3.5	3.5	4.5	4.5
AR2 - 53	CHLOROSIS	3.5wc	5c	5c	5c
		3.5c	5c	4c	5c
		4.5c	4wc	4.5c	4c
AR2 - 54	NO CHLOROSIS	1	2.5	2.5	2.5
		1	3.5	4.5	4
		1	3.5wc	3.5	3.5
AR2 - 56	CHLOROSIS	3c	4.5c	4.5c	4c
		4c	4.5c	4.5c	4.5c
		4c	5c	4.5c	4c
AR2 - 57	CHLOROSIS	3.5c	4c	4.5c	4wc
		4c	4.5c	4.5c	3.5c
		3c	3.5c	4c	4wc
AR2 - 59	CHLOROSIS	3wc	4c	4.5c	4c
		3.5c	4.5c	4.5c	4.5c
		3.5c	45c	4.5c	4c
AR2 - 60	CHLOROSIS	4c	4c	5c	4c
		4.5c	5c	4.5c	4.5c
		4.5c	3.5c	4c	4.5c
AR2 - 61	CHLOROSIS	3.5c	5c	5c	4c
		4.5c	5c	5c	5c
		3.5c	4c	4c	4.5c
AR2 - 63	NO CHLOROSIS	1.5	1.5	3	3
		1	4.5	4	4.5
		1	3.5	4	3
		4.5	3.5	4	4.5
AR2 - 65	CHLOROSIS		4.5c	5c	
		2.5	3.5	3.5	4.5
		3c	4.5c	4c	4
AR2 - 66	NO CHLOROSIS	1	3	4	4
		1.5	4.5	4.5	4
		1.5	4.5	3.5	3.5

Progeny ID	Chlorosis/No-chlorosis	Prosper	6B365	Harry	Jerry
AR2 - 67	NO CHLOROSIS	1.5	2	2.5	3.5
		1	3	4	4.5
		1	3.5	4	4
AR2 - 68	CHLOROSIS	4c	4.5c	5c	4.5c
		4.5c	5c	5c	5c
		4wc	4.5c	4.5c	5c
		4c	3.5c	5c	4.5c
AR2 - 69	CHLOROSIS	3c	4c	4.5c	4c
		3.5c	4.5c	4c	4c
		3c	4.5c	4.5c	4.5c
AR2 - 70	NO CHLOROSIS	1.5	3	3	3
		1.5	3.5	3.5	4
		1	3.5	4	4.5
AR2 - 71	NO CHLOROSIS	1	3	3	3
		2.5	4.5	4	4.5
		1	4	4.5	4
AR2 - 74	CHLOROSIS	2.5wc	3.5wc	3wc	3wc
		3	4c	4c	4.5wc
		1	3.5c	4c	4c
AR2 - 75	NO CHLOROSIS	1.5	2.5	2.5	3
		1	4	3.5	4.5
		1.5	3	4	4
AR2 - 76	NO CHLOROSIS	1	3.5	4	4
		1	2.5	3	3
		1	3	3.5	4.5
AR2 - 77	CHLOROSIS	4c	5c	5c	5c
		3wc	4.5c	4c	4.5wc
		4c	4.5c	4.5c	4.5c
AR2 - 78	CHLOROSIS	4.5c	4.5c	4c	4c
		2.5	4wc	4.5c	4.5
		4c	4c	4.5c	4.5c
AR2 - 80	CHLOROSIS	3c	3c	4c	4
		2.5	4c	3.5c	4.5wc
		4c	2.5c	4.5c	4.5c
AR2 - 81	NO CHLOROSIS	1	3	3.5	4
		2	3.5	4.5	4.5
		2.5	4	4	4.5
AR2 - 82	NO CHLOROSIS	1.5	3	3.5	4
		1	2.5	4	4.5
		2.5	3.5	4	3
		4	3	4	3.5
AR2 - 83	NO CHLOROSIS	1.5	3	4	3
		1.5	4	4.5	4.5
		1	4.5	4.5	4
AR2 - 84	NO CHLOROSIS	1.5	3	3.5	4
		3.5	4	3.5	3.5
		2	3.5	4	4.5
AR2 - 86	NO CHLOROSIS	1.5	3.5	3.5	3.5
		2.5	2.5	4	4.5
		3.5	3.5	4	4.5
AR2 - 87	CHLOROSIS	4.5c	5c	4.5c	5c
		4c	5c	3.5c	4c
		3.5c	4c	4c	4
AR2 - 88	CHLOROSIS	2.5	3.5	3.5wc	4
		3.5wc	4.5	4.5c	5c
		4c	4.5c	4.5c	4c

Progeny ID	Chlorosis/No-chlorosis	Prosper	6B365	Harry	Jerry
AR2 - 89	NO CHLOROSIS	3	4wc	4wc	3.5
		2.5	3.5	4wc	4
		4	4	4.5	4
AR2 - 90	CHLOROSIS	4c	4c	4.5c	4wc
		3.5c	4c	5c	4.5c
		4c	4.5c	3	4
AR2 - 91	NO CHLOROSIS	3	3	4	4
		1.5	3.5	4	4.5
		3	3.5	4	4
AR2 - 94	NO CHLOROSIS	2.5	3	3	3
		3	3.5	3.5	4
		2.5	3.5	3.5	4
AR2 - 98	NO CHLOROSIS	1	3	3.5wc	3.5
		1	4.5	4	4.5
		4.5	3.5	5	4
AR2 - 100	CHLOROSIS	4c	3.5wc	4.5c	4c
		3.5wc	4c	4c	5c
		3.5c	4c	3.5c	3.5c
AR2 - 101	NO CHLOROSIS	2	3.5	3.5	3.5
		2	3.5	4	3.5
		.	3.5	3.5	4.5
		4.5	4.5	5	5
AR2 - 104	NO CHLOROSIS	2	3.5	4	4
		1	3.5	3.5	3
		2	4	5	4.5
AR2 - 105	NO CHLOROSIS	1.5	3.5	3	4
		1.5	3	4	4
		4	3.5	4.5	3.5
		2.5	3.5	4.5	4.5
AR2 - 106	CHLOROSIS	3.5c	5c	4.5c	4.5c
		4c	4.5c	4.5c	4.5c
		4c	4.5c	4.5c	4.5c
		4c	4.5c	4.5c	4c
AR2 - 107	NO CHLOROSIS	1	3	4	4
		1	3.5	3.5	3.5
		3.5	3.5	4.5	5
AR2 - 108	CHLOROSIS	4c	5c	4.5c	4
		3wc	3c	4.5c	4
		4.5c	4c	5c	5c
AR2 - 109	NO CHLOROSIS	2	3	3.5	3
		1	4	4	4.5
		3	3.5	3.5	4.5
AR2 - 110	CHLOROSIS	3.5c	4c	3.5c	3.5
		4c	4.5c	4.5c	4c
		4c	3wc	4wc	4.5c
AR2 - 111	NO CHLOROSIS	1	3	3	3
		4	4	4	4
		3	3	4.5	4
AR2 - 112	NO CHLOROSIS	1.5	3	4	3
		1.5	3.5	4	3.5
		3.5	4	3.5	4
AR2 - 113	CHLOROSIS	3.5c	4c	4c	3.5wc
		2.5wc	4c	3.5c	4c
		4c	3.5wc	4.5c	4c
AR2 - 116	NO CHLOROSIS	1	2.5	3	2.5
		1.5	3.5	4	4
		1	3.5	3.5	4

Progeny ID	Chlorosis/No-chlorosis	Prosper	6B365	Harry	Jerry
AR2 - 118	NO CHLOROSIS	1.5	2.5	2.5	3
		1	4	4.5	4
		4	3.5	4	3.5
AR2 - 120	CHLOROSIS	4c	4.5c	5c	.
		4.5wc	4.5c	4c	sp
		3.5c	4.5c	3.5c	4.5c
AR2 - 121	NO CHLOROSIS	1	2.5	2.5	3
		1	3.5	3	4
		1.5	3.5	3.5	3.5
		1.5	3.5	3	3.5
AR2 - 122	NO CHLOROSIS	1.5	4	4.5	4
		1	4	3.5	4.5
		1.5	4.5	4.5	4.5
AR2 - 123	CHLOROSIS	4c	5c	5c	5c
		2.5	4.5c	3.5c	4c
		3.5c	4.5c	4.5c	3.5c
AR2 - 124	NO CHLOROSIS	1	3	3	3
		2.5	3	4.5	4.5
		2	3.5	4	4
		1	2.5	3	3
AR2 - 128	CHLOROSIS	3c	4c	4c	3c
		3wc	3.5c	4c	3.5c
		3.5wc	4c	3.5c	4
AR2 - 129	CHLOROSIS	4c	5c	4c	.
		3.5c	4c	4c	4.5wc
		3c	4.5c	4.5c	4c
AR2 - 131	CHLOROSIS	3wc	5c	5c	.
		1	3.5c	4wc	4.5c
		4c	4c	5c	4.5c
AR2 - 132	NO CHLOROSIS	2	3	4	.
		1.5	4	4	4
		1.5	3.5	4	2.5
AR2 - 133	NO CHLOROSIS	1	2.5	3.5	3.5
		2.5	4	3.5	3
		3.5	3	4	3.5
AR2 - 134	CHLOROSIS	4c	4.5c	4.5c	4.5c
		4c	4.5c	4.5c	4.5c
		3.5	3.5wc	4c	4.5c
		4.5c	4c	4c	4.5c
AR2 - 135	NO CHLOROSIS	2	3.5	4	.
		1	3	4	3.5
		3.5	3.5	4	4
AR2 - 136	CHLOROSIS	4c	4c	4c	.
		3	4.5c	3.5c	4c
		4c	4c	4.5c	5c
AR2 - 137	CHLOROSIS	4c	5c	4c	.
		1.5	3.5c	3.5wc	4.5
		4c	4.5c	4c	4.5c
AR2 - 138	CHLOROSIS	3.5c	4.5c	4c	.
		3.5wc	4.5c	4c	4.5c
		4c	4c	4c	4c
AR2 - 140	NO CHLOROSIS	1	2.5	3	
		1	4	4.5	4
		1	2.5	3.5	4
		3	3.5	44.5	1

Progeny ID	Chlorosis/No-chlorosis	Prosper	6B365	Harry	Jerry
AR2 - 141	CHLOROSIS	3.5c	4.5c	4.5c	3
		3.5wc	3.5c	4c	4c
		3.5c	5c	4c	4c
		4c	4.5c	5c	5c
AR2 - 143	NO CHLOROSIS	2	3	4	4
		1	2.5	4	3
		4	3.5	3	3.5
AR2 - 144	CHLOROSIS	4.5c	5c	5c	4.5c
		2.5	3.5c	4c	3.5c
		4c	4c	4c	4wc
		4c	4c	4.5c	4c
AR2 - 145	NO CHLOROSIS	1.5	4	4	4
		1.5	4	4	4
		1	3	3	4.5
		3.5	3.5	4.5	3.5
AR2 - 146	CHLOROSIS	4c	5c	5c	4c
		3c	4c	4c	3.5c
		3c	4c	4c	4c

APPENDIX B. GENOTYPE OF THE PROGENY OF AR POPULATION FOR ASSOCIATED SNP MARKERS

Progeny ID	SC1.16.15K	SC1.16.17K	SC1.16.18k	SC1.16.23K	SC1.16.33K	SC1.16.36K	SC1.16.37K	SC1.16.40K	SC1.16.43K	SC1.16.44K	SC1.16.45.2K	SC1.16.51k	SC1.16.69K	SC1.16.78k	SC1.38.18K	SC1.38.20K	SC1.31.43K
AR-2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-7	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
AR-8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-13	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-109	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-17	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	0
AR-18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-21	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1
AR-22	0	0	0	0	0	0	0	0	0	0	0	1	1	3	0	0	0
AR-23	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-25	0	0	0	0	0	0	0	0	0	0	0	1	1	3	0	0	0
AR-26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-28	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-31	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1
AR-32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-35	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-36	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-38	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0
AR-39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-41	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-42	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-48	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1
AR-49	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-51	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1
AR-53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-54	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0
AR-56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-57	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1

Progeny ID	SC1.16.15K	SC1.16.17K	SC1.16.18k	SC1.16.23K	SC1.16.33K	SC1.16.36K	SC1.16.37K	SC1.16.40K	SC1.16.43K	SC1.16.44K	SC1.16.45.2K	SC1.16.51k	SC1.16.69K	SC1.16.78k	SC1.38.18K	SC1.38.20K	SC1.31.43K
AR-60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-61	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
AR-63	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	0
AR-65	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	3
AR-66	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-70	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	0
AR-71	1	1	1	3	1	1	1	1	1	1	1	1	0	0	1	1	3
AR-74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-75	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-76	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0
AR-77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-81	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-82	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-83	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-84	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-86	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0
AR-87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
AR-89	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-90	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
AR-91	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
AR-94	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-98	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-100	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
AR-101	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-104	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
AR-105	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-107	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1
AR-108	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-110	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-111	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-112	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-113	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-116	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-118	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-121	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
AR-122	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-123	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AR-124	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
AR-128	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
AR-129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-131	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
AR-132	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Progeny ID	SC1.16.15K	SC1.16.17K	SC1.16.18k	SC1.16.23K	SC1.16.33K	SC1.16.36K	SC1.16.37K	SC1.16.40K	SC1.16.43K	SC1.16.44K	SC1.16.45.2K	SC1.16.51k	SC1.16.69K	SC1.16.78k	SC1.38.18K	SC1.38.20K	SC1.31.43K
AR-133	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-134	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	1
AR-135	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-136	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
AR-137	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
AR-138	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	1
AR-140	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-141	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	1
AR-143	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-144	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
AR-145	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3
AR-146	0	0	0	0	0	0	0	3	0	0	0	1	1	1	0	0	0
AR-67	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3

APPENDIX C. LIST OF STARP PRIMERS USED IN GENETIC MAPPING OF 86-124ΔMAT1-1-1 × AR CROSSB10ΔMAT1-2-1 POPULATION

Primer name	Primer sequence*
SC10.109kAMAS1	GCAACAGGAACCAGCTATGACGTAAGGACAATCCAATTCTCTG
SC10.109kAMAS2	GACGCAAGTGAGCAGTATGACGTAAGGACAATCCAATTCCATA
SC10.109kRev	TCCCTTAATACACCCGTTATGG
SC10.351kAMAS1	GCAACAGGAACCAGCTATGACTAGAATTAATACGAGAGCCATG
SC10.351kAMAS2	GACGCAAGTGAGCAGTATGACTAGAATTAATACGAGAGCTGTA
SC10.351kRev	ACCGTCGAAGAAGGACAAA
SC10.507kAMAS1	GCAACAGGAACCAGCTATGACTGTGGAGTAGTCCGCTAC
SC10.507kAMAS2	GACGCAAGTGAGCAGTATGACTGTGGAGTAGTCCGTCAA
SC10.507kRev	CAATGGCCTCCACTGATACA
SC10.700kAMAS1	GCAACAGGAACCAGCTATGACACGACGACGACGACGCCG
SC10.700kAMAS2	GACGCAAGTGAGCAGTATGACACGACGACGACGACAACA
SC10.700kRev	CAGTCACTATTCGGCAGGTG
SC10.901kAMAS1	GCAACAGGAACCAGCTATGACAATGGAAAACGCCCCCAC
SC10.901kAMAS2	GACGCAAGTGAGCAGTATGACAATGGAAAACGCCCTTAG
SC10.901kRev	GGACCAAAAATTCGACTTGC
SC10.1086kAMAS1	GCAACAGGAACCAGCTATGACCAGCTGGAAGGGAGTTTC
SC10.1086kAMAS2	GACGCAAGTGAGCAGTATGACCAGCTGGAAGGGAGCCTT
SC10.1086kRev	TAGCAGCACGACAGCAAATA
SC10.1307kAMAS1	GCAACAGGAACCAGCTATGACCGATGCTTGAGAGACTAG
SC10.1307kAMAS2	GACGCAAGTGAGCAGTATGACCGATGCTTGAGAGATCAA
SC10.1307kRev	GTTCTTCTGGTTGCCAATCTT
SC10.1445kAMAS1	GCAACAGGAACCAGCTATGACCTCTCCTGTACAACCTCC
SC10.1445kAMAS2	GACGCAAGTGAGCAGTATGACCTCTCCTGTACAACACCG
SC10.1445kRev	ATATCTTTGGCTGGGCTTGT
SC10.1584kAMAS1	GCAACAGGAACCAGCTATGACCGATGCCATTACCCGAGG
SC10.1584kAMAS2	GACGCAAGTGAGCAGTATGACCGATGCCATTACCCAGGA
SC10.1584kRev	GAGTGGCGATGACGTATATTG
SC33.20kAMAS1	GCAACAGGAACCAGCTATGACGAACTCCCCACTGAGAAG
SC33.20kAMAS2	GACGCAAGTGAGCAGTATGACGAACTCCCCACTGAAGAA
SC33.20kRev	GAGTTCTCGTGGCTCTCGT
SC18.4kAMAS1	GCAACAGGAACCAGCTATGACGAGTTATTAGTACTGTACACCAC
SC18.4kAMAS2	GACGCAAGTGAGCAGTATGACGAGTTATTAGTACTGTACAATAT
SC18.4kRev	ACGAGGAGTTTGAACTTTGATTTAT
SC18.227kAMAS1	GCAACAGGAACCAGCTATGACCTCTGTTGAGCCCCAAG
SC18.227kAMAS2	GACGCAAGTGAGCAGTATGACCTCTGTTGAGCCCTGAA
SC18.227kRev	TTGATTTAGACGGACATTGTGAG
SC18.455kAMAS1	GCAACAGGAACCAGCTATGACGCAATGTTTGGAATGCGCTG
SC18.455kAMAS2	GACGCAAGTGAGCAGTATGACGCAATGTTTGGAATGCAATA
SC18.455kRev	TAGCCTAAACAGCACTAGTC
SC1.4kAMAS1	GCAACAGGAACCAGCTATGACGGCCAGAATGGCGGTGAC
SC1.4kAMAS2	GACGCAAGTGAGCAGTATGACGGCCAGAATGGCGGCAAT
SC1.4kRev	TTCAAGCCAGGTGCTTTCTT
SC1.205kAMAS1	GCAACAGGAACCAGCTATGACTTGTACCGTGTGTATCGC
SC1.205kAMAS2	GACGCAAGTGAGCAGTATGACTTGTACCGTGTGTACTGT
SC1.205kRev	ACTGTGCAGGATCACAAACAT
SC1.432kAMAS1	GCAACAGGAACCAGCTATGACAGGATCGGGCTCCAAG
SC1.432kAMAS2	GACGCAAGTGAGCAGTATGACAGGATCGGGCTCTGAA
SC1.432kRev	AAACTTGACAGAATGACATTGAAAC
SC1.650kAMAS1	GCAACAGGAACCAGCTATGACAGCGGCGTGACGAAACGG
SC1.650kAMAS2	GACGCAAGTGAGCAGTATGACAGCGGCGTGACGAACTGA
SC1.650kRev	GTCGCGCCCAACTAAGGT

Primer name	Primer sequence*
SC1.1026kAMAS1	GCAACAGGAACCAGCTATGACCGACGAAGGCGCTCATGC
SC1.1026kAMAS2	GACGCAAGTGAGCAGTATGACCGACGAAGGCGCTCGCGT
SC1.1026kRev	TCGCCTTCGTAAAGTTAGGC
SC1.1218kAMAS1	GCAACAGGAACCAGCTATGACCGGCCGGAGAGCGGTGTC
SC1.1218kAMAS2	GACGCAAGTGAGCAGTATGACCGGCCGGAGAGCGGCATT
SC1.1218kRev	CACGGAAAGCGTACGTACTTG
SC1.1417kAMAS1	GCAACAGGAACCAGCTATGACTTGAGGGGGCGAGGTCGAGG
SC1.1417kAMAS2	GACGCAAGTGAGCAGTATGACTTGAGGGGGCGAGGTCAGGA
SC1.1417kRev	AACAAACAACCAGCTTCAACG
SC1.1624kAMAS1	GCAACAGGAACCAGCTATGACAACATAGTCAGAGACTTCCC
SC1.1624kAMAS2	GACGCAAGTGAGCAGTATGACAACATAGTCAGAGACTCTCT
SC1.1624kRev	AAAGCTAACTCACGGGATGTC
SC1.1825kAMAS1	GCAACAGGAACCAGCTATGACAAACCACCACCTTTTCCG
SC1.1825kAMAS2	GACGCAAGTGAGCAGTATGACAAACCACCACCTTTCTCA
SC1.1825kRev	CATAATAGCAGGCGTTCACATT
SC1.2025kAMAS1	GCAACAGGAACCAGCTATGACTTACCTTTCGGACCTTTC
SC1.2025kAMAS2	GACGCAAGTGAGCAGTATGACTTACCTTTCGGACCCCTA
SC1.2025kRev	TATAATAGACTCGTGGTGGATGG
SC1.2281kAMAS1	GCAACAGGAACCAGCTATGACCACTAGCACACATACAAAC
SC1.2281kAMAS2	GACGCAAGTGAGCAGTATGACCACTAGCACACATACGCAT
SC1.2281kRev	ATGTCGTGAGGGGATGTTTT
SC1.2474kAMAS1	GCAACAGGAACCAGCTATGACAGATGCTTCTGGAAGAAAG
SC1.2474kAMAS2	GACGCAAGTGAGCAGTATGACAGATGCTTCTGGAAGCGAA
SC1.2474kRev	TAAGTAGGGGTGTATCGCCAAT
SC1.2727kAMAS1	GCAACAGGAACCAGCTATGACTAGCAACGGCGACAGTAG
SC1.2727kAMAS2	GACGCAAGTGAGCAGTATGACTAGCAACGGCGACAACAA
SC1.2727kRev	ACAAAGATGGCCAACGAAAC
SC1.2931kAMAS1	GCAACAGGAACCAGCTATGACGTAATTGTCGAAGGACCCG
SC1.2931kAMAS2	GACGCAAGTGAGCAGTATGACGTAATTGTCGAAGGATTCA
SC1.2931kRev	AAATCTCTACGGTCACGGCTA
SC1.3173kAMAS1	GCAACAGGAACCAGCTATGACAGCCGTCTGTATAATCGTTC
SC1.3173kAMAS2	GACGCAAGTGAGCAGTATGACAGCCGTCTGTATAATCACTA
SC1.3173kRev	AGCCTAACTTATTAAAGTTCTAAGTGTCC
SC1.3672kAMAS1	GCAACAGGAACCAGCTATGACCCGCAGCAGTTGCAATAC
SC1.3672kAMAS2	GACGCAAGTGAGCAGTATGACCCGCAGCAGTTGCAGCAT
SC1.3672kRev	AAGGTCCTAGGAAGGGCAAA
SC1.3898kAMAS1	GCAACAGGAACCAGCTATGACTGCAAGCGATAAGATACTCG
SC1.3898kAMAS2	GACGCAAGTGAGCAGTATGACTGCAAGCGATAAGATATCCA
SC1.3898kRev	TATCGAAGAGCAAGCCAGGT
SC1.4077kAMAS1	GCAACAGGAACCAGCTATGACTTGCTCGTTAGAAAGACCC
SC1.4077kAMAS2	GACGCAAGTGAGCAGTATGACTTGCTCGTTAGAAAGGTCT
SC1.4077kRev	GTCCAGCAGAAGCAACATGA
SC1.4553kAMAS1	GCAACAGGAACCAGCTATGACGGGAAGGAAAGAGGACAC
SC1.4553kAMAS2	GACGCAAGTGAGCAGTATGACGGGAAGGAAAGAGGGTAT
SC1.4553kRev	TCCTCGTCACGCAACTACTG
SC1.4785kAMAS1	GCAACAGGAACCAGCTATGACGTTGCTTCAACAGCACCG
SC1.4785kAMAS2	GACGCAAGTGAGCAGTATGACGTTGCTTCAACAGCCTCA
SC1.4785kRev	AGCCCAACTTTGCTTGCTAC
SC1.5087kAMAS1	GCAACAGGAACCAGCTATGACAAGCCATTATCAACCTACCG
SC1.5087kAMAS2	GACGCAAGTGAGCAGTATGACAAGCCATTATCAACCTCACA
SC1.5087kRev	GGAGCTGCAGGTCTTTCTT
SC1.5253KAMAS1	GCAACAGGAACCAGCTATGACGACTAGATGCGCTAACGC
SC1.5253KAMAS2	GACGCAAGTGAGCAGTATGACGACTAGATGCGCTACAGA
SC1.5253KRev	TGTTCGCTTAAACACTTCCTG

