FURTHER CHARACTERIZATION OF PATHOGEN VIRULENCE AND GENETIC MAPPING OF NEW VIRULENCE GENES IN *PYRENOPHORA TRITICI-REPENTIS*, THE CAUSAL AGENT OF TAN SPOT OF WHEAT

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DOCTOR OF PHILOSOPHY

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ABSTRACT

The ascomycete *Pyrenophora tritici-repentis* (*Ptr*) causes tan spot of wheat, a devastating foliar disease on both common wheat (*Triticum aestivum* L., 2*n*=6*x*=42, AABBDD) and durum (*T. turgidum* ssp. *durum* L., 2n=4x=28, AABB). *Ptr* is known to produce three necrotrophic effectors (NEs), namely Ptr ToxA, Ptr ToxB and Ptr ToxC, to cause disease by interacting with corresponding host sensitivity genes. However, many studies in the last twenty years have suggested that *Ptr* produces additional virulence factors. My Ph.D. research focused on further identification and genetic mapping of new virulence genes in *Ptr*. In Chapter 2, a bi-parental fungal population was developed from a cross between two genetically modified heterothallic fungal strains followed by genotyping, phenotyping and QTL mapping. Two QTLs were identified with one being major and the other being minor, which confer virulence of 86-124 toward a Ptr ToxA-insensitive wheat line. In addition, the multiple copy gene *ToxB* was mapped to two genetically independent loci with one having five copies and the other having a single copy. In Chapter 3, I further characterized virulence of some *Ptr* isolates obtained from Chapter 2 that do not produce any known NEs. These isolates should be classified as race 4 but were found to still cause disease on many common wheat and durum cultivars. Using a common wheat host population, I identified several QTLs associated with the reaction to these isolates, which are different from three known host sensitivity gene loci. In Chapter 4, several natural race 4 isolates collected in North Dakota were shown to cause no or little disease on common wheat genotypes but cause disease on durum and other tetraploid wheat. Using a segregating population, I identified several new QTL associated with disease caused by these race 4 isolates, suggesting the presence of new virulence factors in these isolates. My Ph.D. research greatly advanced the understanding of the genetics of host-pathogen interaction in wheat tan spot and

provided important information to wheat breeding programs aiming to improve tan spot resistance.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

Wheat is a major food crop in the world and mainly includes common wheat (*Triticum aestivum* L., 2n=6x= 42, AABBDD) and durum (*T. turgidium* ssp. *durum* L., 2n=4x=28, AABB) The United States is a major wheat producer and six classes of wheat are produced in US, including hard red winter wheat (HRW), hard red spring wheat (HRS), soft red winter wheat (SRW), soft white wheat (SW), hard white wheat (HW) and durum wheat. North Dakota is the number one wheat producer in the nation for the HRS wheat and durum.

Tan spot, caused by *Pyrenophora tritici-repentis* (Died.) Drechs. [anamorph: *Drechslera tritici-repentis* (Died.) Shoem.], is a devastating foliar disease on both common wheat and durum in almost all wheat growing areas in the world. Yield losses due to tan spot can reach up to 50% (Rees et al. 1982). In North Dakota, tan spot is one of the most common diseases of wheat and can bring significant economic losses if it is not managed (Friskop and Liu 2016). Tan spot can be managed by using cultural practices, such as crop rotation or tillage treatments in a combination with timely fungicide applications. However, utilization of host resistance is the most inexpensive and environmentally friendly way to control disease.

The wheat-*P. tritici-repentis* pathosystem is known to follow an inverse gene-for-gene model where the fungal produced necrotrophic effectors (NEs) interact with host sensitivity genes leading to disease susceptibility. Up to date, three necrotrophic effectors (NEs) have been identified from *Ptr*, namely Ptr ToxA, Ptr ToxB and Ptr ToxC, which interact directly or indirectly with the products of the dominant host genes *Tsn1*, *Tsc2* and *Tsc1*, respectively (reviewed in Ciuffetti et al. 2010; Faris et al. 2013). Based on the production of the three NEs, the *Ptr* isolates have been classified into eight races, all of which have been reported to be present in the world (Lamari et al. 1989a, 1989b; Ali et al. 1999; Strelkov et al. 2002; Ali and

Francl 2003; Lamari et al. 2003). Both Ptr ToxA and Ptr ToxB are proteins and their encoding genes have been cloned from the fungus (Ciuffetti et al. 1997; Martinez et al. 2001, 2004). However, Ptr ToxC was previously characterized as a secondary metabolite and the gene(s) for its production remains unknown (Effertz et al. 2002). Furthermore, many studies in the last two decades have shown or suggested the presence of additional NE-host sensitivity gene interactions, or other types of pathogen virulence factor and host resistance/susceptibility factors (reviewed in Faris et al. 2013; Kariyawasam et al. 2016). To obtain a complete understanding of the disease system, the additional NEs from the fungus and other type of host resistance/susceptibility should be investigated.

To identify new fungal effectors from the fungus, our research group has started using a genetic approach that involves the development of fungal populations followed by genetic linkage mapping (Ameen et al. 2017; Kariyawasam 2019). A fungal cross has been made between 86-124 (race 2, harboring *ToxA*) and DW5 (race 5, harboring *ToxB*) and a small size of the population from the cross has been obtained and reported (Ameen et al. 2017). However, a genetic linkage map has not been developed from this cross. Unlike *ToxA,* which is a single copy gene, *ToxB* is present in race 5 isolates with multiple copies (Martinez et al. 2001, 2004). The genomic locations for these copies are not known yet. Friesen et al. (2003) showed that the race 2 isolate 86-124 can cause disease on a *Tsn1* mutant suggesting that this isolate produce addition virulence factor. The population between 86-124 and DW5 would be useful in identifying the new virulence factor presented in 86-124.

Race 4 is defined as producing no known NEs and being avirulent on wheat (Strelkov et al. 2003). However, we obtained several race 4 isolates from the fields in North Dakota that caused disease specifically on durum. In addition, we obtained some progeny isolates from the

cross between 86-124 and DW5 that do not produce any known NEs. These isolates should be classified as race 4, but they caused disease on many common and/or durum wheat genotypes. Virulence in those race 4 isolates needs to be further characterized.