Primer name	Primer sequence*
SC1.5508KAMAS1	GCAACAGGAACCAGCTATGACCTTTCTTTCTTATTTGCCTAACATC
SC1.5508KAMAS2	GACGCAAGTGAGCAGTATGACCTTTCTTTCTTATTTGCCTAATCTT
SC1.5508KRev	GAGCAGATCGTTCTGTCGAA
SC1.5710KAMAS1	GCAACAGGAACCAGCTATGACCATGCTGTTGGCAGCTAG
SC1.5710KAMAS2	GACGCAAGTGAGCAGTATGACCATGCTGTTGGCAGTCAA
SC1.5710KRev	AGAGATCGGTCAGGTTGAGC
SC1.5935KAMAS1	GCAACAGGAACCAGCTATGACGAAGTGCGACGATGGCCG
SC1.5935KAMAS2	GACGCAAGTGAGCAGTATGACGAAGTGCGACGATGATCA
SC1.5935KRev	GTCAGCCTTTGCAGTTGTGA
SC1.6139KAMAS1	GCAACAGGAACCAGCTATGACCGAAAGTGGTGAAAAATATCCC
SC1.6139KAMAS2	GACGCAAGTGAGCAGTATGACCGAAAGTGGTGAAAAATACTCT
SC1.6139KRev	GGGCATAAGCTTCAAGAGCA
SC1.6351KAMAS1	GCAACAGGAACCAGCTATGACGTGGGGTTGATGAGGAAG
SC1.6351KAMAS2	GACGCAAGTGAGCAGTATGACGTGGGGTTGATGAGAGAA
SC1.6351KRev	CCAGCACAGAACCAACAAAA
SC1.6550KAMAS1	GCAACAGGAACCAGCTATGACGACTAGAGCACTAGGAGC
SC1.6550KAMAS2	GACGCAAGTGAGCAGTATGACGACTAGAGCACTAGAGGA
SC1.6550KRev	TTGAGCTGAGAAAACAGATGC
SC1.6757kAMAS1	GCAACAGGAACCAGCTATGACGAGGGATCGATCAAGAAAG
SC1.6757kAMAS2	GACGCAAGTGAGCAGTATGACGAGGGATCGATCAAGCAAA
SC1.6757kRev	CCCCCATATCGATAAGATCC
SC17.4KAMAS1	GCAACAGGAACCAGCTATGACCGAAGGACTAGCTATCTG
SC17.4KAMAS2	GACGCAAGTGAGCAGTATGACCGAAGGACTAGCTACATA
SC17.4KRev	AAGCCAGTCTCCATGCATTC
SC17.639KAMAS1	GCAACAGGAACCAGCTATGACGCGAGCTAGTCTTAAAAAC
SC17.639KAMAS2	GACGCAAGTGAGCAGTATGACGCGAGCTAGTCTTAAGCAT
SC17.639KRev	AGCACGCGAGGCTAAATACT
SC16.208KAMAS1	GCAACAGGAACCAGCTATGACGATTGGAGGAGGAGAGAC
SC16.208KAMAS2	GACGCAAGTGAGCAGTATGACGATTGGAGGAGGAGGAAT
SC16.208KRev	ACAGGGCCGATGATACTTCC
SC16.504KAMAS1	GCAACAGGAACCAGCTATGACGCTTAGGGAAAGGGTCTG
SC16.504KAMAS2	GACGCAAGTGAGCAGTATGACGCTTAGGGAAAGGGCATA
SC16.504KRev	TCAGCGATCTAGTTCGAGGTT
SC16.692KAMAS1	GCAACAGGAACCAGCTATGACAGCTAGATAGACCAGCCTG
SC16.692KAMAS2	GACGCAAGTGAGCAGTATGACAGCTAGATAGACCAGTTTA
SC16.692KRev	GGCACCTATAGTTCCCGC
SC2.224KAMAS1	GCAACAGGAACCAGCTATGACGAAATGCTGCAATGCATAAC
SC2.224KAMAS2	GACGCAAGTGAGCAGTATGACGAAATGCTGCAATGCACCAT
SC2.224KRev	CAGTTTTGACGGGAGAAAGC
SC2.427KAMAS1	GCAACAGGAACCAGCTATGACCTACAGCTCAAACAAAAAACAC
SC2.427KAMAS2	GACGCAAGTGAGCAGTATGACCTACAGCTCAAACAAAAACAAA
SC2.427KRev	AATTGACGGAGAGTGCCATT
SC2.654KAMAS1	GCAACAGGAACCAGCTATGACCACAGTGTTCTTCCTAGC
SC2.654KAMAS2	GACGCAAGTGAGCAGTATGACCACAGTGTTCTTCCCCGT
SC2.654KRev	TCGACTTGGAGTGCTTTTTG
SC2.870kAMAS1	GCAACAGGAACCAGCTATGACAGCCCCTGATATCGCACG
SC2.870kAMAS2	GACGCAAGTGAGCAGTATGACAGCCCCTGATATCGACCT
SC2.870kRev	ATCGAGGACAACAAGGCTGA
SC2.2861kAMAS1	GCAACAGGAACCAGCTATGACAGCACACTCGCGCTTAAC
SC2.2861kAMAS2	GACGCAAGTGAGCAGTATGACAGCACACTCGCGCTCCAT
SC2.2861kRev	TAGTCGCGTGAGCTGTTTTC
SC2.3124kAMAS1	GCAACAGGAACCAGCTATGACGCACGCAGCCTGACTACC
SC2.3124kAMAS2	GACGCAAGTGAGCAGTATGACGCACGCAGCCTGACCGCA
SC2.3124kRev	TCAGGATGAGAAACGGTCTG

Primer name	Primer sequence*
SC2.3305kAMAS1	GCAACAGGAACCAGCTATGACATTAGGAGTAGTTTAGGTAAAG
SC2.3305kAMAS2	GACGCAAGTGAGCAGTATGACATTAGGAGTAGTTTAGGTCGAA
SC2.3305kRev	GCACTTAATTCAGGGGATCG
SC25.15kAMAS1	GCAACAGGAACCAGCTATGACTGCTTCAGTAGCAGCATC
SC25.15kAMAS2	GACGCAAGTGAGCAGTATGACTGCTTCAGTAGCAGTCTT
SC25.15kRev	AAAATCCATCGAGGGTCTCC
SC25.122kAMAS1	GCAACAGGAACCAGCTATGACGAAGATCTTGTCTCTGCC
SC25.122kAMAS2	GACGCAAGTGAGCAGTATGACGAAGATCTTGTCTCCACT
SC25.122kRev	TCAACAAGCAAGGCAACATC
SC23.0.6kAMAS1	GCAACAGGAACCAGCTATGACTTGATCGCTGCCTCCCGC
SC23.0.6kAMAS2	GACGCAAGTGAGCAGTATGACTTGATCGCTGCCTCTTGA
SC23.0.6kRev	CCAGAGAAGAAGCAGCAAGC
SC23.47kAMAS1	GCAACAGGAACCAGCTATGACTACCTCTACATATGTGCCTG
SC23.47kAMAS2	GACGCAAGTGAGCAGTATGACTACCTCTACATATGTGTTTA
SC23.47kRev	TGAGTCTCGAGCGAGAAAGG
SC23.145kAMAS1	GCAACAGGAACCAGCTATGACGTTAGCGCCCCTGCATC
SC23.145kAMAS2	GACGCAAGTGAGCAGTATGACGTTAGCGCCCCTGACTT
SC23.145kRev	TGCCTCAAGATTACGTGGTG
SC13.95kAMAS1	GCAACAGGAACCAGCTATGACCACGTCCGGACAGCTCTC
SC13.95kAMAS2	GACGCAAGTGAGCAGTATGACCACGTCCGGACAGCCTTT
SC13.95kRev	CCAGCTTTTAGCAGCTCGTC
SC13.213kAMAS1	GCAACAGGAACCAGCTATGACCACTACACCTAAAAATCACCG
SC13.213kAMAS2	GACGCAAGTGAGCAGTATGACCACTACACCTAAAAATCCTCA
SC13.213kRev	GAGCGAGCTTAGGGATAGGC
SC13.397kAMAS1	GCAACAGGAACCAGCTATGACAAGGGCGATGATAGAGAC
SC13.397kAMAS2	GACGCAAGTGAGCAGTATGACAAGGGCGATGATAGGAAT
SC13.397kRev	TGCTTGCAACACGTGTCTAGT
SC13.812kAMAS1	GCAACAGGAACCAGCTATGACTCCTGTGGCTGGTTATTC
SC13.812kAMAS2	GACGCAAGTGAGCAGTATGACTCCTGTGGCTGGTTGCTT
SC13.812kRev	GCCCTTGGGACTTACAACAG
SC13.1044kAMAS1	GCAACAGGAACCAGCTATGACCAGAAGTAGCGCCCATTG
SC13.1044kAMAS2	GACGCAAGTGAGCAGTATGACCAGAAGTAGCGCCCCCTA
SC13.1044kRev	ACGATACCTGCTGGAAGAGG
SC12.0.3kAMAS1	GCAACAGGAACCAGCTATGACCGCTTAAAAACTTTAGAGATATTG
SC12.0.3kAMAS2	GACGCAAGTGAGCAGTATGACCGCTTAAAAACTTTAGAGATCCTA
SC12.0.3kRev	ACGATTCAGATGCGCTGTTT
SC12.143kAMAS1	GCAACAGGAACCAGCTATGACACCGTGACGGAAATACCGC
SC12.143kAMAS2	GACGCAAGTGAGCAGTATGACACCGTGACGGAAATATTGT
SC12.143kRev	CTCCACCATTCCAACATCCT
SC12.363kAMAS1	GCAACAGGAACCAGCTATGACGGAGCCCTGTGACCTTGG
SC12.363kAMAS2	GACGCAAGTGAGCAGTATGACGGAGCCCTGTGACCCCGA
SC12.363kRev	CGCCCGAAGTACATGAAGAT
SC12.569kAMAS1	GCAACAGGAACCAGCTATGACGATTTGGAGGATAAACGGC
SC12.569kAMAS2	GACGCAAGTGAGCAGTATGACGATTTGGAGGATAAATAGT
SC12.569kRev	ACCTCGACGTCGCAGATTAT
SC12.798kAMAS1	GCAACAGGAACCAGCTATGACGCCTGACGTGCACTTACG
SC12.798kAMAS2	GACGCAAGTGAGCAGTATGACGCCTGACGTGCACTCGCA
SC12.798kRev	TGTCGTCTGCGACCTAAATG
SC12.943kAMAS1	GCAACAGGAACCAGCTATGACCTGGGGGTGGATAGGTTG
SC12.943kAMAS2	GACGCAAGTGAGCAGTATGACCTGGGGGTGGATAGACTA
SC12.943kRev	TGTGGCGACTACAACGTCTT
SC12.1189kAMAS1	GCAACAGGAACCAGCTATGACAATTTTCGGTGGTACCCTTC
SC12.1189kAMAS2	GACGCAAGTGAGCAGTATGACAATTTTCGGTGGTACCACTT
SC12.1189kRev	GGCTATCAGCATGGTACACG

Primer name	Primer sequence*
SC14.8kAMAS1	GCAACAGGAACCAGCTATGACTGTAGGCCTGTACTTCGC
SC14.8kAMAS2	GACGCAAGTGAGCAGTATGACTGTAGGCCTGTACTCTGT
SC14.8kRev	ACCTCCCTCCATCCCTTTTA
SC14.245kAMAS1	GCAACAGGAACCAGCTATGACACCTCGATATTGTATCCTTC
SC14.245kAMAS2	GACGCAAGTGAGCAGTATGACACCTCGATATTGTATCTCTT
SC14.245kRev	TCGCGATGATAGCCTTTTCT
SC14.467kAMAS1	GCAACAGGAACCAGCTATGACTTGCCAGTGGGATGCCGC
SC14.467kAMAS2	GACGCAAGTGAGCAGTATGACTTGCCAGTGGGATGTTGT
SC14.467kRev	CCTGCAGGTTTGTTGTGGTA
SC14.749kAMAS1	GCAACAGGAACCAGCTATGACTTGCGTGTGTGTGTATGG
SC14.749kAMAS2	GACGCAAGTGAGCAGTATGACTTGCGTGTGTGTGTGCGT
SC14.749kRev	AGACCAGCCTCCCAGATACC
SC14.986kAMAS1	GCAACAGGAACCAGCTATGACAGAGACGGAAGTTTTTTTCTTG
SC14.986kAMAS2	GACGCAAGTGAGCAGTATGACAGAGACGGAAGTTTTTTTTCTT
SC14.986kRev	TTCATCTCCACGCCTCAAGT
SC3.101kAMAS1	GCAACAGGAACCAGCTATGACGCCCTGAAAGCCGTCGAG
SC3.101kAMAS2	GACGCAAGTGAGCAGTATGACGCCCTGAAAGCCGTAAAT
SC3.101kRev	AACGGGGCTAATCTCCAAGT
SC3.319kAMAS1	GCAACAGGAACCAGCTATGACGTTAACCCTATAAATATATAGTCTG
SC3.319kAMAS2	GACGCAAGTGAGCAGTATGACGTTAACCCTATAAATATATAGCTTA
SC3.319kRev	TCTTTATCGCCTCGCTTATG
SC3.527kAMAS1	GCAACAGGAACCAGCTATGACTTATGCGGCTAGCAAATAAC
SC3.527kAMAS2	GACGCAAGTGAGCAGTATGACTTATGCGGCTAGCAAACCAT
SC3.527kRev	GCGTGCAACCGAGAATAGAT
SC3.762kAMAS1	GCAACAGGAACCAGCTATGACGTATAGTATAACAGCAACACCCT
SC3.762kAMAS2	GACGCAAGTGAGCAGTATGACGTATAGTATAACAGCAACATACA
SC3.762kRev	GCTTTCCTACTCCCACTAGAAGAA
SC3.968kAMAS1	GCAACAGGAACCAGCTATGACCGATAAAAGAGGAAGATACCG
SC3.968kAMAS2	GACGCAAGTGAGCAGTATGACCGATAAAAGAGGAAGATCACA
SC3.968kRev	GGCGAAGATAAAGCTGAACG
SC3.1170kAMAS1	GCAACAGGAACCAGCTATGACTTGGGAAGACGCGAGCGC
SC3.1170kAMAS2	GACGCAAGTGAGCAGTATGACTTGGGAAGACGCGAAAGA
SC3.1170kRev	TTCTCTCCCTCCCTCCCTTA
SC3.1528kAMAS1	GCAACAGGAACCAGCTATGACAAGGGCATATTCCAATTATCAC
SC3.1528kAMAS2	GACGCAAGTGAGCAGTATGACAAGGGCATATTCCAATTACTAT
SC3.1528kRev	GTCGGCGTAACAGTTCCTTG
SC3.1740kAMAS1	GCAACAGGAACCAGCTATGACGGCAGATGACCAATAACG
SC3.1740kAMAS2	GACGCAAGTGAGCAGTATGACGGCAGATGACCAATCGCA
SC3.1740kRev	ATCTCGAAGCTCGAATGGAA
SC3.1945kAMAS1	GCAACAGGAACCAGCTATGACGGCTGTGCCCGGTGTCAC
SC3.1945kAMAS2	GACGCAAGTGAGCAGTATGACGGCTGTGCCCGGTGCTAT
SC3.1945kRev	CGATGTTGCCCTTCTTCAA
SC3.2644kAMAS1	GCAACAGGAACCAGCTATGACGGATCAATTTTCTCATTTTCTACCAG
SC3.2644kAMAS2	GACGCAAGTGAGCAGTATGACGGATCAATTTTCTCATTTTCTAATAT
SC3.2644kRev	CGCCTATACCCGATCTTCCT
SC3.2983kAMAS1	GCAACAGGAACCAGCTATGACATGAGTTGGATGAGAGGG
SC3.2983kAMAS2	GACGCAAGTGAGCAGTATGACATGAGTTGGATGAGGAGT
SC3.2983kRev	CGTCTACTCGAGCTTAACACACA
SC5.0.6kAMAS1	GCAACAGGAACCAGCTATGACCTCCTCTCTACTATACCTC
SC5.0.6kAMAS2	GACGCAAGTGAGCAGTATGACCTCCTCTCTACTATATTTG
SC5.0.6kRev	CAAAGTTTTTGCAGTACCGAGT
SC5.205kAMAS1	GCAACAGGAACCAGCTATGACGCCAGTTTGTGTAGTTAGTC
SC5.205kAMAS2	GACGCAAGTGAGCAGTATGACGCCAGTTTGTGTAGTTGATT
SC5.205kRev	TCAAAAACACCAGCCTCTCC

Primer name	Primer sequence*
SC5.600kAMAS1	GCAACAGGAACCAGCTATGACTGTGAGGGCGGCTATCAG
SC5.600kAMAS2	GACGCAAGTGAGCAGTATGACTGTGAGGGCGGCTACTAT
SC5.600kRev	GCGAGTAGCGAGGATAGTGG
SC5.804kAMAS1	GCAACAGGAACCAGCTATGACCACGGGTTAACGCGCCAC
SC5.804kAMAS2	GACGCAAGTGAGCAGTATGACCACGGGTTAACGCGATAT
SC5.804kRev	CATCCGAGTCCTGGACAAAT
SC5.1016kAMAS1	GCAACAGGAACCAGCTATGACGTATATTGTTTTCGCAATAGATGCTG
SC5.1016kAMAS2	GACGCAAGTGAGCAGTATGACGTATATTGTTTTCGCAATAGATAATA
SC5.1016kRev	TTGTGGACCCTAGATCAGCTC
SC5.1228kAMAS1	GCAACAGGAACCAGCTATGACTCCGCTAAAGCGGATGCC
SC5.1228kAMAS2	GACGCAAGTGAGCAGTATGACTCCGCTAAAGCGGACACT
SC5.1228kRev	TTCCCACCACTCTAGAGCTTTC
SC5.1772kAMAS1	GCAACAGGAACCAGCTATGACCACGTGCGATTTGGATCG
SC5.1772kAMAS2	GACGCAAGTGAGCAGTATGACCACGTGCGATTTGGCCCA
SC5.1772kRev	AATGCAAACAGGAGAGCACA
SC5.2004kAMAS1	GCAACAGGAACCAGCTATGACTTCCTCTTTTCCAGCCAC
SC5.2004kAMAS2	GACGCAAGTGAGCAGTATGACTTCCTCTTTTCCAGTAAA
SC5.2004kRev	TCTGAAAACAGCCAGCTCAA
SC5.2218kAMAS1	GCAACAGGAACCAGCTATGACTTTTGCGTCCCGATTAAC
SC5.2218kAMAS2	GACGCAAGTGAGCAGTATGACTTTTGCGTCCCGATCCAT
SC5.2218kRev	AGTCACGGTACATGCGATCA
SC4.16kAMAS1	GCAACAGGAACCAGCTATGACGTATACTATCAGAACCCCC
SC4.16kAMAS2	GACGCAAGTGAGCAGTATGACGTATACTATCAGAACATCT
SC4.16kRev	GCCTGCTACGTCCTCATCTG
SC4.158kAMAS1	GCAACAGGAACCAGCTATGACCATGCTGTCAATATTCTATACG
SC4.158kAMAS2	GACGCAAGTGAGCAGTATGACCATGCTGTCAATATTCTACCCT
SC4.158kRev	AAGTCCACGTAGGCCATCC
SC4.530kAMAS1	GCAACAGGAACCAGCTATGACATCTTTGTGGACTTGAACG
SC4.530kAMAS2	GACGCAAGTGAGCAGTATGACATCTTTGTGGACTTGCGCA
SC4.530kRev	AAGCACCAAGCGTTTGAGAT
SC4.976kAMAS1	GCAACAGGAACCAGCTATGACCTCAGTCGTGGCCAATTG
SC4.976kAMAS2	GACGCAAGTGAGCAGTATGACCTCAGTCGTGGCCAGCTT
SC4.976kRev	GTTTGGGTATGACGGACACA
SC4.1245kAMAS1	GCAACAGGAACCAGCTATGACTCGAGTCAGCTCGTTTGG
SC4.1245kAMAS2	GACGCAAGTGAGCAGTATGACTCGAGTCAGCTCGTCCGA
SC4.1245kRev	AATCGCTCCTCCTCCTTCTC
SC4.1536kAMAS1	GCAACAGGAACCAGCTATGACATGTGTTAGTCTTTTATCTTTTTCTTC
SC4.1536kAMAS2	GACGCAAGTGAGCAGTATGACATGTGTTAGTCTTTTATCTTTTTTCTG
SC4.1536kRev	ATTTCGCACCCATGCTTCT
SC4.1777kAMAS1	GCAACAGGAACCAGCTATGACGCGGAGGAGGCGGCACAC
SC4.1777kRev	TCTCGTTTTCGGCGGTCT
SC4.1989kAMAS1	GCAACAGGAACCAGCTATGACGCAAAGCGAAGAGGAACCGG
SC4.1989kAMAS2	GACGCAAGTGAGCAGTATGACGCAAAGCGAAGAGGAATAGA
SC4.1989kRev	CTCGGAAACTGCCGTATGAC
SC4.2188kAMAS1	GCAACAGGAACCAGCTATGACCTCGTCATCGTATCTCCCG
SC4.2188kAMAS2	GACGCAAGTGAGCAGTATGACCTCGTCATCGTATCTTACA
SC4.2188kRev	CAGGGAAGAAAGAGGCAAAG
SC4.2375kAMAS1	GCAACAGGAACCAGCTATGACTACCGCCGAGCAATCAGC
SC4.2375kAMAS2	GACGCAAGTGAGCAGTATGACTACCGCCGAGCAATACGT
SC4.2375kRev	AAGTCGTCGCCTTTGATTTC
SC4.2646kAMAS1	GCAACAGGAACCAGCTATGACTTTGAGCCACAGACAACAG
SC4.2646kAMAS2	GACGCAAGTGAGCAGTATGACTTTGAGCCACAGACACTAA
SC4.2646kRev	GGCTTTGAGAAGTCGGGTTA