The objectives for my Ph.D. thesis research were to 1) develop a genetic linkage map, locate *ToxB* loci in *Ptr*, and identify genomic region(s) conferring new virulence in 86-124; 2) characterize virulence of isolates without all three NE genes using pathogenicity test and QTL mapping in a segregating wheat population; 3) characterize virulence of race 4 isolates on durum wheat using pathogenicity test and QTL mapping in two tetraploid wheat populations.

CHAPTER 1. LITERATURE REVIEW

1.1. Wheat

1.1.1. Wheat classification

Wheat is one of the most important food crops in the world and is classified under the family Poaceae. Poaceae is one of the largest plant families including 12 subfamilies, 52 tribes, and 90 subtribes. Wheat is classified under the tribe Triticeae, subtribe Triticinae, and genus *Triticum* (Soreng et al. 2017). Other genera in this tribe include *Aegilops* L. (goatgrass), *Secale* L. (rye) and *Hordeum* L. (barley). Today, around 95% of the total wheat cultivated worldwide is common wheat (*Triticum aestivum* L.), and the remaining 5% is durum (*T. turgidum* ssp. *durum* Desf.) (Shewry 2009). Other species in this genus include emmer wheat (*T. turgidum* ssp. *dicoccom* Schrank.), wild emmer wheat (*T. turgidum* ssp. *dicoccoides* Koern.), Spelt wheat (*T. aestivum* ssp. *spelta* L.) and so on. However, most of them are not cultivated, or rarely so (Classification, USDA).

1.1.2. Wheat evolution

The cultivated wheat species can be at three genome levels, including: diploid (2n=2x=14, AA), tetraploid (2n=4x=28, AABB), and hexaploid (2n=6x=42, AABBDD). It was generally accepted that wheat species evolved from the lower to higher polyploidy levels through two natural hybridization and spontaneously amphiploidization events.

The first hybridization and amphiploidization event took place approximately 300,000 years ago between A and B genome ancestor, which produced the wild emmer wheat *T. turgidum* ssp. *dicoccoides* (2n=4x=28, AABB). The A genome donor to all polyploid wheat species was likely *Triticum urartu* (2n=2x=14, AA), but the B genome donor has not exactly pinpointed (Dvořák et al. 1993). Non-brittle rachis mutation was one of important factors that led

wild emmer wheat (*T. turgidum* ssp. *dicoccoides*) to the cultivated emmer wheat (*T. turgidum* ssp. *dicoccum*) (Feldman and Levy 2012). Common wheat ariose from the second natural hybridization of tetraploid durum wheat with diploid *Aegilops tauschii* Coss. (2n=2x=14, DD), followed by spontaneously amphiploidization event. Common wheat has since undergone several steps of the domestication process and evolved into one of the most important food crops in the world nowadays.

The earliest cultivation of wheats likely taken place in the south-eastern part of Turkey and was then gradually spread to other regions. Those earliest cultivated forms were diploid (genome AA) (einkorn) and tetraploid (genome AABB) (emmer) wheats (Feldman 2001; Reviewed in Shewry 2009). Wheat was first spread into Europe following the route from Anatolia to Greece 8,000 before present years (BP) ago, which then went either to Italy, France and Spain (7,000 BP) or northward from the Balkans to the Danube (7,000 BP). The wheat finally reached the Unite Kingdom and Scandanavia around about 5,000 BP. It was believed that the wheat was first brought to Egypt around 6,000 BP, from where it spread to different regions in the Africa continent. The way of wheat into Asia was thought to be via Iran into central Asia and then reach China around about 3,000 BP. Wheat, mainly bread wheat, was introduced by the Spaniards to Mexico in 1529 and to Australia in 1788.

1.1.3. Wheat production

Common wheat provides approximately 20% of the total calories and proteins for human nutrition, more than any other crops. It is the staple food for over 40% of the world's population (www.faostat.fao.org). Wheat can be grown in a very wide geographic regions in the world, from the latitudes of 60°N to 44°S and from sea level to 3,000 m above sea level (Singh et al. 2011). In the 2019/20 season, wheat was grown in 217.19 million hectares globally with a total production

of 764.39 million metric tons (Foreign Agricultural Service, USDA, Jan. 2020). Wheat has played an important role in human civilization and societal stability by providing enough food. However, the world wheat production is facing serious challenges such as reduced area of arable land, increase human population, and unsustainable environment. Therefore, big efforts need to be taken to increase wheat yield and/or improve quality.

The United States (US) is the 5th largest wheat producer in the world with a production of 52.26 million metric tons in the 2019/20 season (https://www.fas.usda.gov/data/world-agricultural-production). Wheat grown in the United States is divided by color, hardness and growth habit into six classes, including hard red winter wheat (HRW), hard red spring wheat (HRS), soft red winter wheat (SRW), soft white wheat (SW), hard white wheat (HW) and durum wheat. HRW is commonly grown in the Southern and Central Great Plains (Kansas, Oklahoma, Nebraska, Texas, Colorado, South Dakota and Montana), accounting for 40% of the total US wheat production and it has excellent milling and baking characteristics to make daily breads and Asian noodles. The HRS wheat usually has high protein content and is used to make specialty breads such as pan breads or blend with other flours. HRS wheat is cultivated in the Northern Great Plains (North Dakota, Montana, Minnesota, and South Dakota), accounting for 20% of the total US wheat production. SRW wheat, which is mainly for making a wide range of confectionary products like cookies, is grown in Eastern US (Ohio, Missouri, Indiana, Illinois, and Pennsylvania) making up for 15-20% of the total wheat production. SW and HW are grown predominately in the Pacific Northwest, Michigan and New York and accounts for 10-15% of total US production. Durum accounts for 3-5% of the total US wheat production and is mainly produced in North Dakota and Montana (https://www.ers.usda.gov/topics/crops/wheat/wheat-

sector-at-a-glance/). Durum is the hardest of all wheats with a rich amber color and high gluten content and mainly used for making pasta.