Primer name	Primer sequence *
SC11.8kAMAS1	GCAACAGGAACCAGCTATGACTACATGGATCTCACATGC
SC11.8kAMAS2	GACGCAAGTGAGCAGTATGACTACATGGATCTCACCCGA
SC11.8kRev	TCAAGCATTAGGGATGGAATC
SC11.104kAMAS1	GCAACAGGAACCAGCTATGACTTCCTCTCTGCGAAGTAG
SC11.104kAMAS2	GACGCAAGTGAGCAGTATGACTTCCTCTCTGCGAAACAA
SC11.104kRev	AGCTTCCAACTCCACCCTTA
SC11.304kAMAS1	GCAACAGGAACCAGCTATGACGGCGAGTAGCATCCCGAC
SC11.304kAMAS2	GACGCAAGTGAGCAGTATGACGGCGAGTAGCATCCTAAT
SC11.304kRev	GGGGAACTAAGCCATCACTC
SC11.519kAMAS1	GCAACAGGAACCAGCTATGACCAGTTTCGCCATTGTACC
SC11.519kAMAS2	GACGCAAGTGAGCAGTATGACCAGTTTCGCCATTGCCCT
SC11.519kRev	GGCACAGCAACGTCCTACTA
SC11.732kAMAS1	GCAACAGGAACCAGCTATGACCTAAGCCCCCCCCAACGG
SC11.732kAMAS2	GACGCAAGTGAGCAGTATGACCTAAGCCCCCCCCACAGA
SC11.732kRev	TCAGAGGTCTGCCACTACCA
SC11.980kAMAS1	GCAACAGGAACCAGCTATGACGTTCCACCGAAGTCTAAC
SC11.980kAMAS2	GACGCAAGTGAGCAGTATGACGTTCCACCGAAGTCCCAT
SC11.980kRev	ATCAAAACGCAATGATCACC
SC11.1145kAMAS1	GCAACAGGAACCAGCTATGACATGTCACTGTAGCTATCTCG
SC11.1145kAMAS2	GACGCAAGTGAGCAGTATGACATGTCACTGTAGCTATACCT
SC11.1145kRev	ATCCTTGTACGTCCGCTCA
SC11.1283kAMAS1	GCAACAGGAACCAGCTATGACAAGAGCCACTAGCCTTGC
SC11.1283kAMAS2	GACGCAAGTGAGCAGTATGACAAGAGCCACTAGCCCCGT
SC11.1283kRev	TAGGGGCACGTGATCTACAG
SC11.1578kAMAS1	GCAACAGGAACCAGCTATGACGCGGGACACGTGCTCGAC
SC11.1578kAMAS2	GACGCAAGTGAGCAGTATGACGCGGGACACGTGCTAAAT
SC11.1578kRev	GCTTGGAAGGACACAGTCAC
SC15.25kAMAS1	GCAACAGGAACCAGCTATGACGAGGAAGATGAAGTAGATATC
SC15.25kAMAS2	GACGCAAGTGAGCAGTATGACGAGGAAGATGAAGTAGACCTT
SC15.25kRev	GCTAAAGCACTCCCCTGTCT
SC15.193kAMAS1	GCAACAGGAACCAGCTATGACGTACGACTCGAGTAACTG
SC15.193kAMAS2	GACGCAAGTGAGCAGTATGACGTACGACTCGAGTAGTTT
SC15.193kRev	CAGAACCATGGAGAGTCGAG
SC15.377kAMAS1	GCAACAGGAACCAGCTATGACCGCGCGCTCTGGAAGCAG
SC15.377kAMAS2	GACGCAAGTGAGCAGTATGACCGCGCGCTCTGGAAATAA
SC15.377kRev	CCTTCCTCCACACCTACCAC
SC15.529kAMAS1	GCAACAGGAACCAGCTATGACACCCACCCGCGTCCACGC
SC15.529kAMAS2	GACGCAAGTGAGCAGTATGACACCCACCCGCGTCCGTGT
SC15.529kRev	ATGTAGCCGGCTGGGAATTA
SC15.746kAMAS1	GCAACAGGAACCAGCTATGACGGACGAACATTTACTAGTAAG
SC15.746kAMAS2	GACGCAAGTGAGCAGTATGACGGACGAACATTTACTAGCCAA
SC15.746kRev	GGTAGCGCTCATATTCGTAGC
SC15.956kAMAS1	GCAACAGGAACCAGCTATGACGCCATTTTGAGAAGACGG
SC15.956kAMAS2	GACGCAAGTGAGCAGTATGACGCCATTTTGAGAAGCTGA
SC15.956kRev	CCATGTCTCTAGGCTGACCA
SC30.0.1kAMAS1	GCAACAGGAACCAGCTATGACGCAGTATCCTTACCCCAC
SC30.0.1kAMAS2	GACGCAAGTGAGCAGTATGACGCAGTATCCTTACCATAT
SC30.0.1kRev	GCAAAGGGGTAGTGGAACA
SC30.8kAMAS1	GCAACAGGAACCAGCTATGACGCTAGAGATTTCTCGACTG
SC30.8kAMAS2	GACGCAAGTGAGCAGTATGACGCTAGAGATTTCTCGCTTA
SC30.8kRev	ACCGAACAACGCCAAGAGTA
SC30.37kAMAS1	GCAACAGGAACCAGCTATGACGGAAACGACTAGCCAACG
SC30.37kAMAS2	GACGCAAGTGAGCAGTATGACGGAAACGACTAGCCCGCA
SC30.37kRev	TTGACGCGTTGTCTATGTTTG

Primer name	Primer sequence*
SC8.6kAMAS1	GCAACAGGAACCAGCTATGACAAGCTCGAAATTATATAACCGCC
SC8.6kAMAS2	GACGCAAGTGAGCAGTATGACAAGCTCGAAATTATATAACTACT
SC8.6kRev	AGCGCCTATCCCTAAGCTC
SC8.115kAMAS1	GCAACAGGAACCAGCTATGACCACACGCACGAGCACAAC
SC8.115kAMAS2	GACGCAAGTGAGCAGTATGACCACACGCACGAGCAACAT
SC8.115kRev	GTTGTTTTGTGTAGGCGTCTG
SC8.470kAMAS1	GCAACAGGAACCAGCTATGACCTATGGTATCTCATCATCTGG
SC8.470kAMAS2	GACGCAAGTGAGCAGTATGACCTATGGTATCTCATCATACGA
SC8.470kRev	AGAACGGCACAGATCAGGT
SC8.696kAMAS1	GCAACAGGAACCAGCTATGACGACAGAGCAAAGAATATAATTACTG
SC8.696kAMAS2	GACGCAAGTGAGCAGTATGACGACAGAGCAAAGAATATAATTCATA
SC8.696kRev	ACGTTACGTATTGCTGAACCA
SC8.800kAMAS1	GCAACAGGAACCAGCTATGACGAGAAACGACCTGGTCCC
SC8.800kAMAS2	GACGCAAGTGAGCAGTATGACGAGAAACGACCTGGCACA
SC8.800kRev	TCGTTGCTCATTTGGAGTTG
SC8.1433kAMAS1	GCAACAGGAACCAGCTATGACCTGTAAATCACCCTCAATG
SC8.1433kAMAS2	GACGCAAGTGAGCAGTATGACCTGTAAATCACCCTCCGTA
SC8.1433kRev	TGAGCAGCAACTTTATGCAA
SC8.1640kAMAS1	GCAACAGGAACCAGCTATGACAGTTGTCCGACTTCTTTGAC
SC8.1640kAMAS2	GACGCAAGTGAGCAGTATGACAGTTGTCCGACTTCTTCAAT
SC8.1640kRev	ACGCAGACGCAGAGACTAAA
SC8.1758kAMAS1	GCAACAGGAACCAGCTATGACCACCTCCACCACCACTTC
SC8.1758kAMAS2	GACGCAAGTGAGCAGTATGACCACCTCCACCACCATCTA
SC8.1758kRev	GCGATTTGAGTGATTTGGTG
SC24.48kAMAS1	GCAACAGGAACCAGCTATGACGTTGCGCCCCGGGGCACG
SC24.48kAMAS2	GACGCAAGTGAGCAGTATGACGTTGCGCCCCGGGGTGCA
SC24.48kRev	CTTTGGTCACTCGTCCCTGT
SC24.158kAMAS1	GCAACAGGAACCAGCTATGACAGAGGAAGACAGACCGGC
SC24.158kAMAS2	GACGCAAGTGAGCAGTATGACAGAGGAAGACAGACAAGT
SC24.158kRev	TGTCGGCTTCTTTTACTTGC
SC26.45kAMAS1	GCAACAGGAACCAGCTATGACCAAAAGAATATTATAGGAAGAACTG
SC26.45kAMAS2	GACGCAAGTGAGCAGTATGACCAAAAGAATATTATAGGAAGACATA
SC26.45kRev	AAAAAGGGCGATGATCTAGG
SC26.120kAMAS1	GCAACAGGAACCAGCTATGACATCGTTAAACGCTTTAACGTCAT
SC26.120kAMAS2	GACGCAAGTGAGCAGTATGACATCGTTAAACGCTTAACGCTAA
SC26.120kRev	ACATGGGCAAATCGTTTTTA
SC27.41kAMAS1	GCAACAGGAACCAGCTATGACGCTAACGGGTTGCTCCTG
SC27.41kAMAS2	GACGCAAGTGAGCAGTATGACGCTAACGGGTTGCTTTTA
SC27.41kRev	AAGCTGGACATTGGCTTTGT
SC22.0.008kAMAS1	GCAACAGGAACCAGCTATGACACGCAAATTGATCCCCAC
SC22.0.008kAMAS2	GACGCAAGTGAGCAGTATGACACGCAAATTGATCCATAT
SC22.0.008kRev	GAACGGCTTTTGCTTTTGTC
SC22.168kAMAS1	GCAACAGGAACCAGCTATGACTTTCTATGTCGAGTCGGC
SC22.168kAMAS2	GACGCAAGTGAGCAGTATGACTTTCTATGTCGAGTTAGT
SC22.168kRev	TTTGTTGCGCACCTCTGTAG
SC9.5kAMAS1	GCAACAGGAACCAGCTATGACAAAGGAGGGGGGAGTCGG
SC9.5kAMAS2	GACGCAAGTGAGCAGTATGACAAAGGAGGGGGGAGCAGA
SC9.5kRev	TCTTTTTGACATCCGTGTCG
SC9.100kAMAS1	GCAACAGGAACCAGCTATGACCATCCATAAAAATCTGACAATCAG
SC9.100kAMAS2	GACGCAAGTGAGCAGTATGACCATCCATAAAAATCTGACAACTAA
SC9.100kRev	GTGGGGATCGTCGTATTGAC
SC9.377kAMAS1	GCAACAGGAACCAGCTATGACTACATCCACCAACCCAAC
SC9.377kAMAS2	GACGCAAGTGAGCAGTATGACTACATCCACCAACCACAT
SC9.377kRev	TGCTAACCGCTAGTCCCATT

Primer name	Primer sequence*
SC9.553kAMAS1	GCAACAGGAACCAGCTATGACCACGTGGGTCTTTCATAG
SC9.553kAMAS2	GACGCAAGTGAGCAGTATGACCACGTGGGTCTTTCCCAA
SC9.553kRev	AAGAATAGGAGCCTGGCACA
SC9.779kAMAS1	GCAACAGGAACCAGCTATGACTCCTTCCATCGCCCTCGG
SC9.779kAMAS2	GACGCAAGTGAGCAGTATGACTCCTTCCATCGCCCCTGA
SC9.779kRev	GAAGAGCTGGAGGTGCAAGT
SC9.1043kAMAS1	GCAACAGGAACCAGCTATGACCCATTTGCGCCATCCTCC
SC9.1043kAMAS2	GACGCAAGTGAGCAGTATGACCCATTTGCGCCATCTCCT
SC9.1043kRev	TGCAGCATATCGACAAAAGA
SC9.1411kAMAS1	GCAACAGGAACCAGCTATGACATGGAGCTAGAGAGGTAG
SC9.1411kAMAS2	GACGCAAGTGAGCAGTATGACATGGAGCTAGAGAGACAA
SC9.1411kRev	CCCGATGTTCATCATTTGTT
SC24.48kAMAS1	GCAACAGGAACCAGCTATGACGTTGCGCCCCGGGGCACG
SC24.48kAMAS2	GACGCAAGTGAGCAGTATGACGTTGCGCCCCGGGGTGCA
SC24.48kRev	CTTTGGTCACTCGTCCCTGT
SC9.1750kAMAS1	GCAACAGGAACCAGCTATGACTGATAACTCGGTTGGCCG
SC9.1750kAMAS2	GACGCAAGTGAGCAGTATGACTGATAACTCGGTTGAACA
SC9.1750kRev	ATTGTTATCTCCGCCCTACC
SC6.7kAMAS1	GCAACAGGAACCAGCTATGACGTTGTCCAACGCTGTCCG
SC6.7kAMAS2	GACGCAAGTGAGCAGTATGACGTTGTCCAACGCTGCTCA
SC6.7kRev	TATTTTGGGTGGTGGACAAA
SC6.116kAMAS1	GCAACAGGAACCAGCTATGACGTTGCACTACGTAACCAG
SC6.116kAMAS2	GACGCAAGTGAGCAGTATGACGTTGCACTACGTAATTAA
SC6.116kRev	ACAATCGCCAAATCCTCTCT
SC6.220kAMAS1	GCAACAGGAACCAGCTATGACGTTTCTTCATCGTGTAGTTTC
SC6.220kAMAS2	GACGCAAGTGAGCAGTATGACGTTTCTTCATCGTGTAGCCTT
SC6.220kRev	GGCGTCTTCCAGGACTATGT
SC6.587kAMAS1	GCAACAGGAACCAGCTATGACTGTGGGCAAGTAGATTTATTG
SC6.587kAMAS2	GACGCAAGTGAGCAGTATGACTGTGGGCAAGTAGATTTCCTA
SC6.587kRev	GGTGCCTTGAATGAAATCTG
SC6.792kAMAS1	GCAACAGGAACCAGCTATGACGCGACATCGACAAAAGCC
SC6.792kAMAS2	GACGCAAGTGAGCAGTATGACGCGACATCGACAAAGACT
SC6.792kRev	GTTCACTTGCGAACTGGAAG
SC6.1006kAMAS1	GCAACAGGAACCAGCTATGACGCCAAAGATCATTTTGCGCTT
SC6.1006kAMAS2	GACGCAAGTGAGCAGTATGACGCCAAAGATCATTTTGCATTA
SC6.1006kRev	CGCAGAAGCCTTTCAACATA
SC6.1193kAMAS1	GCAACAGGAACCAGCTATGACCAGTGCTTCGTCCGACCG
SC6.1193kAMAS2	GACGCAAGTGAGCAGTATGACCAGTGCTTCGTCCGCACA
SC6.1193kRev	AGGCTGGAAATCGAAGAGAG
SC6.1373kAMAS1	GCAACAGGAACCAGCTATGACTTCTTTCGGTACAGTTAGG
SC6.1373kAMAS2	GACGCAAGTGAGCAGTATGACTTCTTTCGGTACAGTCGGA
SC6.1373kRev	CCCATATCATCACCCCCTAT
SC6.1783kAMAS1	GCAACAGGAACCAGCTATGACGTGAGGGTGTTACTTATC
SC6.1783kAMAS2	GACGCAAGTGAGCAGTATGACGTGAGGGTGTTACTCCTT
SC6.1783kRev	GGTGCAGAAGTGAGGTTTTG
SC6.1956kAMAS1	GCAACAGGAACCAGCTATGACCCATGGGTTCCTGTGAGG
SC6.1956kAMAS2	GACGCAAGTGAGCAGTATGACCCATGGGTTCCTGTAGGA
SC6.1956kRev	TGCTACTCTCCCTCTTCTTCTGT
SC7.212kAMAS1	GCAACAGGAACCAGCTATGACTATGATAGTAAGGAAAAAGACGC
SC7.212kAMAS2	GACGCAAGTGAGCAGTATGACTATGATAGTAAGGAAAAAGCTGA
SC7.212kRev	AGTTATGGCGGACGATTTTT
SC7.664kAMAS1	GCAACAGGAACCAGCTATGACAAAGGGGCGCGAAATTTC
SC7.664kAMAS2	GACGCAAGTGAGCAGTATGACAAAGGGGCGCGAAACCTT
SC7.664kRev	AGGTAGTTCGCGAAAGAAGTAAG

Primer name	Primer sequence*
SC7.891kAMAS1	GCAACAGGAACCAGCTATGACCATTCGAACAGGTGTATAC
SC7.891kAMAS2	GACGCAAGTGAGCAGTATGACCATTCGAACAGGTGTGCAT
SC7.891kRev	GAGTTTAAAACGCGGAGAATC
SC7.1097kAMAS1	GCAACAGGAACCAGCTATGACTTGCGATCGAAACGAAATG
SC7.1097kAMAS2	GACGCAAGTGAGCAGTATGACTTGCGATCGAAACGACGTA
SC7.1097kRev	CCATTCCACGTTCAAAAGAA
SC7.1291kAMAS1	GCAACAGGAACCAGCTATGACGTAAGCGCCGCCTTTCTC
SC7.1291kAMAS2	GACGCAAGTGAGCAGTATGACGTAAGCGCCGCCTTCTTT
SC7.1291kRev	AGACTGCGAAAAAGCATGAA
SC7.1537kAMAS1	GCAACAGGAACCAGCTATGACCCTTCATTCACACATCGG
SC7.1537kAMAS2	GACGCAAGTGAGCAGTATGACCCTTCATTCACACACTGA
SC7.1537kRev	CCGAATGCAGTGGAATAAAA
SC7.1723kAMAS1	GCAACAGGAACCAGCTATGACTGGCCTCTCCTAGCTCTG
SC7.1723kAMAS2	GACGCAAGTGAGCAGTATGACTGGCCTCTCCTAGCCTTT
SC7.1723kRev	GAATGGATGTGCAACTAGGC
SC7.1826kAMAS1	GCAACAGGAACCAGCTATGACTCTGGGGCCAGGATTAAG
SC7.1826kAMAS2	GACGCAAGTGAGCAGTATGACTCTGGGGCCAGGATCGAA
SC7.1826kRev	ACATGTCAGGACAGCCTTGTA
SC19.17kAMAS1	GCAACAGGAACCAGCTATGACACCCCATAAGACCCAAAC
SC19.17kAMAS2	GACGCAAGTGAGCAGTATGACACCCCATAAGACCCGCAT
SC19.17kRev	ACAGTGGCCAAGAATTACGA
SC19.101kAMAS1	GCAACAGGAACCAGCTATGACAATCGTTCGATCCCCCAC
SC19.101kAMAS2	GACGCAAGTGAGCAGTATGACAATCGTTCGATCCCATAT
SC19.101kRev	GATGAAAGACCGTGCAAAAC
SC19.240kAMAS1	GCAACAGGAACCAGCTATGACGCTTAAGTAATCGAGCCTG
SC19.240kAMAS2	GACGCAAGTGAGCAGTATGACGCTTAAGTAATCGAGTTTA
SC19.240kRev	ACGATGAGATGGTACGACGA
SC19.328kAMAS1	GCAACAGGAACCAGCTATGACTCTGGTGGGTGCTGCTGC
SC19.328kAMAS2	GACGCAAGTGAGCAGTATGACTCTGGTGGGTGCTGTCGT
SC19.328kRev	TCTGGGTTCAGTCCAGGGTA
SC20.8kAMAS1	GCAACAGGAACCAGCTATGACCAGTGGGGATAGACTTTC
SC20.8kAMAS2	GACGCAAGTGAGCAGTATGACCAGTGGGGATAGACCCTT
SC20.8kRev	TGCAGCCGCTAGATGTAGTT
SC20.113kAMAS1	GCAACAGGAACCAGCTATGACTTCAACAAACCAACAAAAGCG
SC20.113kAMAS2	GACGCAAGTGAGCAGTATGACTTCAACAAACCAACAAAGACT
SC20.113kRev	GCCCACTTCCTCTTCTTCTC
SC20.238kAMAS1	GCAACAGGAACCAGCTATGACAATAATCACCGAACAGCATC
SC20.238kAMAS2	GACGCAAGTGAGCAGTATGACAATAATCACCGAACAGACTG
SC20.238kRev	TCCTCGTCACCTGCTTACTC
SC21.20kAMAS1	GCAACAGGAACCAGCTATGACCGTTTTTTGCATAAGAGACCGG
SC21.20kAMAS2	GACGCAAGTGAGCAGTATGACCGTTTTTTGCATAAGAGATAGA
SC21.20kRev	TTTCTCTTACCCAAGGGCTTA
SC21.162kAMAS1	GCAACAGGAACCAGCTATGACCAAATCAGACTAGTAACGGC
SC21.162kAMAS2	GACGCAAGTGAGCAGTATGACCAAATCAGACTAGTAAAAGT
SC21.162kRev	ACGACGACAGCGATCTCTTA
SC21.236kAMAS1	GCAACAGGAACCAGCTATGACCGTAGAAGACTATAAACAACTC
SC21.236kAMAS2	GACGCAAGTGAGCAGTATGACCGTAGAAGACTATAAACAGTTT
SC21.236kRev	TGTTTGACACGTTGCATCTC
SC7.1291kAMAS1	GCAACAGGAACCAGCTATGACGTAAGCGCCGCCTTTCTC
SC7.1291kAMAS2	GACGCAAGTGAGCAGTATGACGTAAGCGCCGCCTTCTTT
SC7.1291kRev	AGACTGCGAAAAAGCATGAA

Primer name	Primer sequence*
SC28.14kAMAS1	GCAACAGGAACCAGCTATGACACTTGAAGCACTGCTCCG
SC28.14kAMAS2	GACGCAAGTGAGCAGTATGACACTTGAAGCACTGCCTCA
SC28.14kRev	AGAGGACGCCGACAAGAT
SC28.48kAMAS1	GCAACAGGAACCAGCTATGACTCACGCGCACGGACCCGG
SC28.48kAMAS2	GACGCAAGTGAGCAGTATGACTCACGCGCACGGACATGT
SC28.48kRev	CATAGAACGGCTTAGCCAAA
SC29.53kAMAS1	GCAACAGGAACCAGCTATGACGATAATGTAAGCCTAAATTAGCGTC
SC29.53kAMAS2	GACGCAAGTGAGCAGTATGACGATAATGTAAGCCTAAATTAGAATT
SC29.53kRev	TTCTGGGATTTCTAAGCTCGT
SC32.20kAMAS1	GCAACAGGAACCAGCTATGACAAGGAAAGGGGGTGATGG
SC32.20kAMAS2	GACGCAAGTGAGCAGTATGACAAGGAAAGGGGGTGCCGA
SC32.20kRev	CTTCCTGTTCGCCCTACAAC
SC34.6kAMAS1	GCAACAGGAACCAGCTATGACTAGGTAATCACTGCAAACATG
SC34.6kAMAS2	GACGCAAGTGAGCAGTATGACTAGGTAATCACTGCAAATGTA
SC34.6kRev	GCCTGTAGTGGAGGTTGATG
SC34.213kAMAS1	GCAACAGGAACCAGCTATGACAGAACAGCACCAACCGTC
SC34.213kAMAS2	GACGCAAGTGAGCAGTATGACAGAACAGCACCAACAATT
SC34.213kRev	GTTTGCTCTGCTTGCGTAGT
SC35.26kAMAS1	GCAACAGGAACCAGCTATGACTCAGTTTCCAACGTCTACTC
SC35.26kAMAS2	GACGCAAGTGAGCAGTATGACTCAGTTTCCAACGTCTGTTT
SC35.26kRev	TTCTCTCAACTAAGGCCAGGT
SC35.34kAMAS1	GCAACAGGAACCAGCTATGACGAGGTAAATAGCTTTGCCAG
SC35.34kAMAS2	GACGCAAGTGAGCAGTATGACGAGGTAAATAGCTTTGTTAT
SC35.34kRev	TAGCCTGGTTCGACGAAAT
SC36.15kAMAS1	GCAACAGGAACCAGCTATGACGCGAGACCTTACCTCTAC
SC36.15kAMAS2	GACGCAAGTGAGCAGTATGACGCGAGACCTTACCTACAT
SC36.15kRev	TATCGTGTTTCTTCCGCAAA
SC36.33kAMAS1	GCAACAGGAACCAGCTATGACACTTCTGTAGCTCGACCG
SC36.33kAMAS2	GACGCAAGTGAGCAGTATGACACTTCTGTAGCTCGCTCA
SC36.33kRev	CGAGCAGAAAGCAGCAAC
SC38.1kAMAS1	GCAACAGGAACCAGCTATGACCTTGAGTTATTGCTTCGCC
SC38.1kAMAS2	GACGCAAGTGAGCAGTATGACCTTGAGTTATTGCTTTACT
SC38.1kRev	CCTTGCAAGATATGGACCAG
SC40.6kAMAS1	GCAACAGGAACCAGCTATGACCTGTAGCTTGTTTCCCGC
SC40.6kAMAS2	GACGCAAGTGAGCAGTATGACCTGTAGCTTGTTTCTAGA
SC40.6kRev	TACAAAGCTCAACCGCAAA
SC42.8kAMAS1	GCAACAGGAACCAGCTATGACGCACTATAGCGATGTTCG
SC42.8kAMAS2	GACGCAAGTGAGCAGTATGACGCACTATAGCGATGCCCA
SC42.8kRev	TCCAGAGGTTCTCAATGTCG
SC42.12kAMAS1	GCAACAGGAACCAGCTATGACGTGAGTGTGCCCTCCCTG
SC42.12kAMAS2	GACGCAAGTGAGCAGTATGACGTGAGTGTGCCCTCTATA
SC42.12kRev	AACCCCAACCCAAGACTG
SC1.500kAMAS1	GCAACAGGAACCAGCTATGACCAATCTTGATCTTACCATTCG
SC1.500kAMAS2	GACGCAAGTGAGCAGTATGACCAATCTTGATCTTACCACCCA
SC1.500kRev	GAAGGAGATGGGAGTGCAAA
SC1.583kAMAS1	GCAACAGGAACCAGCTATGACACTCGCATCCACCGCCGC
SC1.583kAMAS2	GACGCAAGTGAGCAGTATGACACTCGCATCCACCGTTGA
SC1.583kRev	CCTTTGAGACGATGCAGGA
SC1.3081kAMAS1	GCAACAGGAACCAGCTATGACTTGTAGAGGCGAGAAGGG
SC1.3081kAMAS2	GACGCAAGTGAGCAGTATGACTTGTAGAGGCGAGAGAGT
SC1.3081kRev	AATCCAACAGACACCGTCCT
SC1.3228kAMAS1	GCAACAGGAACCAGCTATGACAAGTGAGCTATGCTTCACC
SC1.3228kAMAS2	GACGCAAGTGAGCAGTATGACAAGTGAGCTATGCTTACCT
SC1.3228kRev	GCATGGGTCAAGCTCTTTGT