Agriculture is the leading industry in North Dakota. In the 2018/2019 season, North Dakota (ND) led the nation in the production of bread wheat, durum wheat, oats, all dry edible beans, navy beans, pinto beans, canola, dry edible peas, flaxseed, and honey. In ND, wheat is the most grown crop followed by soybean, corn and canola in 2019 (https://www.nass.usda.gov/Statistics_by_State/North_Dakota/Publications/Annual_Statistical_Bulletin/2019/ND-Annual-Bulletin19.pdf). Three classes of wheat are producing in ND, including HRS wheat, durum and HRW wheat, which account for approximately 89.3%, 9.6% and 1.1% of the total wheat production in ND, respectively. The total wheat acreage in 2019 for ND was 7.5 million acres and a total of 320,760,000 bushels of wheat was produced in 2019 with an average yield of 48.5 bu/acre in harvested land. In 2019, the ND wheat crop was valued at $1.4 billion with an average of $4.45 /bu (https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=NORTH%20Dakota, 03/05/2020).

1.1.4. Wheat diseases in North Dakota

The modern cultivated wheats have very narrow genetic variation because of monoculture, which makes them vulnerable to biotic stresses, such as plant diseases (Haudry et al. 2007). Wheat production in North Dakota is often challenged by several diseases. Tan spot, caused by *Pyrenophora tritici-repentis* is the most common leaf disease, and it often occurs with septoria tritici blotch (caused by *Zymoseptoria tritici*) and septoria nodorum blotch (caused by *Parastagonospora nodorum*) as a leaf spotting disease complex. Bacterial leaf streak (BLS), also known as black chaff, caused by the bacterium *Xanthomonas translucens* pv. *undulosa*, has become evident in the state and surrounding areas since 2008.

1.2. Tan spot of wheat

1.2.1. History

The disease was first discovered on the grass *Agropyron repens* in the beginning of the 20th century (Diedicke 1902). The report of the disease on wheat came around the late 1920s and become more often after that (Nisikado 1928; Mitra 1934; Conners 1937; Barrus 1942; Hosford 1971).The disease was originally called yellow spot or yellow leaf blotch because of the production of main chlorosis symptoms but later was described as leaf spots with light brown centers and yellow borders, known as tan spot (Hosford 1982). It was speculated that the fungal pathogen acquired the *ToxA* gene likely around 1940s from another wheat pathogen leading to an increase of its virulence on wheat and establishment of an important disease on wheat (Friesen et al. 2006). Since the 1970s, outbreaks and epidemics of tan spot have been widely reported at the global scale (Hosford 1971; Tekauz 1976; Rees and Platz 1992). This likely correlated with the wide adoption of no or little tillage in agriculture system to maintain soil fertility. Now, tan spot is one of the most common wheat diseases and occurs in almost all wheat growning regions in the world (Faris et al. 2013; Ciuffetti et al. 2014).

1.2.2. Disease symptoms and economic importance

The typical symptoms caused by *Ptr* on susceptible cultivars include tan-colored, elliptical necrotic lesions on infection sites, which are often surrounded by chlorotic halos (Strelkov and Lamari 2003). Under favorable conditions, the lesions can coalesce forming a large area of dead tissue on the leaf, which can reduce leaf photosynthesis activity leading to yield losses. Resistant or partially resistant wheat cultivars can develop pin-size dark spots or smaller necrotic lesions without any chlorosis symptoms. In addition, infection of this pathogen in wheat

spikes can result in the formation of red smudge in kernels, downgrading the grain quality, which is particularly important in durum wheat (Fernandez et al. 1994).

Yield loss caused by tan spot was first reported in 1974 by Hosford and Busch. Since then, many reports on yield losses from tan spot have been available (Rees et al. 1982; Annone 1997; Fernandez et al. 1998; reviewed in Moreno et al. 2012). The disease was reported to reduce both kernel weight and the numbers of grains per head (Schilder and Bergstrom 1990). In addition, the infected kernels have poor seedling emergence which also contributes to the yield loss.

In the US, the annual yield loss caused by tan spot ranges from 2 to 15% but under favorable conditions it can reach up to 49% (Hosford 1982; Evans et al. 1999). In Australia, tan spot is considered as the number one disease on wheat. It causes direct yield losses of $212 million and $461 million for controlling it (Moolhuijzen et al. 2018). In South American, the annual yield loss due to tan spot varies from 20% to 70% (Gamba et al. 2012). In North Dakota, tan spot is a major yield constrain for wheat production with an annual economic loss of multiple million dollars.

1.2.3. Disease cycle

Tan spot is a polycyclic disease. At the beginning of the growth season, mature ascospores are dispersed by wind serving as primary inoculum. Humidity and temperature are very important for the ascospore discharge. Normally, rain and temperature above 10℃ can induce the ascospore discharge. Both conidia and ascospores can cause infections on wheat leaves. A large amount of conidia can be produced from disease lesions, which serve as the secondary inoculum. This cycle can be repeated multiple times during a growth season, which makes disease progress upwards or spread in the field. In the winter, the pathogen survives as

black pinhead-sized pseudothecia on the wheat stubble (Friskop and Liu 2016). *Ptr* can infect other grass species, therefore, the grasses presented around the field can serve as alternative hosts (see host range section). Seeds, wheat stubble and secondary hosts can serve as the principal source of the primary inoculum. Schilder and Bergstron (1994) reported that the fungus can live in the seeds as mycelium and travel for a long distance. (Friskop and Liu 2016).

1.2.4. Disease management

Since the 1970s, minimal tillage practices have been widely practiced in wheat production to avoid soil erosion. However, this practice increased the level of the inoculum through residue build up. This has been believed to be one of main reasons for wheat tan spot becoming a major disease in many places of the world. Therefore, the cultural practices which can reduce the primary inoculum can be used to manage wheat tan spot (Bockus and Claassen 1992). Among them, crop rotation and residue management are two highly recommended cultural practices. Fungicide applications are another important way to manage the disease. Fungicides are available for the application in the early or late growth season (De Wolf et al. 1998; Osborne and Stein 2009). There are two kinds of fungicide, protectant and systemic fungicides. Protectant fungicides contain mancozeb and copper while systemic fungicides contain chemicals belonging to the classes of triazoles, strobilurins and mixture of them. In general, systemic fungicides work better than protectant ones. However, the application of systemic fungicides is not recommended unless certain conditions are met such as wet weather, susceptible plants and large quantity of wheat residues because of the application cost (Friskop and Liu 2016). Although tan spot of wheat can be managed using cultural practices and fungicide applications, these methods are not always practical or cost effective, and developing and deploying genetically resistant cultivars is thought to be the most inexpensive and

environmentally friendly method. Development of resistant cultivars require a good understanding of pathogen virulence, host resistance and how the two interact with each other.

1.2.5. *Pyrenophora tritici-repentis* (*Ptr*)

The causal agent of wheat tan spot is *Pyrenophora tritici-repentis* (Died.) Drechs. (Anamorph: *Drechslera tritici-repentis* (Died.) Shoem.), which is a member of Dothideomycete in ascomycete. The fungus was first isolated from *Agropyron repens* and named as *Pleospora trichostoma* by Diedicke in 1902 and later, it was renamed as *Pleospora tritici-renpentis* (Diedicke 1903). The nomenclature of the fungus has undergone several changes since 1903, including *Helminthosporium gramineum* f. sp. *tritici-repentis*, *H. tritici-vulgaris*, and *H. tritici-repentis* for the anamorph, and *Pleospora trichostoma* and *Pyrenophora trichostoma* *Pyrenophora tritici-repentis* for the teleomorph. The current nomenclature for the fungus has been accepted as teleomorph *Pyrenophora tritici-repentis* (Died.) Drechsler, and anamorph *Drechslera tritidrepentis* (Died.) Shoem (Shoemaker 1962; De Wolf et al. 1998).

The fungus produces light brown conidiophores, each of which bear a single transparent conidium. The conidium is cylindric stick in shape and has five to eight septa. The size of conidia averages 130 μm long and less than 3 μm wide. The secondary conidiophores are absent or rare. The fungus develops one-locule, black, raised fruiting bodies (pseudothecia) to overwinter. Inside a pseudothecium, sac-like asci are formed, and each ascus contains eight sexual spores. Asci are bitunicate with pseudoparaphysis and the median cell of an ascospore has a longitudinal septation (Ellis and Waller 1974). The colony usually has a thick cottony appearance with gray-green or whitish color. A brown pigment forms in the culture media for most isolates (Shoemaker 1962). However, a wide range of morphological variation has been observed for isolates from different geographical regions including the colony color and texture,

mycelial radial growth, conidial length and the number of septa usually varied in isolates (Dos Santos et al. 2002; Banslimane et al. 2017).

1.2.6. Host range

Shoemaker (1962) reported that *P. tritici-repentis* has the widest host range among all species in *Drechalera*. *P. tritici-repentis* was first isolated from the grass species *Agropyron repens* (Diedicke 1902) and later reported on wheat (Nisikado 1928; Mitra 1934). Since then, it was found that the fungus can be isolated and pathogenic on a wide range of graminaceous grasses that belong to the genus of *Agropyron*, *Bromus*, *Dactylis*, *Echinochloa*, *Elymusjunceus*, *Hesperostipa, Lemus*, *Leymus*, *Pascopyrum*, *Psathyrostachys*, *Stipta* and *Thinopyrum* (Hosford 1971; Krupinsky 1987, 1992; Ali and Francl 2003; Ali and Langham 2015). These grasses can serve as alternative hosts for *P. tritici-repentis* providing overseasoning places and sources of inoculum for new infection. Beside those grass species, barley and rye can also be infected by *P. tritici-repentis* (Hosford 1971; Morrall and Howard 1975; Ali and Francl 2001; Abdullah et al. 2017). Several recent studies have been conducted to investigate the specific interactions of barley with *P. tritici-repentis* (Aboukhaddour and Strelkov 2016; See et al. 2019; Wei et al. 2020). However, the fungus was found not to be pathogenic to oats, corn, alfalfa and flax (Horford 1971). In wheat, *P. tritici-repentis* can cause disease on all types of wheat (Horford 1971; Lamari and Bernier 1989; Chu et al. 2008). In addition, accessions in *Aegilops tauschii* (DD genome) were found to be susceptible to the tan spot pathogen (Cox et al. 1992). The wide host range of *P. tritici-repentis* may contribute its success as an important pathogen and pose a challenge for disease management.

1.2.7. Wheat-*P. tritici-repentis* interaction

Genetics of plant pathogen interactions can be mainly described by two models, gene-for-gene model and inverse gene-for-gene model. Gene-for-gene model was first proposed by H.H. Flor (1942) to describe the interaction between plant with biotrophic pathogens. Biotrophic pathogens live on living host and obtain nutrients from their hosts. The result of host pathogen interaction is controlled by a pair of matching genes, the plant resistance (*R*) gene and the pathogen avirulence (*Avr*) gene. The interaction of the matching genes results in resistance reaction, also known as incompatible reaction, while no interaction leads to disease and susceptibility. Inverse gene-for-gene model was used to describe the plant disease caused by necrotrophic pathogens which usually kill plant cells and survive on the dead plant tissues. In this model, the pathogen-produced necrotrophic effector (NE), previously known as host selective toxin (HST), interacts with the product of specific host gene to cause disease and susceptibility (Wolpert et al. 2002; Friesen et al. 2007). The interaction of two genes (NE gene from the pathogen and sensitivity gene from the host) leads to susceptibility rather than resistance.

Tan spot of wheat has been known to follow the inverse gene-for-gene model that involves the fungal-produced NEs and their corresponding wheat dominant sensitivity (susceptibility) genes (Ciuffetti et al. 2010). Up to date, three such interactions, including Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1*, have been identified and characterized in the wheat tan spot system and all these interactions have been shown to play an important role in disease development (Ciuffetti et al. 2010).