Primer name	Primer sequence*
SC1.3338kAMAS1	GCAACAGGAACCAGCTATGACCCCCCCTCCCCCCCCTTC
SC1.3338kAMAS2	GACGCAAGTGAGCAGTATGACCCCCCCTCCCCCCCTCTT
SC1.3338kRev	AGGGTACTAGGCAACCTCCAA
SC1.4914kAMAS1	GCAACAGGAACCAGCTATGACTTTTGGTATCATTGGGAAGC
SC1.4914kAMAS2	GACGCAAGTGAGCAGTATGACTTTTGGTATCATTGGGGCGT
SC1.4914kRev	GCGACTACATTGCCACTTCA
SC1.5377kAMAS1	GCAACAGGAACCAGCTATGACGTGCAATGACCTTTGAATCGT
SC1.5377kAMAS2	GACGCAAGTGAGCAGTATGACGTGCAATGACCTTTGAACTGA
SC1.5377kRev	CGAAGCCATGTTCAGACCTC
SC1.5814kAMAS1	GCAACAGGAACCAGCTATGACCTGTGGTAGCCAGCCCCC
SC1.5814kAMAS2	GACGCAAGTGAGCAGTATGACCTGTGGTAGCCAGCATCT
SC1.5814kRev	TCGATCTCATGTCGCCTTTA
SC1.6475kAMAS1	GCAACAGGAACCAGCTATGACGCTGCGCAGTCGGGCAG
SC1.6475kAMAS2	GACGCAAGTGAGCAGTATGACGCTGCGCAGTCGGATAA
SC1.6475kRev	GCCGTAAGGAACAGGTTCTG
SC1.6551kAMAS1	GCAACAGGAACCAGCTATGACGACTAGAGCACTAGGAGC
SC1.6551kAMAS2	GACGCAAGTGAGCAGTATGACGACTAGAGCACTAGAGGA
SC1.6551kRev	GAAAACAGATGCCGTGGAAC
SC1.6752kAMAS1	GCAACAGGAACCAGCTATGACCTCCTCTATATTCTCTACCC
SC1.6752kAMAS2	GACGCAAGTGAGCAGTATGACCTCCTCTATATTCTCTCACA
SC1.6752kRev	AATAGCAGGAGCATCGCTAGA
SC12.499kAMAS1	GCAACAGGAACCAGCTATGACTAGGAGCAAGAGCACTCG
SC12.499kAMAS2	GACGCAAGTGAGCAGTATGACTAGGAGCAAGAGCATCCA
SC12.499kRev	CGTAGAGTGCCCTGCAAGTT
SC12.1155kAMAS1	GCAACAGGAACCAGCTATGACTTTCGGGTTCGCCTCAC
SC12.1155kAMAS2	GACGCAAGTGAGCAGTATGACTTTCGGGTTCGCCCTAT
SC12.1155kRev	TGCTAATGCCTTGTGAGACG
SC14.10kAMAS1	GCAACAGGAACCAGCTATGACGCTCGCGTCGGTAGTTC
SC14.10kAMAS2	GACGCAAGTGAGCAGTATGACGCTCGCGTCGGTAACTA
SC14.10kRev	GCTTAGTCAGATCGCTGATGC
SC14.114kAMAS1	GCAACAGGAACCAGCTATGACAGCAGGAAAGGGGCGCTG
SC14.114kAMAS2	GACGCAAGTGAGCAGTATGACAGCAGGAAAGGGGCAATA
SC14.114kRev	GGGGATGAGCGAGCTAAGA
SC14.850kAMAS1	GCAACAGGAACCAGCTATGACATGGTGATGCGATGTCATG
SC14.850kAMAS2	GACGCAAGTGAGCAGTATGACATGGTGATGCGATGTTGTA
SC14.850kRev	ACAGGGCAGGAAAGACAGAA
SC14.1022kAMAS1	GCAACAGGAACCAGCTATGACTGTGAGAGTGAAAGCGGC
SC14.1022kAMAS2	GACGCAAGTGAGCAGTATGACTGTGAGAGTGAAAGAAGT
SC14.1022kRev	CGAAGCCGCTAAAACAATTC
SC13.213kAMAS1	GCAACAGGAACCAGCTATGACCACTACACCTAAAAATCACCG
SC13.213kAMAS2	GACGCAAGTGAGCAGTATGACCACTACACCTAAAAATCCTCA
SC13.213kRev	AGCTTGCTTAGCTTGGTTGG
SC13.295kAMAS1	GCAACAGGAACCAGCTATGACAAAAAAAACTCCGGTGACATAGAC
SC13.295kAMAS2	GACGCAAGTGAGCAGTATGACAAAAAAAACTCCGGTGACATGAAT
SC13.295kRev	AACCACCACCCTCACAGAAC
SC3.255kAMAS1	GCAACAGGAACCAGCTATGACAGAGTATGAAGTGGTGATTG
SC3.255kAMAS2	GACGCAAGTGAGCAGTATGACAGAGTATGAAGTGGTGCCTA
SC3.255kRev	CGATCCACGTACAGCCTTCT
SC3.405kAMAS1	GCAACAGGAACCAGCTATGACTGGGATGCTTTCGCCCCG
SC3.405kAMAS2	GACGCAAGTGAGCAGTATGACTGGGATGCTTTCGCTACA
SC3.405kRev	GTTTTCCCCCGAGAAGATTT
SC3.716kAMAS1	GCAACAGGAACCAGCTATGACCATCATCACTCCAACGAC
SC3.716kAMAS2	GACGCAAGTGAGCAGTATGACCATCATCACTCCAATAAT
SC3.716kRev	CTTCATGGCCGAGTTTTCTC

Primer name	Primer sequence*
SC3.2090kAMAS1	GCAACAGGAACCAGCTATGACGAAGCGTGCCGTATCGAC
SC3.2090kAMAS2	GACGCAAGTGAGCAGTATGACGAAGCGTGCCGTATTAAT
SC3.2090kRev	CGCAGAAACCCCACAAATAA
SC3.2199kAMAS1	GCAACAGGAACCAGCTATGACAGATGAGGCTCTGTGCC
SC3.2199kAMAS2	GACGCAAGTGAGCAGTATGACAGATGAGGCTCTGCACG
SC3.2199kRev	GCAACCGGCTATTCCATCTA
SC3.2305kAMAS1	GCAACAGGAACCAGCTATGACGTTTTGCATGGGCTGCGCC
SC3.2305kAMAS2	GACGCAAGTGAGCAGTATGACGTTTTGCATGGGCTGTACT
SC3.2305kRev	CTGGACGTTCGGATAGAAGC
SC3.2836kAMAS1	GCAACAGGAACCAGCTATGACCTCCTGACATATCTACACC
SC3.2836kAMAS2	GACGCAAGTGAGCAGTATGACCTCCTGACATATCTAACCT
SC3.2836kRev	ATCCATGTTCAAAGCCAAGC
SC3.3070kAMAS1	GCAACAGGAACCAGCTATGACCAAACGTTACTCCAAAAGG
SC3.3070kAMAS2	GACGCAAGTGAGCAGTATGACCAAACGTTACTCCAAGCGT
SC3.3070kRev	TGCCTGTCAGTCGAAATGAA
SC5.936kAMAS1	GCAACAGGAACCAGCTATGACCATATTCTCGACCCATCTG
SC5.936kAMAS2	GACGCAAGTGAGCAGTATGACCATATTCTCGACCCAATTA
SC5.936kRev	ACTCATAATGCGGGGGATCT
SC5.1199kAMAS1	GCAACAGGAACCAGCTATGACTTTTGAGTTTACAGCAGCTTC
SC5.1199kAMAS2	GACGCAAGTGAGCAGTATGACTTTTGAGTTTACAGCAGACTG
SC5.1199kRev	AAGTCGCAAGCTGATTGACA
SC5.1349kAMAS1	GCAACAGGAACCAGCTATGACCCCACATCCCCAAAATAC
SC5.1349kAMAS2	GACGCAAGTGAGCAGTATGACCCCACATCCCCAAACCAA
SC5.1349kRev	GATGGTTGGGGGATAGGTGT
SC5.1460kAMAS1	GCAACAGGAACCAGCTATGACGTTTTGGATTCATGACCTTTT
SC5.1460kAMAS2	GACGCAAGTGAGCAGTATGACGTTTTGGATTCATGACCCCTA
SC5.1460kRev	GTTTGGATAGTGGGCGAGTT
SC5.2346kAMAS1	GCAACAGGAACCAGCTATGACTCCACCTTCTCCACTAAC
SC5.2346kAMAS2	GACGCAAGTGAGCAGTATGACTCCACCTTCTCCACCCAT
SC5.2346kRev	GTCTGCGATTTTGCAGAGGT
SC5.2437kAMAS1	GCAACAGGAACCAGCTATGACTGTCTGCACCCTTCTTGG
SC5.2437kAMAS2	GACGCAAGTGAGCAGTATGACTGTCTGCACCCTTCCCGA
SC5.2437kRev	AGTAATGAGGGCGATGGTTG
SC5.2532kAMAS1	GCAACAGGAACCAGCTATGACGAATAATTAGGCGCGTTTTCAC
SC5.2532kAMAS2	GACGCAAGTGAGCAGTATGACGAATAATTAGGCGCGTTTCTAT
SC5.2532kRev	CGTATCAACCCATGGAGAGAA
SC4.272kAMAS1	GCAACAGGAACCAGCTATGACTTTCCATGCTCTTGCATAAAC
SC4.272kAMAS2	GACGCAAGTGAGCAGTATGACTTTCCATGCTCTTGCATGCAT
SC4.272kRev	ATCCGCAAAGAGCTGGAGT
SC4.634kAMAS1	GCAACAGGAACCAGCTATGACCTATAGGGCGTAATAACGG
SC4.634kAMAS2	GACGCAAGTGAGCAGTATGACCTATAGGGCGTAATACAGA
SC4.634kRev	AAGGTAGATGTGATCGCCGTA
SC4.1883kAMAS1	GCAACAGGAACCAGCTATGACTGCTCTCTGCCCCTCACC
SC4.1883kAMAS2	GACGCAAGTGAGCAGTATGACTGCTCTCTGCCCCTACCG
SC4.1883kRev	GGCAAGCTTGACTTGATGG
SC4.2508kAMAS1	GCAACAGGAACCAGCTATGACGAGGAGTCGCAACCCCAG
SC4.2508kAMAS2	GACGCAAGTGAGCAGTATGACGAGGAGTCGCAACCTAAA
SC4.2508kRev	GATGCCGTTTGTGACATTTG
SC11.474kAMAS1	GCAACAGGAACCAGCTATGACATGCAGCAGCAACAGTAG
SC11.474kAMAS2	GACGCAAGTGAGCAGTATGACATGCAGCAGCAACAACAA
SC11.474kRev	GTGTGGCTGAGGAGTGTTCA
SC15.644kAMAS1	GCAACAGGAACCAGCTATGACGCCTGCATCAGACATTTG
SC15.644kAMAS2	GACGCAAGTGAGCAGTATGACGCCTGCATCAGACACCTT
SC15.644kRev	CGTTCCCTTTTCTGTTTGGA

Primer name	Primer sequence*
SC26.75kAMAS1	GCAACAGGAACCAGCTATGACCACAGGCCACGGCTTACG
SC26.75kAMAS2	GACGCAAGTGAGCAGTATGACCACAGGCCACGGCTCCCT
SC26.75kRev	CGCACTGCGAAGACTAACTG
SC8.131kAMAS1	GCAACAGGAACCAGCTATGACTGGAGCATGAGTTTTGAGG
SC8.131kAMAS2	GACGCAAGTGAGCAGTATGACTGGAGCATGAGTTTTAGGA
SC8.131kRev	CCTCCCATACTCCATCTCCA
SC8.262kAMAS1	GCAACAGGAACCAGCTATGACGATGCTTGACAGTGTAGG
SC8.262kAMAS2	GACGCAAGTGAGCAGTATGACGATGCTTGACAGTGCGGA
SC8.262kRev	GAGCACACCTGGGTCAAAGT
SC8.367kAMAS1	GCAACAGGAACCAGCTATGACATGGCTTACTACAGCCAG
SC8.367kAMAS2	GACGCAAGTGAGCAGTATGACATGGCTTACTACAGTAAA
SC8.367kRev	AGGCTGGCAGCTGTACTCAT
SC8.1036kAMAS1	GCAACAGGAACCAGCTATGACAACACGTATGGCGATCC
SC8.1036kAMAS2	GACGCAAGTGAGCAGTATGACAACACGTATGGCGGCCT
SC8.1036kRev	CTCATCCTGCTCAACGTCCT
SC24.171kAMAS1	GCAACAGGAACCAGCTATGACTCTATGTACTCAGCTCAGG
SC24.171kAMAS2	GACGCAAGTGAGCAGTATGACTCTATGTACTCAGCTTGGA
SC24.171kRev	CGCTCAGACCAGACCTCCTA
SC6.676kAMAS1	GCAACAGGAACCAGCTATGACAAGGCAGTCGCAACCCCG
SC6.676kAMAS2	GACGCAAGTGAGCAGTATGACAAGGCAGTCGCAACTACA
SC6.676kRev	TGCTGTGTTACCCGTACCTG
SC7.1422kAMAS1	GCAACAGGAACCAGCTATGACATGAAGCTGCTGGTACG
SC7.1422kAMAS2	GACGCAAGTGAGCAGTATGACATGAAGCTGCTGGCGCA
SC7.1422kRev	GACCTTATCACCCCTGCATT
SC9.700kAMAS1	GCAACAGGAACCAGCTATGACGGTAGGTGCCAGATGTC
SC9.700kAMAS2	GACGCAAGTGAGCAGTATGACGGTAGGTGCCAGACATT
SC9.700kRev	TGCTAACCACATCCAGAACG
SC9.885kAMAS1	GCAACAGGAACCAGCTATGACCTAGCCCCATCAATTCCCC
SC9.885kAMAS2	GACGCAAGTGAGCAGTATGACCTAGCCCCATCAATTTTCT
SC9.885kRev	GTGGATGGGCCAAGCTAATA
SC9.969kAMAS1	GCAACAGGAACCAGCTATGACCCGAAGCAAGAATCACCG
SC9.969kAMAS2	GACGCAAGTGAGCAGTATGACCCGAAGCAAGAATCCACA
SC9.969kRev	ACGCTGAGTCATGGGGATAA
SC9.1319kAMAS1	GCAACAGGAACCAGCTATGACAAGCAAAACATTCCTCCGG
SC9.1319kAMAS2	GACGCAAGTGAGCAGTATGACAAGCAAAACATTCCTTAGA
SC9.1319kRev	TCAACTCAGGGGCAAAAACT
SC9.1515kAMAS1	GCAACAGGAACCAGCTATGACAAAGAACCGACAGACACG
SC9.1515kAMAS2	GACGCAAGTGAGCAGTATGACAAAGAACCGACAGAACCT
SC9.1515kRev	TACGGCATCTGACCACTGAG
SC9.211kAMAS1	GCAACAGGAACCAGCTATGACCGCTCTTCCACCTTGCCG
SC9.211kAMAS2	GACGCAAGTGAGCAGTATGACCGCTCTTCCACCTTAACA
SC9.211kRev	CTTCATCAGCGCCATTGATT
SC24.48k.2AMAS1	GCAACAGGAACCAGCTATGACTTGCGCCCCGGGGCACG
SC24.48k.2AMAS2	GACGCAAGTGAGCAGTATGACTTGCGCCCCGGGGTGCA
SC24.48k.2Rev	CTTTGGTCACTCGTCCCTGT
SC.1.40.7k.2AMAS1	GCAACAGGAACCAGCTATGACCCTGTAGCTTGTTTCCCGC
SC.1.40.7k.2AMAS2	GACGCAAGTGAGCAGTATGACCCTGTAGCTTGTTTCTAGA
SC.1.40.7k.2Rev	GACATAATCACAGCCGCGTA
SC16.9kAMAS1	GCAACAGGAACCAGCTATGACTCAAATTGGTAGACTCATAGAC
SC16.9kAMAS2	GACGCAAGTGAGCAGTATGACTCAAATTGGTAGACTCATGAAG
SC16.9kRev	GCTCAATTGGCTGAGCAAG
SC16.15kAMAS1	GCAACAGGAACCAGCTATGACAAGAGGGGGATTGCCTTG
SC16.15kAMAS2	GACGCAAGTGAGCAGTATGACAAGAGGGGGATTGCACTT
SC16.15kRev	TGGGTACTGCTCTCGCTACC

Primer name	Primer sequence*
SC2.1932kAMAS1	GCAACAGGAACCAGCTATGACATTAGATCCACTTATGCCAGG
SC2.1932kAMAS2	GACGCAAGTGAGCAGTATGACATTAGATCCACTTATGCTGGA
SC2.1932kRev	TGCCATTAGAAGACCAACCA
SC2.2122kAMAS1	GCAACAGGAACCAGCTATGACAAATCCACGCAGGTTATG
SC2.2122kAMAS2	GACGCAAGTGAGCAGTATGACAAATCCACGCAGGTCCTT
SC2.2122kRev	ATTTGCAACCCATGTCCTTC
SC2.2323kAMAS1	GCAACAGGAACCAGCTATGACTGTATAAGTTGCGGAAGG
SC2.2323kAMAS2	GACGCAAGTGAGCAGTATGACTGTATAAGTTGCGGCGGA
SC2.2323kRev	CTGTAGTCTCCCTGCGCTCT
SC2.2506kAMAS1	GCAACAGGAACCAGCTATGACCCGAAGAAATGTGGTTCC
SC2.2506kAMAS2	GACGCAAGTGAGCAGTATGACCCGAAGAAATGTGGCCCT
SC2.2506kRev	GTACGTGTTTGCATGGGATG
SC2.2616kAMAS1	GCAACAGGAACCAGCTATGACCCCAACTAAACACCAACGTC
SC2.2616kAMAS2	GACGCAAGTGAGCAGTATGACCCCAACTAAACACCAATATT
SC2.2616kRev	GGGGGTGGAAAGACATAAAG
SC2.2733kAMAS1	GCAACAGGAACCAGCTATGACTTCAGCGGGGCGAGTCAC
SC2.2733kAMAS2	GACGCAAGTGAGCAGTATGACTTCAGCGGGGCGAGCTAT
SC2.2733kRev	TCGAAAAAGGGCGATAGAAG
SC7.303kAMAS1	GCAACAGGAACCAGCTATGACACGTTGCTGAGAGCCCGC
SC7.303kAMAS2	GACGCAAGTGAGCAGTATGACACGTTGCTGAGAGCTTGA
SC7.303kRev	CATTGAATCCTTCGCTCCAT
SC7.1692kAMAS1	GCAACAGGAACCAGCTATGACGAGACGGTCGTGTTAAG
SC7.1692kAMAS2	GACGCAAGTGAGCAGTATGACGAGACGGTCGTGTCGAA
SC7.1692kRev	TGGGACATATCAGCCTCCAC
SC6.1373kAMAS1	GCAACAGGAACCAGCTATGACTCTTTCGGTACAGTTAGG
SC6.1373kAMAS2	GACGCAAGTGAGCAGTATGACTCTTTCGGTACAGTCGGA
SC6.1373kRev	ATGAGGGCCCATATCATCAC
SC6.1424kAMAS1	GCAACAGGAACCAGCTATGACTCAGTGGCACGCAAGC
SC6.1424kAMAS2	TCAGTGGCACGCCGGA
SC6.1424kRev	CACGTCCCAGAGAATGGTC
SC6.1596kAMAS1	GCAACAGGAACCAGCTATGACACGTCGCCGTCGCCAC
SC6.1596kAMAS2	GACGCAAGTGAGCAGTATGACACGTCGCCGTCGTTAT
SC6.1596kRev	CTGGGGTCAATGGAGGTGTA
SC6.1668kAMAS1	GCAACAGGAACCAGCTATGACGAGGAACGTATACAAGTTAAC
SC6.1668kAMAS2	GACGCAAGTGAGCAGTATGACGAGGAACGTATACAAGTCCAT
SC6.1668kRev	AGTCGTCTGGTCGTGAATCC
SC5.1530kAMAS1	GCAACAGGAACCAGCTATGACCCATGGCCGTGTATTCG
SC5.1530kAMAS2	GACGCAAGTGAGCAGTATGACCCATGGCCGTGTACCCA
SC5.1530kRev	GGCCCTTCGATGGATAGAGT
SC5.1052kAMAS1	GCAACAGGAACCAGCTATGACCATGGCATGTCAGTTCGG
SC5.1052kAMAS2	GACGCAAGTGAGCAGTATGACCATGGCATGTCAGTCTGA
SC5.1052kRev	CTCTTCGGCATCATCATCAG
SC.1.9.351kAMAS1	GCAACAGGAACCAGCTATGACATCGTCTCGGCGAGCATG
SC.1.9.351kAMAS2	GACGCAAGTGAGCAGTATGACATCGTCTCGGCGAGTGTA
SC.1.9.351kRev	TGTCCTCTCCCAAACTGCTC
SC.1.7.362kAMAS1	GCAACAGGAACCAGCTATGACCTCTGGAATGCATCCACC
SC.1.7.362kAMAS2	GACGCAAGTGAGCAGTATGACCTCTGGAATGCATCACCT
SC.1.7.362kRev	AACAGAACTGGACACGGTCA
SC.1.7.996kAMAS1	GCAACAGGAACCAGCTATGACCTAGACATGTATGGCCATG
SC.1.7.996kAMAS2	GACGCAAGTGAGCAGTATGACCTAGACATGTATGGCTGTA
SC.1.7.996kRev	GCGCACATGTCAGATTATGC
SC.1.7.1127kAMAS1	GCAACAGGAACCAGCTATGACACTCCACGCATGAATAAC
SC.1.7.1127kAMAS2	GACGCAAGTGAGCAGTATGACACTCCACGCATGAACCAT
SC.1.7.1127kRev	CAGAGGACCGTGACGAGATT

Primer name	Primer sequence*
SC.1.7.1220kAMAS1	GCAACAGGAACCAGCTATGACTCTCGTGACAAGAATCATAAC
SC.1.7.1220kAMAS2	GACGCAAGTGAGCAGTATGACTCTCGTGACAAGAATCACGAA
SC.1.7.1220kRev	CGTCGACTCAGTTGCTACCC
SC.1.8.586kAMAS1	GCAACAGGAACCAGCTATGACTCAAAGCTGCCCGTATC
SC.1.8.586kAMAS2	GACGCAAGTGAGCAGTATGACTCAAAGCTGCCCGCCTT
SC.1.8.586kRev	TCGTGAACGTCTGAGGGTAA
SC.1.11.474kRev	GTTCAAGTCGGCTCCGTAGA
SC7.212kRev2	CAGTTATGGCGGACGATTTT
SC7.1537kRev2	TACACAGGGAGGCCATCAAT
SC9.100kRev2	TTTGATGACTCTTCGGTTCG
SC9.779kRev2	GAGCTGGAGGTGCAAGTGAT
SC9.1411kRev2	TGCATTGTGATACCCGATGT
SC35.26kRev2	GGGCCACTTGCAGAGATTT
SC38.1kRev2	AACGGCAATCCAAAACAGTC

*Red color basepairs indicate the difference of the two AMAS primers.