1.2.7.1. Pathogen race classification

The physiological races of a fungal pathogen represent the biotypes capable of causing disease on specific genotypes of a susceptible host. Lamari and Bernier (1989a, 1991, 2003) reported the physiologic difference in the tan spot pathogen using diverse wheat genotypes and found that symptoms of necrosis and chlorosis induced in the host by *P. tritici-repentis* infection were genetically distinct. These milestone discoveries led to establishment of a *Ptr* race system as well as the development of a rating scale largely based on lesion types, all of which have been widely adopted in the tan spot research community.

Eight races have been described according to the production of the three necrotrophic effecters (NEs) and virulence on a set of wheat differential lines. Current differential lines for wheat tan spot disease include hexaploid wheat genotypes Glenlea, 6B365, 6B662 and Salamouni. Races 2, 3 and 5 produced only one NE and are virulent on single differential line Glenlea (producing necrosis due to Ptr ToxA), 6B365 (producing chlorosis due to Ptr ToxC) and 6B662 (producing chlorosis due to Ptr ToxB), respectively. Races 1, 6, and 7 produce two NEs and have a combination of virulence with race 1 combining virulence of races 2 and 3, race 6 combining virulence of race 3 and 5, and race 7 combining virulence of races 2 and 5. Race 8 combines virulence of races 2, 3, and 5 (Strelkov and Lamari 2003; Faris et al. 2013). However, race 4 produces no known NE and is avirulent on all the differentials.

Studies have showed that races 1 to 5 are present in North American with races 1 being predominant (Lamari et al. 1989a, 1989b, 1998; Ali et al.1999, 2003). The predominance of race 1 has also been reported in elsewhere of the world (Lamari and Bernier 1989a). Race 5, which produce Ptr ToxB, was first identified from Algeria, then it was found in Canada and US (Ali et al. 1999; Strelkov et al. 2002). Race 6 was found in eastern Algeria (Strelkov et al. 2002), and

races 7 and 8 were identified near Azerbaijan and the Turkish-Syrian border (Lamari et al. 2003; Ali and Francl 2002), but they have not been reported in North America (Lamari and Strelkov 2010).

However, the current race classification of *Ptr* has been challenged by the identification of isolates that do not conform to any of the eight races. Ali et al. (2010) obtained a number of *Ptr* isolates from Arkansas which lack the *ToxA* gene but still caused necrosis on the Ptr ToxA differential line Glenlea. Abdullah et al. (2017) evaluated a set of *Ptr* isolates from South Dakota and also found some isolates induce necrosis on Glenlea (race 2) without the presence of the *ToxA* gene. Interestingly, Benslimane et al. (2018) identified some Algerian isolates failed to cause disease on Glenlea despite containing the *ToxA* gene. Most recently, Kamel et al (2019) found some isolates from Tunisia that induced the disease like race 7 but they don't have the *ToxA* gene. All these suggested that *Ptr* produces additional NEs.

1.2.7.2. NEs of Ptr

1.2.7.2.1. Ptr ToxA

Ptr ToxA is a small protein (~13.2 KDa), which is encoded by a single copy gene *ToxA* (Ciuffetti et al. 1997). It is produced by the majority of *P. tritici-repentis* isolates worldwide. Friesen et al. (2006) showed that *Ptr* likely acquired the *ToxA* gene from the *Parastagonospora nodorum* through a horizontal gene transfer event, leading to the increase in virulence for *Ptr.* The Ptr ToxA has a β-barrel and a loop containing an Arg-Gly-Asp (RGD) domain, which has been shown to be important for its biological activity (Meinhardt et al. 2002; Sarma et al. 2005). Ptr ToxA is likely internalized into plant cells after surface recognition followed by the transportation to chloroplast where it disrupts photosynthesis systems and induce reactive oxygen species (ROS) accumulation (Manning and Ciuffetti 2005; Manning et al. 2009).

Recently, Ptr ToxA was shown to physically bind to PR-1 protein suggesting that it may counteract plant defense system (Lu et al. 2013).

1.2.7.2.2. Ptr ToxB

Ptr ToxB is the second proteinaceous NE identified in *Ptr*, which was first characterized from race 5 isolates (Orolaza et al. 1995). Mature Ptr ToxB was found to be a heat stable protein with a molecular mass of 6.61 kDa (Strelkov et al. 1999). Ptr ToxB is capable of degrading chlorophyll and inducing defense responses in sensitive genotypes (Strelkov et al. 1998). Figueroa et al. (2015) showed that Ptr ToxB mainly stays in the apoplast of wheat leaves and acts extracellularly. The *ToxB* gene, encoding Ptr ToxB, was shown to have an open reading frame of 261 bp and be present as multiple copies (Martinez et al. 2001, 2004). The function of the *ToxB* gene was validated through transforming the *ToxB* gene into a non-pathogenic isolate that lacked *ToxB* (Strelkov et al. 2002; Ciuffetti et al. 2010). The copy number of the *ToxB* gene in different isolates ranges from 2 to10 copies and the level of chlorosis production is proportional to the copy number (Martinaz et al. 2004; Amaike et al. 2008). Strelkov et al. (2003) reported that the race 3 isolate D308 carried a copy of *ToxB* gene but had no Ptr ToxB activity. A single copy of *toxb* is present in race 4 isolates which shares 86% similarity with *ToxB* (Martinez et al. 2004). *toxb* is transcriptionally active but does not produce chlorosis (Amaike et al. 2008). In addition, Andrie et al (2008) reported that a highly similar *ToxB* gene is present in *Pyrenophora bromi* and occurs as a multi-copy gene.

1.2.7.2.3. Ptr ToxC

Different from other NEs, Ptr ToxC, a chlorosis-inducing NE, is not a protein and it has been characterized as a nonionic, polar, low molecular mass molecule that might be derived from

secondary metabolism (Effertz et al. 2002). However, Ptr ToxC has not been completely purified and fully characterized and its chemical structure remains unknown.