APPENDIX D. LIST OF SIMPLE SEQUENCE REPREAT PRIMERS USED IN GENETIC MAPPING OF THE86-124ΔMAT1-1-1 × AR CrossB10ΔMAT1-2-1 POPULATION

Primer name	Primer sequence [a]
PtrSSR/AAC003F	CACGACGTTGTAAAACGACTTGTGGAGATGGGCGTTG
PtrSSR/AAC003R	GCTTCTTTGGTGTCTGCAGAA
PtrSSR/AAC004F	CACGACGTTGTAAAACGACTGGTGGATTCGTTGTTGTTGT
PtrSSR/AAC004R	CGCAATATCAAAACCAAGCC
PtrSSR/AAC008F	CACGACGTTGTAAAACGACTTCTGCTGTCTTCTTTGCCA
PtrSSR/AAC008R	TTGTATAGCCAGGTACGTCCG
PtrSSR/AAC010F	CACGACGTTGTAAAACGACAACGGACAAACGGTCCTTCA
PtrSSR/AAC010R	TTTGGACTTGCAGCAGTGAA
PtrSSR/AAC011F	CACGACGTTGTAAAACGACTACATGCACACGGTCATGTC
PtrSSR/AAC011R	TTCCGTACAATTGACCACGA
PtrSSR/AAC013F	CACGACGTTGTAAAACGACCGGCCTCGATTACTTCTTTT
PtrSSR/AAC013R	ACTGAACTACGGTCATGAGCA
PtrSSR/AAC015F	CACGACGTTGTAAAACGACTTGTGGTGGATTCGGGTGT
PtrSSR/AAC015R	TATCTGTACCACGGAAAGCGA
PtrSSR/AAC017F	CACGACGTTGTAAAACGACGCCACTGTGGTGGATTCTCTT
PtrSSR/AAC017R	TCGGTTCTCGTCTTGATGGAT
PtrSSR/AAG021F	CACGACGTTGTAAAACGACTCATCGCACTGTGGTGTATTC
PtrSSR/AAG021R	TTAGCCTCGGTGCCAAGAA
PtrSSR/AAG023F	CACGACGTTGTAAAACGACACTGCACTTTGACACGCAAT
PtrSSR/AAG023R	TGGTGCTGCCTCCACTGTT
PtrSSR/AAG026F	CACGACGTTGTAAAACGACCTTGGGGGTTGCGTTAAAAT
PtrSSR/AAG026R	AAGCGCATTCCTCACCTCTTT
PtrSSR/AAG040F	CACGACGTTGTAAAACGACTGGTCAATGTGGTGGATTCT
PtrSSR/AAG040R	AAGGGGGTCATGATGTATGGA
PtrSSR/AAG041F	CACGACGTTGTAAAACGACAGAGGCGTTCAAGTGGGATA
PtrSSR/AAG041R	TTAGCCTCGGTGCCAAGAA
PtrSSR/AAG045F	CACGACGTTGTAAAACGACTGGATTCCGCGATATGAAG
PtrSSR/AAG045R	ATACCGTAGCAGTCTCGCGTT
PtrSSR/AAG046F	CACGACGTTGTAAAACGACAGCTCATGTGGTGGATTCTGT
PtrSSR/AAG046R	AGTACACCAGCCATGCATGTT
PtrSSR/AAG048F	CACGACGTTGTAAAACGACACATTGTGGTGGATTCTCGTC
PtrSSR/AAG048R	TTATGCATGCTCGCCTTGAT
PtrSSR/AAG049F	CACGACGTTGTAAAACGACAAAGCATGATTCCCCCTGTT
PtrSSR/AAG049R	TCTTTGCTTGCTTGCTTGCT
PtrSSR/AAG050F	CACGACGTTGTAAAACGACCCCCTTGACTCATCACACTCT
PtrSSR/AAG050R	ATGTTCTCGAAGCATGTGCG
PtrSSR/AAG051F	CACGACGTTGTAAAACGACACGATGTGGTCGGTTATTAGG
PtrSSR/AAG051R	ACGGAAAAGCGTAGTTTGCA
PtrSSR/AAG054F	CACGACGTTGTAAAACGACCTGGGAGAACGAACGTATGAA
PtrSSR/AAG054R	GTTTAAAATCCCCAAAATCCA
PtrSSR/AAG055F	CACGACGTTGTAAAACGACCGCAAACATAAAAAACCGCC
PtrSSR/AAG055R	TCTGCTTTTGTGGTGCTTCA
PtrSSR/AAG056F	CACGACGTTGTAAAACGACATCGCCACTGTGGTGGATT
PtrSSR/AAG056R	ATCGTCATCGCGGGAAGA
PtrSSR/AAG057F	CACGACGTTGTAAAACGACCAATCAAAATTCCCACTCGG
PtrSSR/AAG057R	CCCACATCTGCGACAACAATA
PtrSSR/AAT001F	CACGACGTTGTAAAACGACCGATACATCTCAACAACGCGA
PtrSSR/AAT001R	TTCGGCCAAAGTCCTACAT
PtrSSR/AAT002F	CACGACGTTGTAAAACGACCCGAAGAAACCACCCATAGAA
PtrSSR/AAT002R	TTGCGCAGAGCTTAGGTGTA
PtrSSR/AAT003F	CACGACGTTGTAAAACGACTAGGCCGAAGTCTTGCATAGT
PtrSSR/AAT003R	GGCGTGGAGGCATTATGTG

Primer name	Primer sequence [a]
PtrSSR/AAT004F	CACGACGTTGTAAAACGACTCTGGCTCGCTACTAATCAAA
PtrSSR/AAT004R	TACACCTAAGCTCTGCGCAA
PtrSSR/AAT006F	CACGACGTTGTAAAACGACTAAGCGGGAAGCTTGGTCTAA
PtrSSR/AAT006R	AAGTTGCAAAAGTTGGTGGG
PtrSSR/AAT007F	CACGACGTTGTAAAACGACTATCGTGTCGAGTGTCTCCC
PtrSSR/AAT007R	AAGTCGGGCCGAAGTGTCAA
PtrSSR/AAT008F	CACGACGTTGTAAAACGACTGTGGTGGATTCTAAATGGAA
PtrSSR/AAT008R	CTGCTTCATTTGAAGGCACA
PtrSSR/AAT009F	CACGACGTTGTAAAACGACGTTGTTGAGATGCATCGCTT
PtrSSR/AAT009R	GCGGGAAGCTTGGTCTAACTA
PtrSSR/AAT011F	CACGACGTTGTAAAACGACTAGAGCCTGCCGAGATTGTTT
PtrSSR/AAT011R	GCCAAAGCCACAATTAGCAA
PtrSSR/AAT012F	CACGACGTTGTAAAACGACGCAAATCATCCCCCAAATTG
PtrSSR/AAT012R	CCTTCGTAGCAGCTATGTTCG
PtrSSR/AAT014F	CACGACGTTGTAAAACGACGCGAACCATACCAGAAAACCA
PtrSSR/AAT014R	CCCACTGTCTGCTTGTTATCA
PtrSSR/AAT015F	CACGACGTTGTAAAACGACCGAACCATACCAGAAAACCA
PtrSSR/AAT015R	CGATACATCTCAACAACGCGA
PtrSSR/AAT016F	CACGACGTTGTAAAACGACTCCTTCTGCTCGGGCTTACT
PtrSSR/AAT016R	ATGTAGCAGCGGGCTCTTTT
PtrSSR/AAT018F	CACGACGTTGTAAAACGACCGAACATAGCTGCTACGAAGG
PtrSSR/AAT018R	CCATCCAGCTCTTGCTCACTA
PtrSSR/AAT020F	CACGACGTTGTAAAACGACTCAGCACTAGCGCCTACTCTA
PtrSSR/AAT020R	ACCAATACCCAGTCACCAGAA
PtrSSR/AAT022F	CACGACGTTGTAAAACGACAAGGTGAGTAAAGTTGGTGGG
PtrSSR/AAT022R	TACAAGGCCTTCCAACAAGCT
PtrSSR/AAT025F	CACGACGTTGTAAAACGACCGATACATCTCAACAACGCGA
PtrSSR/AAT025R	TTAGCACCTGGCAACAAACA
PtrSSR/AC001F	CACGACGTTGTAAAACGACTGAACTGTGCCAACGCAAA
PtrSSR/AC001R	TTTCAGCGAGATAGCATGAGC
PtrSSR/AC003F	CACGACGTTGTAAAACGACAACCCCCCATGTCGCATAA
PtrSSR/AC003R	TCGCTAGATACGCCTTTTGTG
PtrSSR/AC004F	CACGACGTTGTAAAACGACATGAATGGCGTTCAGATGCA
PtrSSR/AC004R	TAACCTTGGAAACTCCGCAC
PtrSSR/AC005F	CACGACGTTGTAAAACGACTCTGTGGCTTGGTATTCGGT
PtrSSR/AC005R	CACCCATTTTTTAGGCCCTT
PtrSSR/AC006F	CACGACGTTGTAAAACGACATTGCCTGCGGGTCGATCTA
PtrSSR/AC006R	GTTTGACGGGCCTTTTGTTA
PtrSSR/AC007F	CACGACGTTGTAAAACGACTTTTCTTGTGACCGCGAAAG
PtrSSR/AC007R	AAAGGCGGACTAGGGGCTA
PtrSSR/AC008F	CACGACGTTGTAAAACGACATGCTTGCTGACCGACATAT
PtrSSR/AC008R	CCGTTGGAGAAGCTTGAAA
PtrSSR/AC009F	CACGACGTTGTAAAACGACAGCCACGGAGACAGACGTCAT
PtrSSR/AC009R	TCGCTGATCGATGAGGTAAGT
PtrSSR/AC010F	CACGACGTTGTAAAACGACTGGTGCATTCTGCATCTTCA
PtrSSR/AC010R	TGAACAACTGTGTGTGCGTT
PtrSSR/AC011F	CACGACGTTGTAAAACGACGTTTTCCGCGGTTCAACTTT
PtrSSR/AC011R	CATTAGGCGGGTTAAATTCC
PtrSSR/AC012F	CACGACGTTGTAAAACGACTCGCCCATCCATGTCCACT
PtrSSR/AC012R	CGCTCTCGAAGCATTTGTG
PtrSSR/AC013F	CACGACGTTGTAAAACGACTGTTGGCGTAAGTAGAGGGGT
PtrSSR/AC013R	CGGTCTCGTCTGATGGATAGT
PtrSSR/AC014F	CACGACGTTGTAAAACGACAAATCTCGGCGTGAACTCTT
PtrSSR/AC014R	AGGCAGTCTTGTCAACGCA
PtrSSR/AC015F	CACGACGTTGTAAAACGACCGAAGATAGCCGGAGATGTAT
PtrSSR/AC015R	ATGCAAGAATGGGAGGGTG
PtrSSR/AC016F	CACGACGTTGTAAAACGACTACTTACAGAGCTGCAGACGC
PtrSSR/AC016R	GCAACAGTGTGGAGAAGTGAA

Primer name	Primer sequence [a]
PtrSSR/AC017F	CACGACGTTGTAAAACGACTGTCATGAGTCATGTGGTGGA
PtrSSR/AC017R	CAACGGTCTTTGTTCTTTGC
PtrSSR/AC019F	CACGACGTTGTAAAACGACTGGACCTTCCGAATAAGGTCA
PtrSSR/AC019R	CAGTCCGGTCTCGGACTTT
PtrSSR/AC020F	CACGACGTTGTAAAACGACTGCATAACGCATTCTGTCTTG
PtrSSR/AC020R	AGGGATCCGGGATGATGTTTA
PtrSSR/AC021F	CACGACGTTGTAAAACGACTTCTCCTTTTCGGCCTTCTTC
PtrSSR/AC021R	AAAGACGGGGAAGGACAAACA
PtrSSR/AC022F	CACGACGTTGTAAAACGACTCACATTCCCATCTCAACCCT
PtrSSR/AC022R	AAACACGTATAGAGCGGGTGC
PtrSSR/AC024F	CACGACGTTGTAAAACGACAAACAAAACCGACAGCTGCA
PtrSSR/AC024R	CCCGGTCTGGACAATCATG
PtrSSR/AC025F	CACGACGTTGTAAAACGACTCCATCATGAGCTCTTTCCA
PtrSSR/AC025R	GCCTGCTGGTGATATCGTGTA
PtrSSR/AC026F	CACGACGTTGTAAAACGACTGGAAATCTGCTGCTAAGCA
PtrSSR/AC026R	TCGCTAGATACGCCTTTTGTG
PtrSSR/AC027F	CACGACGTTGTAAAACGACTTCGCGGAGTGTCAGTTGAGT
PtrSSR/AC027R	CGGGCCCATTTTTAAGCATT
PtrSSR/AC028F	CACGACGTTGTAAAACGACTACTTGTCTTGTGACCGCGA
PtrSSR/AC028R	AAAGGCGGACTAGGGGCTA
PtrSSR/AC029F	CACGACGTTGTAAAACGACATGGGAGTGAGGATACATGGG
PtrSSR/AC029R	GGTCTGGCTTGCATAGCGA
PtrSSR/AC031F	CACGACGTTGTAAAACGACACCACTGCCTGGCAATTATT
PtrSSR/AC031R	ACTCCAGCCACACAGACCTAT
PtrSSR/AC032F	CACGACGTTGTAAAACGACTGGAAATCTGCTGCTAAGCA
PtrSSR/AC032R	TCTTTACTCTCTCCCACGTCC
PtrSSR/AC033F	CACGACGTTGTAAAACGACATACCACACACGCACGCAATT
PtrSSR/AC033R	TGATGTGGCAAAGGAGATGA
PtrSSR/AC034F	CACGACGTTGTAAAACGACACCTCCCATCACCAGACAGA
PtrSSR/AC034R	GGACTTTTTGGCTTGGTGTT
PtrSSR/AC035F	CACGACGTTGTAAAACGACCATTGTGGTGGAATTCGAGA
PtrSSR/AC035R	TTTTATCGCCCTTCTTCCCT
PtrSSR/AC036F	CACGACGTTGTAAAACGACACATTCCCGTCTGTCCCTTTT
PtrSSR/AC036R	ACATTCCCGTCTGTCCCTTTT
PtrSSR/AC038F	CACGACGTTGTAAAACGACTTTGGAGTACATTGTGGTGGA
PtrSSR/AC038R	TTTTTCTTGTAGGCAGCGCA
PtrSSR/AC039F	CACGACGTTGTAAAACGACCACCACCTTAACGCCCAA
PtrSSR/AC039R	GTCGGTTCCGAAATCGATAGT
PtrSSR/AC040F	CACGACGTTGTAAAACGACGGAGTGAGGATACATGGGGTA
PtrSSR/AC040R	TTGCATAGCGATGGCGTTT
PtrSSR/AC041F	CACGACGTTGTAAAACGACGCTGTTTGAATACGGTTGATG
PtrSSR/AC041R	TAATGACGTGTCTGGCGACTA
PtrSSR/AC043F	CACGACGTTGTAAAACGACTTGATACATCGCTCCCCATT
PtrSSR/AC043R	AAATCCCGCTTACAAACCCA
PtrSSR/AC044F	CACGACGTTGTAAAACGACAGGGTCCTGTCTTGCACTGA
PtrSSR/AC044R	AACAACACCACTGGCGGTGTA
PtrSSR/AC045F	CACGACGTTGTAAAACGACATGCCTCCTTACTGACTTTGG
PtrSSR/AC045R	TGCTGATATCGACACCAACA
PtrSSR/AC047F	CACGACGTTGTAAAACGACACCTCCCACCCTCAAACATAT
PtrSSR/AC047R	GGGGAGAACAAGCAAACTTAA
PtrSSR/AC048F	CACGACGTTGTAAAACGACCAATCCCACCCCATTACAAA
PtrSSR/AC048R	CTGCGGTTTTTTCTCCTTCT
PtrSSR/AC050F	CACGACGTTGTAAAACGACACATCGGACGTCTGCTCACA
PtrSSR/AC050R	TCTTTTTAGGAGCAGGTGCTG
PtrSSR/AC051F	CACGACGTTGTAAAACGACCAAGCATAGCGGTGACGACT
PtrSSR/AC051R	TTGCATTGGCATTTTGTCCC

Primer name	Primer sequence [a]
PtrSSR/AC053F	CACGACGTTGTAAAACGACATCATCGCACTGGGTGGATT
PtrSSR/AC053R	ATGCCCATTACACCCTAATGC
PtrSSR/AC055F	CACGACGTTGTAAAACGACATATGATGGGTGTGATGGGGA
PtrSSR/AC055R	TACGTCGTTCATCACGTTCTG
PtrSSR/AC056F	CACGACGTTGTAAAACGACTACGCGATTGGACTGCTGAA
PtrSSR/AC056R	TTTTTGCGTGCGCGTGTA
PtrSSR/AC057F	CACGACGTTGTAAAACGACTCATCGCACTGTGGTGGATT
PtrSSR/AC057R	CGGTCTTGGAAAAGTCACAA
PtrSSR/AC058F	CACGACGTTGTAAAACGACTCATTCAGTCCCAACCCAAAC
PtrSSR/AC058R	ATGGATCGCGGTTGTTCTG
PtrSSR/AC059F	CACGACGTTGTAAAACGACCCAGAAACTAGGTCCAAAGGA
PtrSSR/AC059R	TGAATGAATCGAGGACGACA
PtrSSR/AC060F	CACGACGTTGTAAAACGACTAAATAGCCTAATGGGCCCCT
PtrSSR/AC060R	AACCACGTAACCCCACACTTT
PtrSSR/AC061F	CACGACGTTGTAAAACGACTCGCCTCAATACTGCCTGTAA
PtrSSR/AC061R	TGGAGGAGGTCGCTTCTGTT
PtrSSR/AC062F	CACGACGTTGTAAAACGACGAGGGTCAAGTTGTGCAGAGT
PtrSSR/AC062R	TCGGATATGCTCCGTACAACA
PtrSSR/AC063F	CACGACGTTGTAAAACGACATCATCGCACTGTGTGTGTGT
PtrSSR/AC063R	CTCTGGAAATTGCGGTTGAA
PtrSSR/AC065F	CACGACGTTGTAAAACGACATAACTTGCCACAGCCCCTAT
PtrSSR/AC065R	ATTCCTATCCCGTCCAAACA
PtrSSR/AC066F	CACGACGTTGTAAAACGACTCCGAATGACATATCGTGGTG
PtrSSR/AC066R	ATAGCCTAGCATCTTCCCTCA
PtrSSR/AC067F	CACGACGTTGTAAAACGACGTTTGAGGAGGACGAGGAGGT
PtrSSR/AC067R	AAGCCAGGTGTGTGTGTGTGT
PtrSSR/AC063F	CACGACGTTGTAAAACGACATCATCGCACTGTGTGTGTGT
PtrSSR/AC063R	CTCTGGAAATTGCGGTTGAA
PtrSSR/AC065F	CACGACGTTGTAAAACGACATAACTTGCCACAGCCCCTAT
PtrSSR/AC065R	ATTCCTATCCCGTCCAAACA
PtrSSR/AC066F	CACGACGTTGTAAAACGACTCCGAATGACATATCGTGGTG
PtrSSR/AC066R	ATAGCCTAGCATCTTCCCTCA
PtrSSR/AC067F	CACGACGTTGTAAAACGACGTTTGAGGAGGACGAGGAGGT
PtrSSR/AC067R	AAGCCAGGTGTGTGTGTGTGT
PtrSSR/AC069F	CACGACGTTGTAAAACGACGCAAGGGTGTTAAGATCGACA
PtrSSR/AC069R	TTATCCCGAGCCGGTCTTTA
PtrSSR/AC070F	CACGACGTTGTAAAACGACTTACAAAGTTCGAGCGAGAGC
PtrSSR/AC070R	GCACCGCCCTCTGAATCTT
PtrSSR/AC071F	CACGACGTTGTAAAACGACTTGTTTGGTACGATATCGGC
PtrSSR/AC071R	TTTGCACATGATCCGACCTT
PtrSSR/AC072F	CACGACGTTGTAAAACGACTATTCATCGCACTGTGGTGGA
PtrSSR/AC072R	AAGGACGCGTTTTTCGTGTA
PtrSSR/AC073F	CACGACGTTGTAAAACGACATTCAACGCGGCGTAATAGA
PtrSSR/AC073R	GGCATCCACATCCAGACGT
PtrSSR/AC075F	CACGACGTTGTAAAACGACTGGTGAGAAGTGAATGCATGG
PtrSSR/AC075R	TTGCCAAGGCACTAGCTACG
PtrSSR/AC076F	CACGACGTTGTAAAACGACAGGGATGGGAGTGTGAGTGTG
PtrSSR/AC076R	TGGGTTGATGGGGAGAATA
PtrSSR/AC077F	CACGACGTTGTAAAACGACAGAGAAGAAGCGTGCCAAGAT
PtrSSR/AC077R	TCAGAAGGGTCTGCTTTGTCA
PtrSSR/AC078F	CACGACGTTGTAAAACGACCAAAGGCAGTCTTTCCGAAA
PtrSSR/AC078R	ACTGTGAGCGGGGGTTGTT
PtrSSR/AC079F	CACGACGTTGTAAAACGACATTTGGGCATAGGAAACGGA
PtrSSR/AC079R	CGTAGGATTCAGTCGGTACCT
PtrSSR/AC080F	CACGACGTTGTAAAACGACTGTTCAGAACCATCGGAAAAG
PtrSSR/AC080R	AAGGAGACCAAGACGTAGCAT
PtrSSR/AC081F	CACGACGTTGTAAAACGACTGGATTCTTGGTTTATGCGG
PtrSSR/AC081R	AACAACCAACCTCCTAAACCC

Primer name	Primer sequence [a]
PtrSSR/AC082F	CACGACGTTGTAAAACGACTGGATTCAACACGCTGTAGTC
PtrSSR/AC082R	TACACTTTCCTTGGACGGGAT
PtrSSR/AC083F	CACGACGTTGTAAAACGACCCAAGTGTATCCGCAGCAA
PtrSSR/AC083R	TCGGTGTAGAGGGTAATGTGG
PtrSSR/AC084F	CACGACGTTGTAAAACGACTAATCATCGCCACTGTGGTG
PtrSSR/AC084R	ATAACGTCGTTTGGGGTGCA
PtrSSR/AC086F	CACGACGTTGTAAAACGACTGAAGAAGAGTCGCGATGAA
PtrSSR/AC086R	ACCATCATGATTACGTCCTCC
PtrSSR/AC087F	CACGACGTTGTAAAACGACTCAGAGAGAGGGAGCATCGAC
PtrSSR/AC087R	GGAGACAGACGGATGGACAGA
PtrSSR/AC088F	CACGACGTTGTAAAACGACTTGTACGCCGAGAAGAGGGA
PtrSSR/AC088R	TGGCTCATTATAACAAACGCA
PtrSSR/AC090F	CACGACGTTGTAAAACGACTATCGCACGCTTGATACCAA
PtrSSR/AC090R	TTCGACGCGTTCATATGGA
PtrSSR/AC091F	CACGACGTTGTAAAACGACAGGCGAGCGTAGTTCGGTATA
PtrSSR/AC091R	GGGAACGGGAGATTTTGGTA
PtrSSR/AC092F	CACGACGTTGTAAAACGACCAAGTTTCCCAGGAAGGTAAA
PtrSSR/AC092R	AGATGGCCAGTGGCGTATT
PtrSSR/AC093F	CACGACGTTGTAAAACGACCCTCCCCAAAAAAGCTTGAT
PtrSSR/AC093R	TGCCCAAACCGAACCGAA
PtrSSR/AC094F	CACGACGTTGTAAAACGACTACATGGTAGGCCTGGTTGTG
PtrSSR/AC094R	TGAAAAGCCATTCCAAGACG
PtrSSR/AC095F	CACGACGTTGTAAAACGACTACATTGTGGTGGATTCCCTG
PtrSSR/AC095R	AACAAAAATCCTCTCCGGCA
PtrSSR/AC096F	CACGACGTTGTAAAACGACATTCCCATCACCAACGAAGA
PtrSSR/AC096R	AGCTTGTTCTTGTGGATGAGG
PtrSSR/AC097F	CACGACGTTGTAAAACGACACATGCTACCGTCATTTTGAA
PtrSSR/AC097R	TCTTTTCGAGGAAGCCGATT
PtrSSR/AC098F	CACGACGTTGTAAAACGACATCATACCCCAGCATGAATGG
PtrSSR/AC098R	CTGCAAGCTAACCTGGGAAA
PtrSSR/AC099F	CACGACGTTGTAAAACGACACCTCCCCCTTGTTCACTTT
PtrSSR/AC099R	CGGGGAAAGCATTTCATGT
PtrSSR/AC100F	CACGACGTTGTAAAACGACCCGTCCCTGACTTTACTCAAA
PtrSSR/AC100R	AAAGTTACGCAGAGGGGTTT
PtrSSR/AC101F	CACGACGTTGTAAAACGACTGGATTCGAGATAAGGTGGGT
PtrSSR/AC101R	TTTAGTACAGACGCCCGCAA
PtrSSR/AC102F	CACGACGTTGTAAAACGACCATGATTCGCTTAGACGGG
PtrSSR/AC102R	TCCAACCCTTCTCTACACACA
PtrSSR/AC103F	CACGACGTTGTAAAACGACTGGATTCTGTGCGTGTGTGT
PtrSSR/AC103R	ATCTTGGAAGTCTTGCAGCCT
PtrSSR/AC104F	CACGACGTTGTAAAACGACGTGAGCGCGAAGTTTCATTA
PtrSSR/AC104R	CCATTCCCTTGTTTGGGTAA
PtrSSR/AC105F	CACGACGTTGTAAAACGACCATTGTGGTGGATTCGACAT
PtrSSR/AC105R	TTCCACCAAGACTTTGCCTA
PtrSSR/AC106F	CACGACGTTGTAAAACGACTCAGGCATTTGGTAATACGG
PtrSSR/AC106R	TCAAATTTTCAACCCCAGGC
PtrSSR/AC107F	CACGACGTTGTAAAACGACAGTCTCCCACTGCACTGCAAT
PtrSSR/AC107R	TGTGTGTGCGTTAGTTGAGCT
PtrSSR/AC108F	CACGACGTTGTAAAACGACCAGAAAAACCCCACTTGTTG
PtrSSR/AC108R	TAAGGAAAGGCCCGATGTGTA
PtrSSR/AC111F	CACGACGTTGTAAAACGACATTGTGGTGGATTCTTCCCTG
PtrSSR/AC111R	TGTGAGGAAGGACCGTTAAGC
PtrSSR/ACC001F	CACGACGTTGTAAAACGACATTTCTCACTTGCCGCTGTT
PtrSSR/ACC001R	TCTGCTCTCGTAGTTGGGC

Primer name	Primer sequence [a]
PtrSSR/ACC002F	CACGACGTTGTAAAACGACATCGAGATCGTCTCTCTTGCT
PtrSSR/ACC002R	AGCGCGAGGAAGAAGAGAAGA
PtrSSR/ACC003F	CACGACGTTGTAAAACGACTAACACATAAACCCAGGCGA
PtrSSR/ACC003R	CGTTACGCGAGTTTGGTTTT
PtrSSR/ACC004F	CACGACGTTGTAAAACGACATTCATGGGGGTCCGTTTGTT
PtrSSR/ACC004R	TGCTAGTTCGCCGAGATGTA
PtrSSR/ACG001F	CACGACGTTGTAAAACGACGCTTGAAAGATGGCTTTCCT
PtrSSR/ACG001R	GTTCCAAAAGAGCTTAGCCAA
PtrSSR/ACG002F	CACGACGTTGTAAAACGACTTGTGGTGGATTCATGACTGG
PtrSSR/ACG002R	ACTGGGCGCTGCATGAAAA
PtrSSR/AG001F	CACGACGTTGTAAAACGACAGCGACGAACAGCTAGGAAAT
PtrSSR/AG001R	CTCAACGATAACCACCAGGAT
PtrSSR/AG002F	CACGACGTTGTAAAACGACCCGAAATCCCATGTTTGGTA
PtrSSR/AG002R	TGCATGGAACAAGGCCAGTA
PtrSSR/AG003F	CACGACGTTGTAAAACGACCCGAAATCCCATGTTTGGTA
PtrSSR/AG003R	AAAGGCATGCTAGGAACACA
PtrSSR/AG004F	CACGACGTTGTAAAACGACTAGGACTGAAAGACCCATGGA
PtrSSR/AG004R	GAAGGGCATAAGCAAAAGGA
PtrSSR/AG006F	CACGACGTTGTAAAACGACGGGATCCACATGACTGACTCA
PtrSSR/AG006R	ATAAGATGGTCGGAGGCACA
PtrSSR/AG007F	CACGACGTTGTAAAACGACGGGATCCACATGACTGACTCA
PtrSSR/AG007R	TGCTCGGAAACGATTCAAGA
PtrSSR/AG008F	CACGACGTTGTAAAACGACGTTTGTTTCATTTGGGCCAG
PtrSSR/AG008R	AATCATGCCATCTCCTTCCC
PtrSSR/AG009F	CACGACGTTGTAAAACGACTGATGGATCCGATCTGATGGT
PtrSSR/AG009R	TGCAGACACTTCCATCCATCT
PtrSSR/AG010F	CACGACGTTGTAAAACGACATCCCTTTTTCTCGCCTTCT
PtrSSR/AG010R	TTTATGCATTAGCTGGCCTG
PtrSSR/AG011F	CACGACGTTGTAAAACGACAACTTGCTCATGTCATGCACC
PtrSSR/AG011R	AGCGCCATGTATTTGTCGAT
PtrSSR/AG012F	CACGACGTTGTAAAACGACTTTTGGCGGTACCTTCGGA
PtrSSR/AG012R	TATGGCACTGGAACTGGGATT
PtrSSR/AG015F	CACGACGTTGTAAAACGACTCGTAAGCTGTTCGGTAACG
PtrSSR/AG015R	TCATTCTCCTGCCCTTCTACT
PtrSSR/AG021F	CACGACGTTGTAAAACGACATCCCAACTGCTGCTCTACA
PtrSSR/AG021R	AGCATTCGGATAACATCAGCC
PtrSSR/AG022F	CACGACGTTGTAAAACGACTGACCTCATTCAGCCCGTT
PtrSSR/AG022R	ACTAGGCTCCCCAGAAGTGA
PtrSSR/AG027F	CACGACGTTGTAAAACGACTAACTAACGCCAGTCGAGTGG
PtrSSR/AG027R	AATGACGGGGTGGTGATAACA
PtrSSR/AG028F	CACGACGTTGTAAAACGACTGACATAGCTACGATGGTGGG
PtrSSR/AG028R	ATAGACGAACGGGGTTTTGGT
PtrSSR/AG030F	CACGACGTTGTAAAACGACGACATGGGAAGCAGAAATGA
PtrSSR/AG030R	ATTGAGGGGGTTATCGAGAA
PtrSSR/AG031F	CACGACGTTGTAAAACGACATGCACGGCAACACAACAA
PtrSSR/AG031R	ATCCTCTCAGCCAACACCG
PtrSSR/AG032F	CACGACGTTGTAAAACGACGGACCACGGGAATAGGAAGA
PtrSSR/AG032R	AGGGACGATTGCACTGAGAAA
PtrSSR/AG033F	CACGACGTTGTAAAACGACTACCTCGAGCGACGCTAAACA
PtrSSR/AG033R	CCGCGCTATGATATACCCTCT
PtrSSR/AG034F	CACGACGTTGTAAAACGACGCGTTTTTTTTGTGCTGTGC
PtrSSR/AG034R	AAACTTACCATGTCATGCCCC
PtrSSR/AG036F	CACGACGTTGTAAAACGACTCACACCAACAATCATGCCA
PtrSSR/AG036R	GTTTGTTTCATTTGGGCCAG

Primer name	Primer sequence [a]
PtrSSR/AG038F	CACGACGTTGTAAAACGACATCGCGAGCAGGTACACCTT
PtrSSR/AG038R	CAAGTCGCTTGACCCACAA
PtrSSR/AG040F	CACGACGTTGTAAAACGACCCTGAGGAACGGTCTTGATAA
PtrSSR/AG040R	ATTCTTTTCCTGGAGTAGCGG
PtrSSR/AG041F	CACGACGTTGTAAAACGACTTGCATGGTCCGAAGGGGT
PtrSSR/AG041R	ATCGCAGCCATCCAAGCA
PtrSSR/AG042F	CACGACGTTGTAAAACGACTTCAAGGACCACGATTGGAT
PtrSSR/AG042R	TGGGGAGGAAGAATAGGAGAA
PtrSSR/AG043F	CACGACGTTGTAAAACGACAACGCGCTGGACCTCAGATTT
PtrSSR/AG043R	TGGGCCGCTTATTTATGCTA
PtrSSR/AG045F	CACGACGTTGTAAAACGACTCGTCTTTGTACGGCAAAAG
PtrSSR/AG045R	AACAGGACCCCACAAATTCA
PtrSSR/AG046F	CACGACGTTGTAAAACGACAGGTGTTCAGCGGGGAATTA
PtrSSR/AG046R	TCCTCTCTCTCCTCCTCCTCT
PtrSSR/AG047F	CACGACGTTGTAAAACGACCAGGCTCGAGGGAATCAAA
PtrSSR/AG047R	TTCGAGAAGCCTTTCACTCCA
PtrSSR/AG048F	CACGACGTTGTAAAACGACTTGACTCATGTGGTGGATTCA
PtrSSR/AG048R	TTTCTTTGCCTGTGCCATCT
PtrSSR/AG050F	CACGACGTTGTAAAACGACGCTGCGTAAAACGGTCGTATA
PtrSSR/AG050R	TCATTTTCTTTCCCTCCCTC
PtrSSR/AG052F	CACGACGTTGTAAAACGACGAACGAGCAGTCATAACGTGA
PtrSSR/AG052R	AAGACAAAGGTTGGGCCGA
PtrSSR/AG053F	CACGACGTTGTAAAACGACTGAACCGAACCGAACCGTA
PtrSSR/AG053R	TAATGTATAGCCGAGGCGAA
PtrSSR/AG054F	CACGACGTTGTAAAACGACATGAGCTCTGGGAGCTTTCTG
PtrSSR/AG054R	CCATCTCGCTTTTGAGATACC
PtrSSR/AG055F	CACGACGTTGTAAAACGACGTTCCTTGCTAGGGGGTCAAT
PtrSSR/AG055R	TTTGGCCGGATTCCATGAT
PtrSSR/AG056F	CACGACGTTGTAAAACGACAGGGATGGGAGGAAAAGAA
PtrSSR/AG056R	CTGCGATGGTGATTTATCAAA
PtrSSR/AG057F	CACGACGTTGTAAAACGACACCATGTTGCACGGCTGATA
PtrSSR/AG057R	GGAGTGGACGAAGTCTGTTCT
PtrSSR/AG059F	CACGACGTTGTAAAACGACTTGTATAGGGCGGTGGTAGAT
PtrSSR/AG059R	GGCGTTTTAAAATGGGATGG
PtrSSR/AG060F	CACGACGTTGTAAAACGACTCGGAGTAACCATTGTGGATT
PtrSSR/AG060R	AAAGCTTAGGGTCGGGCTTAA
PtrSSR/AG062F	CACGACGTTGTAAAACGACTACCGTCGTTCCCCCATAGTT
PtrSSR/AG062R	GCTGGGTCAGTCACAGTCAT
PtrSSR/AG063F	CACGACGTTGTAAAACGACTTGCTTGGAAGGTCGTGAGA
PtrSSR/AG063R	AACCTTGAAAACGCCTTGCT
PtrSSR/AG064F	CACGACGTTGTAAAACGACCCGTCCGACACTAGATCATCA
PtrSSR/AG064R	CCGTGGTGATTGCTTGGT
PtrSSR/AG065F	CACGACGTTGTAAAACGACATCCAACCCCACGTTGAT
PtrSSR/AG065R	TGCGTGGAGTAGCGTCAAGT
PtrSSR/AG066F	CACGACGTTGTAAAACGACCCATGTAACAACAATACGCCC
PtrSSR/AG066R	CGTTTCGTCGCGAGAGAGA
PtrSSR/AG067F	CACGACGTTGTAAAACGACTTGTGGTGGATTCCATCAGA
PtrSSR/AG067R	GCAGCTTTTGGCGAAGTTTT
PtrSSR/AG069F	CACGACGTTGTAAAACGACATGCTTCTTGGCCGAATGTT
PtrSSR/AG069R	GCCTCTATGTGCCGAGAGAGA
PtrSSR/AG070F	CACGACGTTGTAAAACGACATAGCCAGCTCAAACGTTCC
PtrSSR/AG070R	TTGTCACATGGTTCAGTCGG
PtrSSR/AG072F	CACGACGTTGTAAAACGACTTGACGAGCGGGTTCTAGAAA
PtrSSR/AG072R	AAAATACCAGCGCTACCATCC
PtrSSR/AGC002F	CACGACGTTGTAAAACGACACATCTCAAGCTCGACGCCT
PtrSSR/AGC002R	TGACAGCTGGTCCGAACTGTA

Primer name	Primer sequence [a]
PtrSSR/AGC003F	CACGACGTTGTAAAACGACTCAGAAAGTCTTTTGGGGCT
PtrSSR/AGC003R	TCATATCCCGCACCCGTT
PtrSSR/AGG002F	CACGACGTTGTAAAACGACTCGCCACTGTGGTGGATTATT
PtrSSR/AGG002R	CAAGTCCCCATTCTACAGCAT
PtrSSR/AGG003F	CACGACGTTGTAAAACGACTCGCTAGATCTATCGCTTGGG
PtrSSR/AGG003R	ACAAAGCAGATGACGAGGAGA
PtrSSR/AGG004F	CACGACGTTGTAAAACGACAGACGACAAAGCAGATGACGA
PtrSSR/AGG004R	CGGCAGGTATCTCGCTAGAT
PtrSSR/AGG005F	CACGACGTTGTAAAACGACTCGCTAGATCTATCGCTTGGG
PtrSSR/AGG005R	ACAAAGCAGATGACGAGGAGA
PtrSSR/AGG007F	CACGACGTTGTAAAACGACAATGTGGTGGAATTCCCTCCT
PtrSSR/AGG007R	CAACACTTACCTCACCGGTCT
PtrSSR/AGG010F	CACGACGTTGTAAAACGACACTACGTTGAGAGCACTGCTG
PtrSSR/AGG010R	AAGAGAAAGCTAGGGAGGGAA
PtrSSR/AGG011F	CACGACGTTGTAAAACGACTCAGCTTCAAGAATGGTGGT
PtrSSR/AGG011R	ACAGTCTCGTCCTGTTCGG
PtrSSR/AGT008F	CACGACGTTGTAAAACGACTGATGCGCAACATGTCGAGTA
PtrSSR/AGT008R	TAAGGGGCAGGACTTGGAAA
PtrSSR/AGT009F	CACGACGTTGTAAAACGACGGGGATCCACACTAGTCAACG
PtrSSR/AGT009R	GCGAACGAACTCGAGATTGA
PtrSSR/AGT014F	CACGACGTTGTAAAACGACTGCCGTAAGCAGTCCAAGC
PtrSSR/AGT014R	AAATCAGACGCCCAGTAGTCA
PtrSSR/AGT015F	CACGACGTTGTAAAACGACTGTCTTGAAGCCTGCTCTGAA
PtrSSR/AGT015R	CAGTTAAGCTAGCGGGGTAAA
PtrSSR/AGT017F	CACGACGTTGTAAAACGACATCCTGCATTCTGCTGCATA
PtrSSR/AGT017R	TCCTCGCAGCCTAGTGGTAGT
PtrSSR/AGT020F	CACGACGTTGTAAAACGACCCGTTAGGGTCGTGAGAAAAA
PtrSSR/AGT020R	TGCCATGAACTATACTCCGGT
PtrSSR/AGT021F	CACGACGTTGTAAAACGACTGTGGTGGATTCGGATTGATT
PtrSSR/AGT021R	TGGACATGATGCGCAACAT
PtrSSR/AT004F	CACGACGTTGTAAAACGACTGCTTGGTGCCGTAGTACTTG
PtrSSR/AT004R	ACCCCCTATAGCCGAATATCT
PtrSSR/AT005F	CACGACGTTGTAAAACGACTACGGTCAATAGGGCTTCGAT
PtrSSR/AT005R	GACGCTCGCAAAGTCTACATA
PtrSSR/AT006F	CACGACGTTGTAAAACGACCGGATTTTTGGAGGGGTTAT
PtrSSR/AT006R	GCTCCATATGCCAGTATGGAT
PtrSSR/AT007F	CACGACGTTGTAAAACGACAAGGGTGTCCGTCACTGTAAA
PtrSSR/AT007R	ACATCCAGATCATCTCGACGA
PtrSSR/AT012F	CACGACGTTGTAAAACGACACGGCCTTCGGCAAGTCTA
PtrSSR/AT012R	CCTGTAGCGTGAGGCTGATAT
PtrSSR/AT014F	CACGACGTTGTAAAACGACGCAAGGGTCAGTAGCGTAAAA
PtrSSR/AT014R	ATGGTTTGATTATTCCCGCC
PtrSSR/AT016F	CACGACGTTGTAAAACGACGCACGTGACATGCGCAATA
PtrSSR/AT016R	AACAGCAATCAATCAGCGG
PtrSSR/AT017F	CACGACGTTGTAAAACGACAAACCAACACTGATCACCCCT
PtrSSR/AT017R	AACAGCAATCAATCAGCGG
PtrSSR/AT018F	CACGACGTTGTAAAACGACAGGATTGCAGCTGGTAGCTTT
PtrSSR/AT018R	TTTAGAAGGCTGCGAGGTGG
PtrSSR/AT019F	CACGACGTTGTAAAACGACAGCTGCACGCAACACGGAT
PtrSSR/AT019R	ACGCCATCCACACAAATCT
PtrSSR/AT021F	CACGACGTTGTAAAACGACTGAAGAAGAGTCGCGATGAA
PtrSSR/AT021R	ACCATCATGATTACGTCCTCC
PtrSSR/ATG002F	CACGACGTTGTAAAACGACCAGTGGCTTTGCCACTAAAA
PtrSSR/ATG002R	TAGGTCGCTCGACGACATGA
PtrSSR/ATG003F	CACGACGTTGTAAAACGACATGTTGCAGAGCCTCGACA
PtrSSR/ATG003R	AAGCGTCTTTGGGCGAAGA

Primer name	Primer sequence [a]
PtrSSR/ATG004F	CACGACGTTGTAAAACGACAACTTTGCTCGACCGTCAA
PtrSSR/ATG004R	TTACCGTCGTCTGCTCGATAA
PtrSSR/ATG005F	CACGACGTTGTAAAACGACTCATCGCACTGTGGTGGATT
PtrSSR/ATG005R	TTGCTAGTCTTTTCCCCATCC
PtrSSR/AAC016F	CACGACGTTGTAAAACGACATAGTCTAGATGGGCGAGCGT
PtrSSR/AAC016R	ATAAGACGGTGGCGTAGCGT
PtrSSR/AAC018F	CACGACGTTGTAAAACGACTACTTAAGCATTCGAAGCCG
PtrSSR/AAC018R	AGCAGCGTTCTTGAGGAAGA
PtrSSR/AAG044F	CACGACGTTGTAAAACGACTGTGGTGGATTCCCATTTCA
PtrSSR/AAG044R	GCGAAGTAGAGTAGAGGGCCT
PtrSSR/AAT005F	CACGACGTTGTAAAACGACTAGAGCCTGCCGAGATTGTTT
PtrSSR/AAT005R	GCCTTCGGCCAAAGTCATA
PtrSSR/AAT023F	CACGACGTTGTAAAACGACCCCGCTGCTACATCTCTTTCT
PtrSSR/AAT023R	GGAGAACCTAGGCTTCTGAAA
PtrSSR/AC018F	CACGACGTTGTAAAACGACTGCAGTAAGCACAGTGAAGGC
PtrSSR/AC018R	ATCGGCGGAGAAGATCATTT
PtrSSR/AC023F	CACGACGTTGTAAAACGACCACAAAGGCCACCAAGGGA
PtrSSR/AC023R	AACCCGCTTTGTAGTGGCTT
PtrSSR/AC030F	CACGACGTTGTAAAACGACGGGAGACGACCATCCGTTATA
PtrSSR/AC030R	CAACCGCGGAAAATATCTTC
PtrSSR/AC037F	CACGACGTTGTAAAACGACTACCACTAACCGCCCCTTAA
PtrSSR/AC037R	TAAGCGAGGGAGGGATGTG
PtrSSR/AC042F	CACGACGTTGTAAAACGACAGCCACAGCATTACACACACA
PtrSSR/AC042R	TTTGCACTCTCTTTACACCCA
PtrSSR/AC046F	CACGACGTTGTAAAACGACGGGTAAATGAAAAACGCAGA
PtrSSR/AC046R	AACGATGGGCACAGAGCAGTA
PtrSSR/AC049F	CACGACGTTGTAAAACGACTGATTCATTCGCACTGTGGT
PtrSSR/AC049R	ATGTCCCACACTCAACACGT
PtrSSR/AC054F	CACGACGTTGTAAAACGACATTCCCGTCGTACCTGTATGA
PtrSSR/AC054R	ACGCACTACACGGCAATGA
PtrSSR/AC064F	CACGACGTTGTAAAACGACAAGGTCGTGGGTTTTTGCTT
PtrSSR/AC064R	AGCGGGGTGTATCAAAGTCAA
PtrSSR/AC068F	CACGACGTTGTAAAACGACGCATTCCAAAGTCCGGCT
PtrSSR/AC068R	TGCCACTGCACCTGTATTTCT
PtrSSR/AC074F	CACGACGTTGTAAAACGACCGTTGTCTTGTTTTGAGCGA
PtrSSR/AC074R	GACACACCTCCAAACGCTATT
PtrSSR/AC085F	CACGACGTTGTAAAACGACTGTAGATGCGAGTGCGGGTA
PtrSSR/AC085R	GGAATCTTTTCATCCAATTGC
PtrSSR/AC089F	CACGACGTTGTAAAACGACTATCGGCGTTCTTGATGTGTC
PtrSSR/AC089R	ATTGCTTGTTGCTAGTCACCG
PtrSSR/AC109F	CACGACGTTGTAAAACGACGCGGCTGATAGAGTACATGTG
PtrSSR/AC109R	TGTGAAGGATTTGGCGAAGA
PtrSSR/AG025F	CACGACGTTGTAAAACGACTCTTGCGCTCGTTTGTTTTC
PtrSSR/AG025R	GAACGACACGTCTCCACGATA
PtrSSR/AG044F	CACGACGTTGTAAAACGACTTTTGTCTGAGTAACCACGGC
PtrSSR/AG044R	TCAACCATACGGCAGCCAT
PtrSSR/AGC001F	CACGACGTTGTAAAACGACAGAAGAGGAAGGAGCAGCAA
PtrSSR/AGC001R	TGTTGAAGACCTGGAGGAGTT
PtrSSR/AGG009F	CACGACGTTGTAAAACGACTGGATTCAAGCAGCCAAAGA
PtrSSR/AGG009R	TAACCCACGTCCAGTCCATCA
PtrSSR/AGT012F	CACGACGTTGTAAAACGACAACTGTGGTGGATTCAGTGGT
PtrSSR/AGT012R	AGCATAAATACGTTGACCCCC
PtrSSR/AT015F	CACGACGTTGTAAAACGACAAAGAGCAGGGTTATGGTGGA
PtrSSR/AT015R	GCAACCAGTCCAATCATATTG
PtrSSR/ATG006F	CACGACGTTGTAAAACGACGCACAGATATCTCCATTCCCA
PtrSSR/ATG006R	AAGCGACCATCGTGGCAAT