1.2.7.2.4. Other NEs

Ptr likely produces other NEs or virulence factors in addition to the three previously described. The existence of putative Ptr ToxDs has been reported in two conference papers (Meinhardt et al. 2003; Ciuffetti et al. 2003), but there has been no formal journal publication. In addition, many studies have suggested that *Ptr* produce additional NEs. First, as mentioned above, many isolates were identified that cannot conform to the current race classification system indicating the presence of new virulence factors. Secondly, the current designated races produce more NEs than just the three (Ptr ToxA, Ptr ToxB and Ptr toxC). Friesen et al. (2003) showed the race 2 isolate 86-124 (produce Ptr ToxA) still had the ability to cause disease on Kulm *Tsn1* mutants. Third, the *ToxA* knockout strains were shown to cause disease on various wheat genotypes, likely through interacting with new susceptibility loci (Moffat et al. 2015; Manning and Ciuffetti 2017; Kariyawasam et al. 2018; Liu et al. 2020).

1.2.7.3. Host susceptibility genes

The corresponding host susceptibility genes for three NEs have been mapped and their interaction with NE have been evaluated in various wheat genotypes. One of sensitivity genes have been cloned and characterized for functions and expressions.

The corresponding host gene conferring sensitivity to Ptr ToxA is *Tsn1*. Faris et al. (1996) first conducted restriction fragment length polymorphism (RFLP) mapping of *Tsn1* and it was located on the long arm of chromosome 5B. The diagnostic marker *Xfcp623* for *Tsn1* has developed (Faris et al. 2010) and its reliability for identifying wheat genotypes with resistance to the *P. tritici-repentis* and insensitivity to Ptr ToxA has been confirmed by recent research

(Kokhmetova et al. 2019). The *Tsn1* gene was isolated from wheat using a map-based cloning strategy (Haen et al. 2004; Lu and Faris 2006; Faris et al. 2010). The cloned *Tsn1* gene harbors serine/threonine protein kinase (S/TPK), nucleotide binding (NB), and leucine-rich repeat (LRR) domains, which resembles the structure of a resistance (R) gene. The transcription of *Tsn1* is regulated by the circadian clock and light suggesting the interaction between Ptr ToxA and *Tsn1* may be associated with the photosynthesis pathway. The yeast two-hybrid experiment shows that the two proteins do not interact directly (Faris et al. 2010).

Many QTL mapping studies have indicated that the Ptr ToxA-*Tsn1* interaction has a significant role in the disease development of wheat tan spot. Some studies showed that the interaction is highly significant being a major factor in disease (Lamari and Bernier 1989b) while others revealed the interaction only played a minor role (Friesen et al. 2004; Chu et al. 2008; Singh et al. 2008a; Faris et al. 2012) or no role at all in hexaploid wheat (Faris and Friesen 2005; Kariyawasam et al. 2016). In tetraploid wheat, the Ptr ToxA-Tsn1 interaction has never been shown to be important in tan spot development (Virdi et al. 2016; Galagedara et al. 2020). In addition, the presence of race non-specific resistance QTL may have an epistatic effect on the Ptr ToxA-*Tsn1* interaction (Kariyawasam et al. 2016). Very recently, Liu et al. (2017) reported that the ToxA-*Tsn1* and ToxC-*Tsc1* interactions have an additive effect on disease development of tan spot.

Friesen and Faris (2004) first mapped the wheat gene *Tsc2,* which confers sensitivity to Ptr ToxB, to the distal end of the short arm of chromosome 2B using the International Triticeae Mapping Initiative (ITMI) mapping population. Abeysekara et al. (2010) confirmed the location of *Tsc2* and demonstrated that *Tsc2* is a single dominant gene. The study also delineated the *Tsc2* gene to a 3.3 cM genetic interval with the EST-based marker *XBE444541* co-segregating with the

gene. The Ptr ToxB-*Tsc2* interaction has been shown to be important in disease development in all wheat populations whenever it is present (Abeysekara et al. 2010).

Faris et al. (1997) conducted QTL analysis of reaction to a *Ptr* race 1 and identified a major QTL (*QTsc.ndsu-1A*) on the short arm of chromosome 1A which is associated with chlorosis induction due to Ptr ToxC. Effertz et al. (2002) partially purified Ptr ToxC and map the *Tsc1* gene conferring sensitivity to Ptr ToxC to the distal end of the chromosome arm 1AS. Further linkage analysis suggests that the sequence-tagged site (STS) marker *XksuD14* on the proximal side of *Tsc1* might be useful for selecting against the functional *Tsc1* allele. The Ptr ToxC-*Tsc1* interaction was reported to play a key role in disease development (Effertz et al. 2002; Kariyawasam et al. 2016, 2018; Liu et al. 2017).

1.2.7.4. Qualitative resistance

In addition to the three NEs sensitivity genes, several qualitative genes conferring resistance to a specific race or isolate have also been identified. Singh et al. (2006) evaluated a set of LDN-DIC disomic chromosome substitution lines and identified *Tsr2* on the chromosome arm 3BL which confers recessive resistance to necrosis caused by the race 3 isolate 331-9. Tadesse et al. (2006a) identified a recessive resistant gene (*Tsr3*) on the chromosome 3D with the race 1 isolate ASC1b using a population derived from three resistant synthetic hexaploidy wheat lines and susceptible cultivar Chinese Spring. In another study, Tadesse et al. (2006b) conducted monosomic analysis to investigate resistance against ASC1a in Salamouni using Chinese Spring cytogenetic stocks. A recessive gene was identified on 3A, which was designated as *Tsr4*. Singh et al. (2008b) mapped *Tsr5* which is also on the chromosome 3B conferring recessive resistance to DW13 (race 5) in durum wheat. Most recently, a single dominant gene on chromosome 3B from the wild emmer accession IsraelA was identified by Faris et al. (2020) through chromosome

substitution line analysis, which is designated as *Tsr7*. The gene was found to confer resistance to races 1, 2, 3, and 5. This gene is likely the same as the race nonspecific QTL previously identified in hexaploid wheat (Faris and Friesen 2005; Kariyawasam et al. 2016).

1.2.7.5. Quantitative resistance

Faris et al. (1997) carried out the first QTL mapping for tan spot using the ITMI population (W-7984 × Opata 85) with isolates Pti2 (race 1), 86-124 (race 2) and D308 (race 3). A major QTL on 1AS (*QTsc.ndsu-1A*) and a minor QTL on chromosome 4AL were identified. Since then, many QTL and association mapping studies have been conducted for wheat tan spot, which have led to the identification of many genomic regions associated with resistance/susceptibility to tan spot caused by different races or isolates. Some of these QTLs likely corresponded to the three NE sensitivity loci indicating the three NE-host sensitivity gene interactions played an important role in disease development. However, other QTLs were mapped to the genomic locations different from the three sensitivity loci indicating new NE-sensitivity gene interaction or other type of host pathogen interactions.