Primer name	Primer sequence [a]
PtrSSR/AG035F	CACGACGTTGTAAAACGACTTGCAGGTCGACTCTAGAGGA
PtrSSR/AG035R	CGCACCCCAAACCTATACCTA
PtrSSR/AAT010F	CACGACGTTGTAAAACGACAGCGAACCATACCAGAAAACC
PtrSSR/AAT010R	CCCCTGAGTACCCTACTACGA
PtrSSR/AAG042F	CACGACGTTGTAAAACGACGGCTAAGAATGGAAATGACGA
PtrSSR/AAG042R	TGCGAGGTGAAAGAAAGAAGA
PtrSSR/AC052F	CACGACGTTGTAAAACGACATTCACCCATCGTATCGCAT
PtrSSR/AC052R	TTTGAGCTCTTCGAATTCGG
PtrSSR/AAG043F	CACGACGTTGTAAAACGACCGCCACTGTGGTGGATTCTAT
PtrSSR/AAG043R	TTGAGAGCACTGCTGCCCA
PtrSSR/AAT017F	CACGACGTTGTAAAACGACAGCCAAAAACCATATTAGCGA
PtrSSR/AAT017R	AATACAACGCCAGAGTCCCCT
PtrSSR/AAT021F	CACGACGTTGTAAAACGACTTACTCCACGAACCTCACCTC
PtrSSR/AAT021R	TAGTCTTCTGTTGGCGCAGGT
PtrSSR/AAT024F	CACGACGTTGTAAAACGACTCCCGGACTTCTGCTTCTTCT
PtrSSR/AAT024R	ACCACCCAAAATATGCCCAT
PtrSSR/AG020F	CACGACGTTGTAAAACGACTAACGTGTTGCCACATGTTG
PtrSSR/AG020R	TTGCGCTCCGACACTAAACTT
PtrSSR/AGT011F	CACGACGTTGTAAAACGACTATCGGGGGAAACGCAAA
PtrSSR/AGT011R	TACAAGATCTTCCGACGGGC
PtrSSR/AC002F	CACGACGTTGTAAAACGACGCCCTGGTATCTCACCAAGAA
PtrSSR/AC002R	TCAGCCAGGCATCCATTATT
PtrSSR/AAT019F	CACGACGTTGTAAAACGACACGGGGGACCCACATTATTAT
PtrSSR/AAT019R	TTCGGACGACCTTCACATCTT
PtrSSR/AAT013F	CACGACGTTGTAAAACGACAGCCAAACAAACACCCAAAC
PtrSSR/AAT013R	TGAGGCCGCACACAATAGAT
PtrSSR/AAC014F	CACGACGTTGTAAAACGACATTCATCGCTCGGGGGTTT
PtrSSR/AAC014R	TACTTCGACCGTCACGCAA
PtrSSRAAC001F	CACGACGTTGTAAAACGACTATCGCGGTAGGATTGTGGTT
PtrSSRAAC001R	TCAAGGCGGATCGGAAATTA
PtrSSRAAC002F	CACGACGTTGTAAAACGACATCGCTTTCGTTGCGTCTGT
PtrSSRAAC002R	ACGGCTGAACTGGCACAGTA
PtrSSRAAC005F	CACGACGTTGTAAAACGACTGACTTGGGGGATCGTCTAT
PtrSSRAAC005R	TGGCAGTCTAGGGGTTGTG
PtrSSRAAC006F	CACGACGTTGTAAAACGACTGATCGTGCATTTGCGATG
PtrSSRAAC006R	ATTCCAGGCCACAGTCAAGTA
PtrSSRAAC007F	CACGACGTTGTAAAACGACTGTGGTGGATTCTTGTTGTTG
PtrSSRAAC007R	AAATTACTTTGGCGCCCC
PtrSSRAAC009F	CACGACGTTGTAAAACGACTTTGGGAGATGGGGGAAA
PtrSSRAAC009R	TTGCGTCTGTGCGACATG
PtrSSRAAC012F	CACGACGTTGTAAAACGACACCATTTTGCATGGCCCT
PtrSSRAAC012R	TCCCTTTCTAAGCCAGAAGCA
PtrSSRAAC019F	CACGACGTTGTAAAACGACACGACCACTTTAGGGGAGAAT
PtrSSRAAC019R	CGGCAAGGAGTATGATGAGTT
PtrSSRAAG002F	CACGACGTTGTAAAACGACCGGGTACAATTGTGGTGGATT
PtrSSRAAG002R	GGGTGTTTAAAAGTCAACGCA
PtrSSRAAG003F	CACGACGTTGTAAAACGACTAGAGGACCCACGTGCAA
PtrSSRAAG003R	AGCGTCAGGAAGAAGCCTTG
PtrSSRAAG004F	CACGACGTTGTAAAACGACTTCGCCTGCAGATATGGGA
PtrSSRAAG004R	TAACGTGTGACCTCGACACTT
PtrSSRAAG005F	CACGACGTTGTAAAACGACATCAGTAGGTGCCGCAATCTT
PtrSSRAAG005R	TTCATCGCGGTGAACTGAA
PtrSSRAAG006F	CACGACGTTGTAAAACGACACTCTAGAGGATCCCACCACG
PtrSSRAAG007F	CACGACGTTGTAAAACGACCCGTTCCCTATAAGCAAAAGC
PtrSSRAAG007R	GCCAGAGAGATCTTCGAGTCA

Primer name	Primer sequence [a]
PtrSSRAAG008F	CACGACGTTGTAAAACGACCTGCGGCCAAAAAAAGATTG
PtrSSRAAG008R	TATTCCCACAATAGCTCTGCC
PtrSSRAAG009F	CACGACGTTGTAAAACGACAAATCATCGCACTGTGGTGG
PtrSSRAAG009R	TATCCCAGGATTCCCGGAAA
PtrSSRAAG010F	CACGACGTTGTAAAACGACGGTTCAATGTGGTGGATTCTG
PtrSSRAAG010R	GAAGGAATGCCTGTATGCAA
PtrSSRAAG011F	CACGACGTTGTAAAACGACCGGTACATGCATGACGTTGTT
PtrSSRAAG011R	GCACGCTTATATGCGCTTTT
PtrSSRAAG012F	CACGACGTTGTAAAACGACCGGCACAGTAAGGAGCTAGAT
PtrSSRAAG012R	CCGTTAGGTCCTAGTGAATGC
PtrSSRAAG013F	CACGACGTTGTAAAACGACTTGATGCTGTTCTGCGTGA
PtrSSRAAG013R	GCCCTTATCCGATCACCTAGT
PtrSSRAAG014F	CACGACGTTGTAAAACGACCGCCAACATAGTTGCGAATT
PtrSSRAAG014R	AGGGTGTTTAAAAGTCAACGC
PtrSSRAAG015F	CACGACGTTGTAAAACGACACTGTGGTGGATTCGTTGAA
PtrSSRAAG015R	TTCTTAGCTCCGCCTAGGTG
PtrSSRAAG016F	CACGACGTTGTAAAACGACGCGTGATCTCGCCACATATTA
PtrSSRAAG016R	CGACGTCCTTCACAGGATTTA
PtrSSRAAG017F	CACGACGTTGTAAAACGACATCATCGCACTGTGGTGGATT
PtrSSRAAG017R	GGCGGCTTCATATTACTGAA
PtrSSRAAG018F	CACGACGTTGTAAAACGACAGCGTCTGGAAGAAGCCTTG
PtrSSRAAG018R	TCTCTGCATTTGTGGTGCAA
PtrSSRAAG019F	CACGACGTTGTAAAACGACCTGCGGCCAAAAAAAGATTG
PtrSSRAAG019R	TTTTCGGCCCACAGGGTTT
PtrSSRAAG020F	CACGACGTTGTAAAACGACATTAGATATGAGTCCGGGCTG
PtrSSRAAG020R	CGTAGGAGACCTGGTATCTCG
PtrSSRAAG022F	CACGACGTTGTAAAACGACTCGTGGGTATAAAACGGCTCT
PtrSSRAAG022R	TTCGGCGGCTTCATATTACT
PtrSSRAAG024F	CACGACGTTGTAAAACGACCGCCAACATAGTTGCGAATT
PtrSSRAAG024R	TCCGCCTCTGTAGGTGTTAAA
PtrSSRAAG025F	CACGACGTTGTAAAACGACAAGAGGTGTCGACTAGCGTTT
PtrSSRAAG025R	GGCTTAATTTTAAGCGCGTG
PtrSSRAAG027F	CACGACGTTGTAAAACGACCAAATCATCGCCACTGTGGT
PtrSSRAAG027R	TCGCCTTATCAATACGCCAT
PtrSSRAAG028F	CACGACGTTGTAAAACGACTTAGCGAGATCACGCGTTAT
PtrSSRAAG028R	GTGCATTCCAGAAGCCTAAA
PtrSSRAAG029F	CACGACGTTGTAAAACGACCGGAGTACATTGTGGTGGATT
PtrSSRAAG029R	CGGTACATGCATGACGTTGTT
PtrSSRAAG030F	CACGACGTTGTAAAACGACCCATTAAAATCCAGTCCACCC
PtrSSRAAG030R	AGCCAACTGCATCGTAGTTTG
PtrSSRAAG031F	CACGACGTTGTAAAACGACTGGATTAGACGGGGACATTGT
PtrSSRAAG031R	TAGCCACCTGCATCGTATTCT
PtrSSRAAG032F	CACGACGTTGTAAAACGACTAGACGGGTACATTGTGGTGG
PtrSSRAAG032R	ACCGTTGTGGGTGTATACCAA
PtrSSRAAG033F	CACGACGTTGTAAAACGACACCCCACCCTCCCATTAAA
PtrSSRAAG033R	TTGCCCAGAAGAGGTTGAA
PtrSSRAAG034F	CACGACGTTGTAAAACGACGCCCTTATCCGATCACCTAGT
PtrSSRAAG034R	CGGCCAAGTCCATTGAATCTA
PtrSSRAAG035F	CACGACGTTGTAAAACGACGCACGCTTATATGCGCTTTT
PtrSSRAAG035R	ACACATGACACACGCTTGTTT
PtrSSRAAG036F	CACGACGTTGTAAAACGACTTACGGATGTCGGAGGGTGTT
PtrSSRAAG036R	TATACCAAGCTCTCCGCCTCT
PtrSSRAAG037F	CACGACGTTGTAAAACGACATCGCCACTGTGGTGGATT
PtrSSRAAG037R	ATTGCCGTGGGGTGAGAGT
PtrSSRAAG038F	CACGACGTTGTAAAACGACTGCATTTGTGGTGCAAGATC
PtrSSRAAG038R	AGAAGCCTTGGCCATTTTCA

Primer name	Primer sequence [a]
PtrSSRAAG039F	CACGACGTTGTAAAACGACCGCCAACATAGTTGCGAATT
PtrSSRAAG039R	CGCCTCTGTAGGGTGTTTAAA
PtrSSRAAG047F	CACGACGTTGTAAAACGACACATCGACACGTCGACCGT
PtrSSRAAG047R	TATGGTGAGGGATCGAGGGTA
PtrSSRAAG052F	CACGACGTTGTAAAACGACGCCGGAAATAACCAATCAGA
PtrSSRAAG052R	ACGGATACGGTTCAAGCAAT
PtrSSRAAG053F	CACGACGTTGTAAAACGACCAAGTGCATTCCAGAAGCCTA
PtrSSRAAG053R	TCCTTCACAGGATTTAGCGA
PtrSSRAAG058F	CACGACGTTGTAAAACGACGGTCCGTTAGGTCCTAGTGAA
PtrSSRAAG058R	GGCTTAATTTTAAGCGCGTG
PtrSSRAAG059F	CACGACGTTGTAAAACGACAGCTCATGTGGTGGATTCCTT
PtrSSRAAG059R	TTTTTTTGGAAAGCCCCAGG
PtrSSRAC110F	CACGACGTTGTAAAACGACCCTCTGCGCTCTTCTTCTTAG
PtrSSRAC110R	CCAGAACATCAACAACACCGT
PtrSSRAC112F	CACGACGTTGTAAAACGACAGCACAGCAGGCAAAGGAA
PtrSSRAC112R	GCATTGCGGTAGCTGTGTCTA
PtrSSRAG005F	CACGACGTTGTAAAACGACTCACCATGTCTGCTTCTGCAT
PtrSSRAG005R	TAATCGCCCAAGTAGCATCGT
PtrSSRAG013F	CACGACGTTGTAAAACGACTCTGCATTTGTGGTGCAAGA
PtrSSRAG013R	TAACTTGATTTGAGCGCGTG
PtrSSRAG014F	CACGACGTTGTAAAACGACAATCGTCACGTAGATCGAACC
PtrSSRAG014R	TTAGCACCTAAGACGTCGATG
PtrSSRAG016F	CACGACGTTGTAAAACGACTGTGTAGGGGACCGGAATG
PtrSSRAG016R	TGAATGAGGAACGGCATTTG
PtrSSRAG017F	CACGACGTTGTAAAACGACTTCGCCTGCAGATATGGGA
PtrSSRAG017R	ACGGGTAGCTCTATGTGAGGA
PtrSSRAG018F	CACGACGTTGTAAAACGACACGTCCCTGCTTACGGATGT
PtrSSRAG018R	ATACCAAGCTTCTCCGCCTCT
PtrSSRAG023F	CACGACGTTGTAAAACGACTCAAACTCAGAAATCCGCCT
PtrSSRAG023R	TTTCCCACTCTCCTGCCCTAT
PtrSSRAG024F	CACGACGTTGTAAAACGACTCAGGTTTTCGAATTCCCGT
PtrSSRAG024R	GCGAGCATTTTTCAGGTTTAC
PtrSSRAG026F	CACGACGTTGTAAAACGACAAAGATACCTCCACAGCAGCA
PtrSSRAG026R	TGACGCTCAGACGTGGTTT
PtrSSRAG029F	CACGACGTTGTAAAACGACAAGAGGGAGCCACTGCAAAT
PtrSSRAG029R	AGGGAAGACGGAGTGCCTGTA
PtrSSRAG037F	CACGACGTTGTAAAACGACTTGGAAGTGCATGGCAAACT
PtrSSRAG037R	GGTGTGAGGGATTATTCTGCT
PtrSSRAG039F	CACGACGTTGTAAAACGACTTTGTCCCGCACAACACAA
PtrSSRAG039R	ACATGGAGAGTGAGTGCAGTG
PtrSSRAG049F	CACGACGTTGTAAAACGACACCACCACTGACCCATCTTTT
PtrSSRAG049R	TCACGGTGATGGCACATATA
PtrSSRAG051F	CACGACGTTGTAAAACGACCCCGACCAGGTACTAAGAAGA
PtrSSRAG051R	GGCTTGGTTCTTTTTTCGGA
PtrSSRAG058F	CACGACGTTGTAAAACGACTGTCCGCCTTCATGTACCTA
PtrSSRAG058R	TGGGGGTGAGTTTTACGTTT
PtrSSRAG061F	CACGACGTTGTAAAACGACCAAGCTGTTCAACGCAAGAGT
PtrSSRAG061R	ATGCGCGTAGAGACAGAAGG
PtrSSRAG068F	CACGACGTTGTAAAACGACGCAGAGGAGGTTGTGATTCCA
PtrSSRAG068R	TTATGCGTCGTCTTGGAGTT
PtrSSRAG071F	CACGACGTTGTAAAACGACTGAACCAGAGAGTGAGTGGAA
PtrSSRAG071R	TCCGTTCGTGGACGTTGA
PtrSSRAGG001F	CACGACGTTGTAAAACGACGGAGGAGAAGGAGGAGGAGAA
PtrSSRAGG001R	ACGATCTGCTGTCTCATCTCA

Primer name	Primer sequence [a]
PtrSSRAGG006F	CACGACGTTGTAAAACGACTGGAGACCGATAGTTGAGGAT
PtrSSRAGG006R	TCACTGTCCTCAACCACCG
PtrSSRAGG008F	CACGACGTTGTAAAACGACGCGAGTTAGAAACGAGGCAA
PtrSSRAGG008R	ATATGGTGGTTGCTTGCGCTA
PtrSSRAGT001F	CACGACGTTGTAAAACGACCCACGCTCATCACTTTGTCTA
PtrSSRAGT001R	CCACTTGATTGAGTTTTGCG
PtrSSRAGT002F	CACGACGTTGTAAAACGACTTACGATGCGCTCACACTAGA
PtrSSRAGT002R	ATGGCCCGCTTGTAGTCTTTA
PtrSSRAGT003F	CACGACGTTGTAAAACGACATCCACCTCCGTTGCAGTT
PtrSSRAGT003R	CTGATTTCACCGCGAAAACA
PtrSSRAGT004F	CACGACGTTGTAAAACGACTTACGCCCCGTTCGTAGATAT
PtrSSRAGT004R	TGTTCAATCTTGCGTCGATG
PtrSSRAGT005F	CACGACGTTGTAAAACGACCCACTGGCTCATCACTTTGT
PtrSSRAGT005R	ACCACTTGATTGAGTTTGCG
PtrSSRAGT006F	CACGACGTTGTAAAACGACTTCGCTACCACACGACTGTAT
PtrSSRAGT006R	CGAACGAGGCAGCCTAGTATT
PtrSSRAGT007F	CACGACGTTGTAAAACGACCCATGAGGAGAAGTATTCCCA
PtrSSRAGT007R	ACCGCGAAACATCTGTGAGA
PtrSSRAGT010F	CACGACGTTGTAAAACGACTGGGGTGGTGTGATGTAAAT
PtrSSRAGT010R	CCTGATTTCACGCGAAAACA
PtrSSRAGT013F	CACGACGTTGTAAAACGACAGCAACCAATCAGCGTAGAGA
PtrSSRAGT013R	AATGCAACGCAGTCCTCCT
PtrSSRAGT016F	CACGACGTTGTAAAACGACATTGAGGCTCTGTCATCTTGC
PtrSSRAGT016R	ACCCGGTGGTCGTAGTACTAG
PtrSSRAGT018F	CACGACGTTGTAAAACGACTTGTATGCGAGTGAGTATGGG
PtrSSRAGT018R	CCGCCGCTTAAGCTTGTATTA
PtrSSRAGT019F	CACGACGTTGTAAAACGACAATCGCACAAGCACGTGTCA
PtrSSRAGT019R	GTTTTGCGTTTTCCTTGTAGC
PtrSSRAGT022F	CACGACGTTGTAAAACGACACTCCCATCCTTTTCTAGCGA
PtrSSRAGT022R	TTCTGTATTCCCAACCACAGC
PtrSSRAT001F	CACGACGTTGTAAAACGACTGATACCGGCCATGTGTATA
PtrSSRAT001R	GGATGGGTGTAGACCCACATA
PtrSSRAT002F	CACGACGTTGTAAAACGACCTAAGCGGGAAGCTTGGTCTA
PtrSSRAT002R	GCATCGCTTAAGGTGCAAAA
PtrSSRAT003F	CACGACGTTGTAAAACGACTTATATATATGGCGGTCCCCG
PtrSSRAT003R	CACGAATAGTATATGTCGCGG
PtrSSRAT008F	CACGACGTTGTAAAACGACTGTGGGTATTCGCATCTGAT
PtrSSRAT008R	CGCTACGGATGCCTAGACATA
PtrSSRAT009F	CACGACGTTGTAAAACGACGGGGTGGTGTAAAATGTTGA
PtrSSRAT009R	ACACGGGTATGGATACGGTAT
PtrSSRAT010F	CACGACGTTGTAAAACGACTCGTCACACGTTGTCTCGTTA
PtrSSRAT010R	CCCCTCCTCACATATCGGTAT
PtrSSRAT011F	CACGACGTTGTAAAACGACGGGACCCCGTGTGTATATAT
PtrSSRAT011R	TGGAGATAGAGTCCTTTGTGG
PtrSSRAT013F	CACGACGTTGTAAAACGACGGGGATATCATACATCCAGCA
PtrSSRAT013R	GGTGCATGTACACGTGTGCAT
PtrSSRAT020F	CACGACGTTGTAAAACGACAGGGGAAAGGGGTTTGGTTAT
PtrSSRAT020R	TCACGAAAGCATTTCAGGGA
PtrSSRAT022F	CACGACGTTGTAAAACGACTGCATCCAAGCTCGTCGA
PtrSSRAT022R	CGCAGATCTTGCATGTGAGAT
PtrSSRAT023F	CACGACGTTGTAAAACGACTCCTGAATGATGCACAGACGA
PtrSSRAT023R	TCCCAATAACCGCCCATATA
PtrSSRATG001F	CACGACGTTGTAAAACGACTCCTGACCTTACCCAGAATCA
PtrSSRATG001R	GATCCGATACCACGGAGATT

[a]Forwared primer for each pair was modified by add the M13 sequence (CACGACGTTGTAAAACGAC)

APPENDIX E. GENOME ANNOTATION STATISTICS OF AR CROSSB10 ASSEMBLY

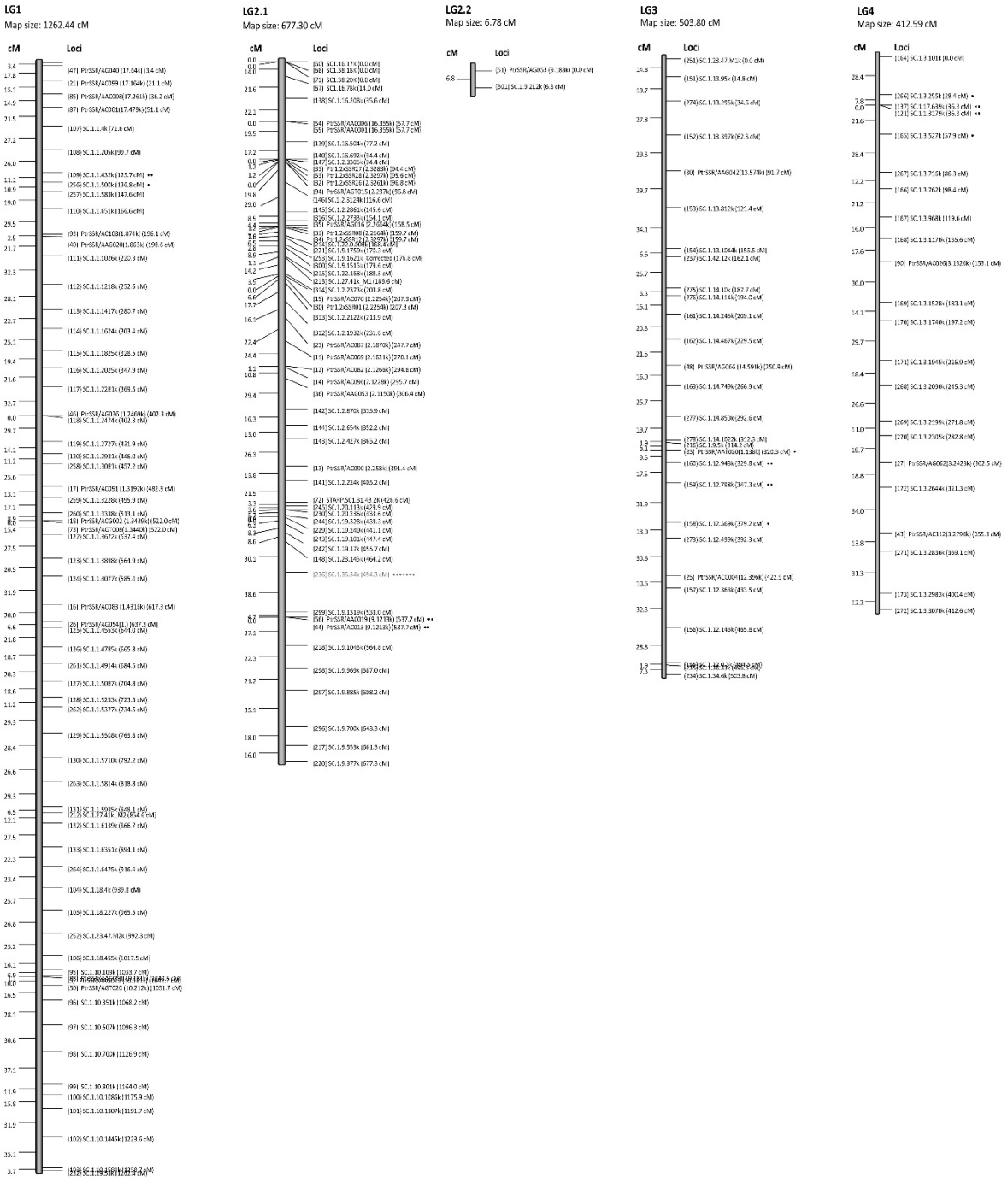
Table E1. Annotated gene properties

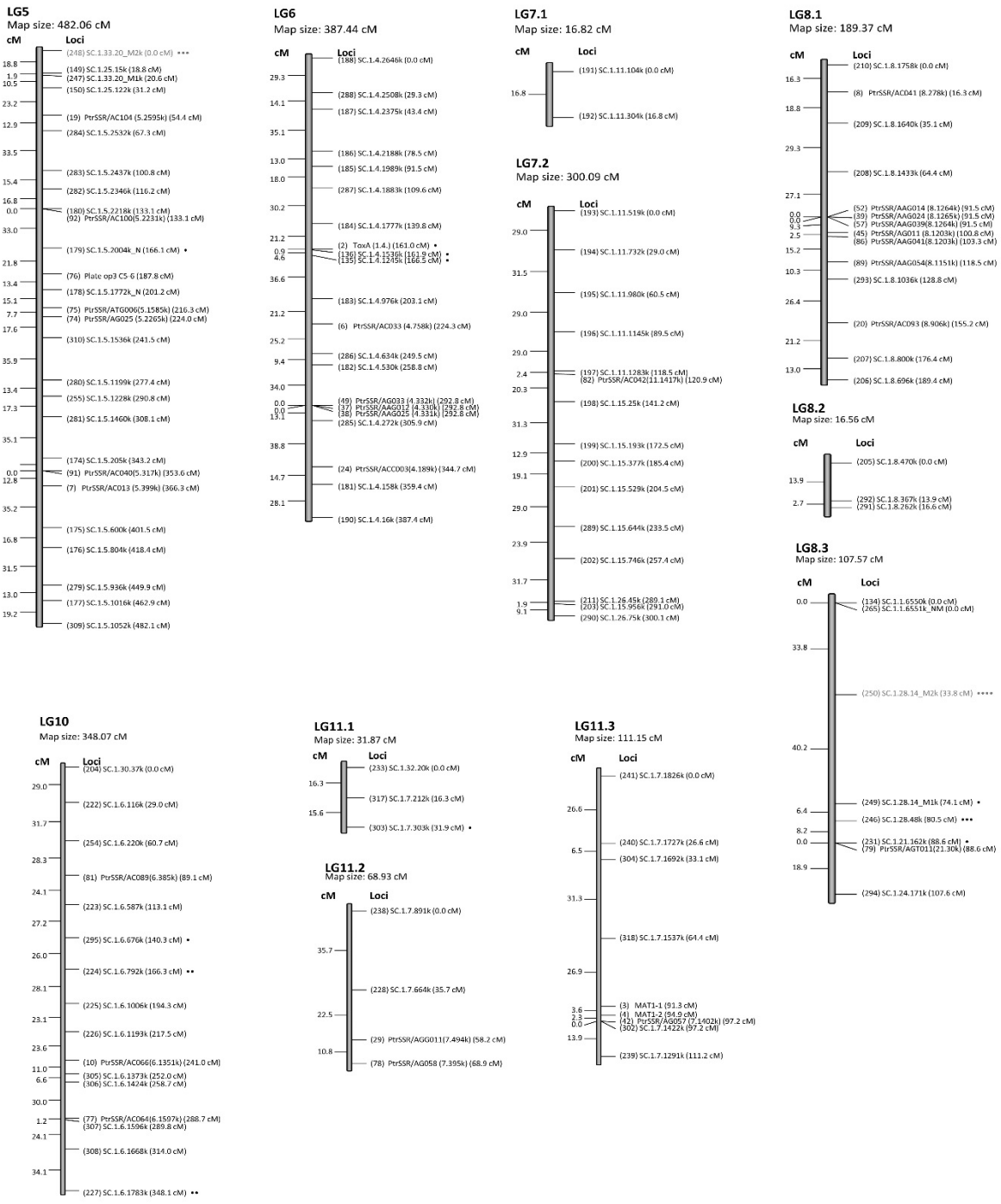
Parameter	Value
Annotated genes	13,768
Mean gene length (bp)	1741.2
Mean exon/gene	2.8
Predicted secreted proteins	1,221
Predicted effectors	312

Table E2. Repetitive content of AR CrossB10

Repeat	Number of elements	Length occupied in the genome (bp)	Percentage of the genome (%)
DNA transposons	2397	2,418,282	6.02
LTR retrotransposon	2306	3,076,648	7.66
LINEs	499	697,178	1.74
SINEs	0	0	0
Unclassified	4563	1,340,251	3.34
Total	9765	7,567,509	18.85

APPENDIX F. GENETIC LINKAGE MAPS FOR AR POPULATION




LG5
Map size: 482.06 cM
LG6
Map size: 387.44 cM
LG7.1
Map size: 16.82 cM
LG7.2
Map size: 300.09 cM
LG8.1
Map size: 189.37 cM
LG8.2
Map size: 16.56 cM
LG8.3
Map size: 107.57 cM
LG10
Map size: 348.07 cM
LG11.1
Map size: 31.87 cM
LG11.2
Map size: 68.93 cM
LG11.3
Map size: 111.15 cM

APPENDIX G. 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 HARRY×WESLEY POPULATION

RIL ID.	RIL No.	Pti2	86-124	86-124ΔToxA	331-9	DW5	AR CrossB10
HW- 8	WH1	3.67	2.5	4	4.33	3	3.33
HW- 9	WH2	2.17	1.5	3.17	4.33	1.17	3.5
HW- 11	WH3	3.17	2.5	3.33	4.5	3.17	3.67
HW- 12	WH4	2	1.67	2.67	4	2.17	3.5
HW- 13	WH5	2.5	2.5	1.83	4.17	1.33	3.5
HW- 15	WH6	3.67	2.83	4.17	2.83	3.33	3.17
HW- 16	WH7	3.33	2.67	3	2.83	2.83	2.83
HW- 17	WH8	3.17	3	3.17	4	2	3.33
HW- 18	WH9	2.67	2	2.67	4	2.33	3
HW- 20	WH11	2.67	2.5	3.33	2.5	2.17	2.67
HW- 24	WH12	2.83	2.5	3.33	2.67	2.5	2.17
HW- 25	WH13	2.17	2.5	2.33	2.83	2.33	2.5
HW- 27	WH15	3.17	2.17	2.33	4.67	3.5	3.67
HW- 29	WH16	2.5	2.5	2.5	2.33	2	2.17
HW- 30	WH17	4.17	3.33	3.5	4.33	2.83	3.5
HW- 31	WH18	2.67	2.83	2.33	2.33	2.17	2.33
HW- 37	WH19	3.17	3.33	3.33	3.83	2.67	3.83
HW- 40	WH21	3.17	3.17	3.17	2.5	3.33	2.17
HW- 41	WH22	3.33	3.67	3.33	3.67	3	4.17
HW- 43	WH23	4.17	3.83	3	3.5	3.5	3.17
HW- 44	WH24	2.67	2.83	2.67	2.67	2.5	2.33
HW- 46	WH25	3.17	3.67	3.17	3	2.83	2.67
HW- 47	WH26	3	3.5	3	2.83	3.33	3.17
HW- 48	WH27	2.5	2.17	1.67	4	2.17	4.25
HW- 52	WH28	1.67	2.5	2.67	2.17	2.33	2.5
HW- 53	WH29	4	3.83	4.17	4	3.83	3.83
HW- 54	WH30	2.5	3.17	2.83	2.83	2.33	2
HW- 56	WH31	4	3	2.17	4.67	3.67	3.83
HW- 62	WH32	2.67	2.83	3.67	2.83	3	2.5
HW- 64	WH33	3	2.67	2	3.67	1.75	2.5
HW- 65	WH34	3.17	3.5	3.17	3.83	3.5	3.67
HW- 66	WH35	3.5	3.5	3.17	4.17	3.5	3.83
HW- 67	WH36	3	2.83	4.17	4.33	3	2.83
HW- 68	WH37	2.33	3	2.5	3.17	2.5	2.83
HW- 69	WH38	2.5	2	2.17	3.17	3.17	2.83
HW- 70	WH39	3	3.33	2.33	3.83	2.33	4
HW- 71	WH40	2.83	3.33	3	2.67	2.17	3
HW- 72	WH41	3.33	2.5	2.5	3.5	2	3.5
HW- 75	WH42	3.83	3.33	3.83	4.17	2.5	3.33
HW- 76	WH43	2	2.17	2.5	1.83	2	2.33

RIL ID.	RIL No.	Pti2	86-124	86-124ΔToxA	331-9	DW5	AR CrossB10
HW- 78	WH44	2.83	3	3.17	1.83	3.5	2.5
HW- 79	WH45	3.83	3.5	3.33	3.67	3.17	3.67
HW- 80	WH46	2.67	2.5	3	3.83	2.67	4
HW- 82	WH47	2.25	2.5	4	3	2.17	2.5
HW- 83	WH48	3.83	3.17	2.33	3.67	2.33	3
HW- 84	WH49	3.33	3.5	2.33	4.33	3	3.33
HW- 85	WH50	2.5	2.17	2.5	3.5	2	3
HW- 86	WH51	2.33	1.67	2.17	2.67	2.33	2.83
HW- 87	WH52	1.5	1.33	1.83	2	2	2.67
HW- 88	WH53	3.17	3.17	2.67	4.17	2.33	3.67
HW- 89	WH54	2.5	2.33	4	2.67	2.83	3
HW- 90	WH55	3.5	3.83	4.5	2.75	2.67	2.5
HW- 92	WH56	3	3	2.5	2.83	2.5	2.5
HW- 93	WH57	2.33	2.17	2.67	2.17	2.33	2.67
HW- 97	WH58	2.25	2.83	4	2.75	2.83	3.5
HW- 98	WH59	2.67	2.5	2.67	2.17	1.5	3.33
HW- 100	WH60	2.5	2	2	3.83	2	2.67
HW- 102	WH61	3.17	2.33	2	3	2.5	3.17
HW- 103	WH62	1.83	2	2.17	2	3	2.83
HW- 104	WH63	3.17	3	3.17	2.17	4.5	2.83
HW- 106	WH64	2.5	2.83	3.83	2.17	4.17	3.67
HW- 109	WH65	2.5	2.67	2.17	3	3	3.17
HW- 110	WH66	3	3.67	2.83	3.67	3.5	3.67
HW- 113	WH67	3.83	2.83	3.67	2	3	3.33
HW- 115	WH68	3	3.5	3.83	2	2.83	2.33
HW- 117	WH69	3	3	3.17	3.67	3.17	3.5
HW- 118	WH70	3.17	3.33	3.33	2	4	2.33
HW- 119	WH71	2.33	3.67	2.17	2.33	3	2.67
HW- 120	WH72	3.67	3	2.33	2.67	3	2.5
HW- 121	WH73	2.83	3.33	2.67	3.5	3.17	3.67
HW- 122	WH74	2.67	3	3	4.5	3.17	3.5
HW- 123	WH75	2.17	2.83	2.67	2	3	2.5
HW- 124	WH76	3.33	3.17	1.83	3.83	2.67	3.5
HW- 125	WH77	3.5	3.5	2	4	3.17	3.33
HW- 127	WH78	2.33	3	3.67	4	2.83	3.33
HW- 128	WH79	3.33	2.83	3.33	4.33	2.67	3.33
HW- 130	WH80	2.83	3.17	3.17	4.17	3	3.67
HW- 131	WH81	3.5	3.5	2	4	2.5	2.5
HW- 132	WH82	3.25	3.17	4	4	3.5	3.83
HW- 133	WH83	2.5	2.67	2.67	4	3	3

RIL ID.	RIL No.	Pti2	86-124	86-124ΔToxA	331-9	DW5	AR CrossB10
HW- 135	WH84	3.83	3.83	3.5	4.17	3.67	3.67
HW- 136	WH85	2.83	3.33	3.17	3.67	3	3.17
HW- 137	WH86	3.5	3.83	2.33	3.67	3.67	3
HW- 138	WH87	3.5	3.33	3.5	3	4.17	3.17
HW- 139	WH88	3.5	2.83	3.33	4	3.17	3.67
HW- 140	WH89	2.5	3.17	3.17	2.5	2.5	3.33
HW- 141	WH90	3.17	3.5	3.17	3.33	3.17	3.5
HW- 142	WH91	3.67	3.5	3.17	4.33	4.17	4.17
HW- 144	WH92	3	3.17	4	2.25	3.17	2.5
HW- 147	WH93	3.17	2.33	2	3.67	3.17	3.83
HW- 148	WH94	3.33	4	3.33	3	3.17	3.67
HW- 149	WH95	3.67	3.5	3.83	3.17	3.67	3.83
HW- 152	WH96	2.67	3	2.33	2.33	3.17	2.5
HW- 153	WH97	1.83	2.5	2.67	1.83	2	2.17
HW- 154	WH98	4	3.5	2.83	3.5	2.67	3.5
HW- 155	WH99	3.17	3.33	3.17	2.67	2.67	3.5
HW- 157	WH100	4.33	3.5	3	4.5	3.67	3.5
HW- 159	WH101	3.67	2.83	2	2.17	2.5	2.83
HW- 160	WH102	3.33	3.83	3.33	4	3.33	3.83
HW- 164	WH103	3.33	3.5	2.17	3.67	2.67	3.33
HW- 165	WH104	3.17	2.83	1.67	3.33	2.67	3
HW- 167	WH105	2.67	2.83	1.33	4.75	2.33	4
HW- 170	WH107	2.5	2.83	1.83	1.83	2.17	2.33
HW- 172	WH108	4.17	3.67	2.33	3.17	2.5	3.67
HW- 173	WH109	2.5	2.5	2	1.67	2.67	1.83
HW- 174	WH110	4.17	3.33	2.83	3.5	2.33	3.67
HW- 175	WH111	2	3.17	2.83	3.67	2.83	3.17
HW- 176	WH112	2.33	2.83	2.17	2.5	2.67	2.17
HW- 177	WH113	2.83	1.83	2.5	3.33	2.33	2.83
HW- 178	WH114	4	3.5	3.5	3.67	3.17	3.33
HW- 179	WH115	2.83	3.17	2.5	2.83	2.83	2.67
HW- 181	WH116	2.83	1.83	2.17	3.33	3	2.5
HW- 182	WH117	3.17	2.17	2.83	2	2.83	2
HW- 184	WH118	2.83	3.17	3.33	2	2.67	2.5
HW- 187	WH120	2	3	1.17	1.83	1.83	2.5
HW- 192	WH122	2.67	2.83	3.17	2.17	2.83	3.17
HW- 193	WH123	4	3.5	3.83	4	2.33	3.5
HW- 194	WH124	3.17	3	3	2.83	3.33	3.5
HW- 195	WH125	2.33	2.33	2.5	3.5	2.67	3.5
HW- 197	WH126	2.83	2.83	3.17	3.67	2.67	3
HW- 198	WH127	2.83	2.67	3	2.17	2.17	3

RIL ID.	RIL No.	Pti2	86-124	86-124ΔToxA	331-9	DW5	AR CrossB10
HW- 199	WH128	3.5	3.5	3.67	4	3.67	3.17
HW- 200	WH129	1.67	2	2.67	2.17	1.67	2
HW- 201	WH130	2.83	3.33	3.33	2.5	3.5	3
HW- 203	WH131	1.67	2.67	3	1.83	2.33	3.17
HW- 206	WH132	3.5	3.33	2.5	2.17	2.5	2.67
HW- 207	WH133	1.33	1.67	1.33	2.17	1.33	2.33
HW- 208	WH134	2.83	2.67	2.17	3.17	2.83	2.83
HW- 209	WH135	3.5	3.83	3.5	3.33	3.67	2.83
HW- 210	WH136	2.67	2.67	2.67	3.67	1.5	3.33
HW- 211	WH137	3	3.67	3.5	3.75	2.83	3.17
HW- 212	WH138	4	4.17	4	4.25	2.67	4
HW- 213	WH139	3.67	2.67	3	2.67	2.33	2.33
HW- 215	WH141	2.25	2.83	1.5	4	2.67	3.75
HW- 216	WH142	2.75	2.66	3.5	3.75	2.83	4
HW- 217	WH143	2.33	3.83	3	2.83	2.83	3.83
HW- 218	WH144	3.75	3.5	3.5	2.75	3.33	3
HW- 219	WH145	3.83	3.83	3.83	3.5	3.33	3.5
HW- 221	WH146	3.25	3.33	2.5	2.5	2.5	3.33
HW- 222	WH147	2.25	3.5	4	2.5	2.5	3
HW- 224	WH148	3.83	4.17	3.67	3.33	3.5	2.83
HW- 225	WH149	3.25	3.83	3.5	2	2.67	3
HW-226	WH150	3.25	3.33	4	4.75	3.17	4
HW-227	WH151	2.67	3.5	3.67	4.17	2.5	4
HW-228	WH152	3.25	3.67	2.5	4.25	2.83	4.17
HW-229	WH153	2.5	3.67	2	3	3.33	3
HW-230	WH154	2.83	2.83	3.17	2.33	2.33	3.33
HW-231	WH155	2.75	3	2.5	2.25	2.5	2.83
HW-233	WH157	3.5	3.83	2.5	3.83	2.83	4.33
HW-234	WH158	2.33	2.83	3	3.83	2.5	3.67
HW-235	WH159	3.5	3	3.5	2	2.83	3.67
HW-236	WH160	3.17	3.33	3.33	2.5	2.5	3.17
HW-237	WH161	3	3.17	3	3.75	1.83	3.33
HW-238	WH162	3.83	3.5	3.17	4.17	3.17	3.5
HW-239	WH163	3	3.33	3.5	2	2.5	2.83
HW-240	WH164	3.75	3.67	4.5	4	3.17	3.67
HW-241	WH165	3.67	3.33	3.17	3.83	2.5	3.67
HW-242	WH166	2.17	2.5	2.17	3.17	1.5	3.17
HW-243	WH167	3.5	3.33	2.83	3.67	1.83	4.17
HW-244	WH168	2.33	3.17	2.83	3.5	1.83	3.67
HW-245	WH169	3.67	3.5	2.33	3.83	3.33	3.67
HW-246	WH170	3.67	3.33	3.83	3	3.5	3

RIL ID.	RIL No.	Pti2	86-124	86-124ΔToxA	331-9	DW5	AR CrossB10
HW-247	WH171	3	3.17	2.67	2.67	1.83	2.5
HW-248	WH172	2.25	2.5	3	3	1.67	2.67
HW-249	WH173	2.17	2.67	2.67	3.67	2	2
HW-250	WH174	3	3.17	3.17	3.83	2.33	2.83
HW-251	WH175	3.67	3	3	4	3	3.67
HW-252	WH176	3.83	3.33	3.5	4.5	2.67	4
HW-253	WH177	1.5	2	2.33	2.17	2	1.83
HW-254	WH178	3.17	2.83	3.5	2.67	2.83	3.33
HW-255	WH179	3	3	2.5	3.5	2.67	3.67
HW-256	WH180	2.67	2.83	2.5	2.17	2.5	2.83
HW-257	WH181	2	2.5	3	2.5	2.5	2.5
HW-258	WH182	2.67	3.17	3.33	4	3.5	4.17
HW-259	WH183	3	2.83	2.33	2.17	2.17	2.17
HW-263	WH187	2.67	2.67	2.67	3.83	2.83	4
HW-264	WH188	3.83	3.67	3.83	3	3.5	3.5
HW-265	WH189	3.33	3.17	2.83	2.33	3	2.33
HW-266	WH190	3.5	2.33	3.5	4.5	2.83	3.67
HW-267	WH191	3.33	3.17	3.17	3	3.67	2.83
HW-268	WH192	3.5	3.5	3.5	4.33	3	4
HW-269	WH193	2.33	3	3	2.83	1.83	3.33
HW-270	WH194	2.83	3	3.33	2.67	2.33	3
HW-271	WH195	2.5	2.67	2.83	3.67	1.83	3.17
HW-272	WH196	2	3.17	2.5	2.5	2.67	2.5
HW-273	WH197	3.33	3.67	4	3.33	3.5	3.33
HW-275	WH199	3.17	3.33	3.5	3	2.5	2.67
HW-276	WH200	4	3.17	2	4.17	2.5	3
HW-277	WH201	3	3	2.83	3.67	3.33	3
HW-278	WH202	2.5	2.33	2.67	3.17	2.5	2.67
HW-279	WH203	3	3	3.67	3.83	2.67	3.17
HW-280	WH204	2.33	3.33	3.17	2.67	2.33	3
HW-281	WH205	3	3.17	2.33	3	2.17	3.33
HW-282	WH206	3.5	3.17	3.67	2.33	3.33	2.17