The wheat chromosomes that have been identified to carry a QTL for tan spot disease include 1A (Sun et al. 2010), 1B (Faris and Friesen 2005), 1D (Gurung et al. 2011), 2A (Friesen and Faris 2004; Liu et al. 2017), 2B (Friesen and Faris 2004), 2D (Faris et al. 1997), 3A (Chu et al. 2010; Singh et al. 2008b), 3B (Chu et al. 2010; Faris and Friesen 2005; Kariyawasam et al. 2016), 3D (Stadlmeier et al. 2019), 4A (Faris et al. 1997; Chu et al. 2008), 4B (Stadlmeier et al. 2019, Dinglasan et al. 2019), 4D (Hu et al. 2019), 5A (Chu et al. 2010; Hu et al. 2019), 5B (Chu et al. 2008), 5D (Faris et al. 2012), 6A (Kalia et al. 2018), 6B (Singh et al. 2008; Hu et al. 2019), 6D (Hu et al. 2019), 7A (Kariyawasam et al. 2018), 7B (Faris et al. 2012; Chu et al. 2010) and

7D (Faris et al. 2012; Kariyawasam et al. 2018). Most of these QTLs were identified for a specific race or isolate.

A few QTLs were identified to confer resistance to multiple races; therefore, they were called race-nonspecific resistance QTL. The race-nonspecific resistance QTL was first reported by Faris and Friesen (2005) using a wheat population derived from the cross between Brazilian breeding line 'BR34' and the NDSU cultivar 'Grandin' for reaction to races 1, 2, 3 and 5. Two QTLs (*QTs.fcu-1BS* on 1BS and *QTs.fcu-3BL* con 3BL) were identified for all the races. Kariyawasam et al. (2016) confirmed the presence of the race-nonspecific resistance QTL on 3BL in the soft white spring wheat cultivars 'Penawawa'. Most recently, *Tsr7* was published in tetraploid wheat as a race-nonspecific resistance gene (Faris et al. 2020). The race-nonspecific resistance QTL/gene will be very useful in breeding program to develop wheat cultivars with resistance to all races.

The high-throughput genotyping technologies and genome wide association studies (GWAS) have been used to identify tan spot resistance QTL. Gurung et al. (2011) conducted GWAS on a collection of 567 spring wheat landraces for reaction to tan spot with diversity arrays technology (DArT) markers, and identified QTLs on chromosomes 1D, 2A, 2B, 2D, 4A, 6A, and 7D. Using a GWAS approach, Liu et al (2015) identified genomics regions on the chromosome arms 3BL, 3DS, 4AL, 5DL and 7DL significantly associated with resistance to tan spot in a panel of the North American winter wheat cultivars and breeding lines. Singh et al. (2016) conducted an association mapping on 170 lines of historical bread wheat germplasm developed at CIMMYT and revealed some previously identified as well as novel regions for tan spot resistance.

1.2.8. The genome of *P. tritici-repentis*

P. tritici-repentis was diverse in genetic variation in virulence. Studying genome structure and sequence information is important for us to understand virulence variation in *Ptr*. The genome of *Ptr* was first studied by using clamped homogeneous electric field (CHEF) gel analysis (Lichter et al. 2002). It was revealed that chromosome size varied among non-pathogenic and pathogenic isolates and also within the pathogenic isolates. Lichter et al. (2002) was the first to locate the *ToxA* gene to a 3.0 Mb chromosome and identified the homologous chromosome in non-pathogenic isolates, which has a size of 2.75 Mb. Aboukhaddour et al. (2009) revealed a total of 29 karyotypes from 47 isolates of different races. The number of chromosomes of these karyotypes ranged from 8-11 and the size of the genome ranged from 25.5 to 48 Mb. In the same study, the authors determined the chromosomal locations of the *ToxA* and *ToxB* genes in different races. The multiple copy gene of *ToxB* was located to either one chromosome or two different chromosomes.

The first genome sequence and optical map of *P. tritici-repentis* was published by Manning et al. (2013). In this study, the authors sequenced the genomes of three different *P. tritici-repentis* isolates and assembled a reference genome on the race 1 isolate Pt-1C-BFP, which was collected from North American. Forty-seven scaffolds (or supercontigs) were assembled with approximately 40 Mb in size and a total of 12,141 predicted genes. Among all the supercontigs, 26 were further constructed into 11 chromosomes by using an optical mapping approach. Comparative genomics showed nonpathogenic race (race4-SD20) can be clearly separated from pathogenic ones (race 5-DW7 and race1- Pt-1C-BFP). The study also identified single nucleotide polymorphism (SNPs) in different genomes by referring to reference genome, which are 73,190 SNPs for SD20 and 7,429 SNPs for DW7.

Moolhuijzen et al. (2018) recently published a new reference genome sequence with an optical map of *P. tritici-repentis* using the Australian race 1 isolate M4. The reference genome consisted of 50 supercontigs with a size of 40.9 Mb and 13,797 predicted genes. Approximately 39.9 Mb (98%) of the assembled sequence was mapped to 10 chromosomes. Genome comparison between M4 and BFP revealed some structural variations including translocations, inversions and fusions. In addition, Moolhuijzen et al. (2018) reported draft genome sequences for seven other isolates belonging to races 1 (134, 239, 5213, and 11,137), 2 (86-124), 5 (DW5) and a new race (AR CrossB10). Very recently, Moolhuijzen et al. (2019) generated a new high-quality genome sequence on the Australian race 1 isolate V1. Sequence alignment of V1 to M4 (Moolhuijzen et al. 2018) and Pt-1C-BFP (Manning et al. 2013) identified the chromosomal rearrangements on chr1, chr2, chr3 and chr7.

The availability of high-quality genome sequences for *P. tritici-repentis* is important for us to mine genes conferring virulence. Putative effector genes can be identified through size estimation and signal peptide prediction. Moolhuijzen et al. (2018) identified more than 200 putative effectors in M4 genome by looking for gene models that encodes for small secreted proteins with cysteine residues and signal molecules. In addition to proteins, gene clusters which related to secondary metabolites were also identified. All these would provide important information for us to identify addition NEs in *Ptr*.

1.2.9. Genetic tools to dissect pathogen virulence

Like in plant, genetic mapping has been a powerful tool to identify genes or QTL conferring virulence in fungal pathogens. Zhong et al. (2002) successfully map the virulence gene *VHv1* in *Cochliobolus sativus*, the causal agent of spot blotch of barley. Linkage mapping was also applied to identify avirulence genes in fungal pathogens, such as the *Mycosphaerella*

graminicola (Kema et al. 2002) and *P. teres* f. *teres* (Lai et al. 2002; Koladia et al. 2017). Genetic linkage and high-quality genome sequence has facilitated the cloning of avirulence/virulence genes. For example, Zhong et al. (2017) cloned the *AvrStb6,* the first avirulence gene to be functionally validated in *Zymoseptoria tritici* using a genetic map developed from two Swiss strains.

Genetic linkage mapping requires the development of segregating fungal population through sexual reproduction. Sexual reproduction in ascomycetes is regulated by the *MAT* locus which has idiomorphs of *MAT1-1* and *MAT1-2.* Some ascomycetes have either one of the idiomorphs (heterothallic) whereas other carry both idiomorphs (homothallic) (Turgeon and Yoder 2000). For heterothallic fungi, bi-parental population can be developed by using two natural isolates with opposite mating types. However, bi-parental population development in homothallic is difficult because all the isolates carry both mating type genes allowing them to self instead of outcrossing. One way to solve the problem is through genetic modification of mating type locus, which can convert homothallic strains to heterothallic strains (Wirsel et al. 1996; Lee et al. 2003; Debuchy et al. 2010).

Ptr is a homothallic fungus. Lepoint et al. (2010) reported that *P. tritici-repentis* contains both *MAT1-1-1* and *MAT1-2-1* in tandem spanning 4.5 kb. Very recently, Ameen et al. (2017) created heterothallic strains of *P. tritici-repentis* by deleting one of the *MAT* genes using a split marker strategy. The research showed that both *MAT1-1-1* and *MAT1-2-1* are required for sexual reproduction and that the cross between two genetic modified heterothallic strains produce progeny. This work opened the possibility to use genetic mapping approach to identify new NEs in *Ptr*. Using this approach, Kariyawasam (2019) developed a *Ptr* bi-parental population that

segregates for Ptr ToxC production and have successfully map the locus controlling Ptr ToxC production.

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CHAPTER 2. DEVELOPMENT OF A GENETIC LINKAGE MAP AND MAPPING OF THE TOXB LOCI AND NEW QTLS CONFERRING PATHOGEN VIRULENCE IN *PYRENOPHORA TRITICI-REPENTIS*

2.1. Abstract

The ascomycete *Pyrenophora tritici-repentis* (*Ptr*) is the causal agent of tan spot of wheat, a common and economically important wheat disease worldwide. Three necrotrophic effectors (NE), including Ptr ToxA, Ptr ToxB and Ptr ToxC, have been identified from the fungal pathogen that act as important virulence factors by interacting with corresponding host sensitivity genes. However, many studies have suggested the fungal pathogen produces additional NEs. To identify new NEs from the race 2 isolate 86-124, we developed a fungal population from a cross between this isolate and the race 5 isolate DW5. Because this population also segregates for the multiple copy *ToxB* gene, the second objective was to map individual *ToxB* loci. The population was subjected to genotyping with SNP and SSR markers as well as *ToxA*, mating type genes and each *ToxB* locus. For phenotyping, each progeny was inoculated onto the Ptr ToxA-insensitive line CDC-Osler which is susceptible to 86-124, but resistant to DW5. The constructed genetic map consisted of 11 linkage groups (LG) which corresponded to the 11 *P. tritici-repentis* chromosomes (Ch.) of the optical map of the reference genome Pt-1C-BFP. Five of six *ToxB* copies are tightly linked with each other residing at the distal end of Ch.11 while the sixth copy was located to the distal end of Ch.5. Virulence of 86-124 toward CDC-Osler, designated as *VirOsler*, mapped to two genomic regions with one being a major QTL (*VirOsler1*) on Ch.2 and the other being a minor QTL (*VirOsler2*) on Ch.7. The identification of new virulence factors is a significant step to further understand fungal virulence and host pathogen interaction in wheat tan spot system.

2.2. Introduction

Pyrenophora tritici-repentis (*Ptr*) is an ascomycete fungal pathogen that cause tan spot on both common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* ssp. *durum*). The fungal pathogen was first isolated from the grass *Agropyron repens* (Diedicke 1902) and was later identified as a wheat pathogen (Nisikado 1928; Mitra 1934; Conners 1937; Barrus 1942; Hosford 1971). In the last century, tan spot had evolved from a minor problem to a major wheat disease worldwide (Hosford 1982; Faris et al. 2013). Two possible reasons may have contributed to this change: 1) the fungus acquired a new virulence gene from another wheat pathogen (Friesen et al. 2006); 2) a wide adoption of no or little tillage agriculture practice led to the buildup of primary inoculum in the fields (reviewed in Faris et al. 2013).

The fungus infects wheat leaves and causes necrosis and/or chlorosis symptoms by producing necrotrophic effectors. The fungal infection can lead to dysfunction of photosynthesis on flag leaves, thus affecting grain filling and reduce the yield (Friskop and Liu 2016). Under favorable conditions, tan spot can cause significant yield losses up to 50% on highly susceptible cultivars, and it can also negatively affect grain quality (Rees et al. 1982; De Wolf et al. 1998). Although fungicide application and cultivar practice can be used to manage tan spot, development and deployment of resistant cultivars is the most cost-effective and environmentally friendly way to control disease. Developing resistant cultivars required a thorough understanding of pathogen virulence.

The fungal pathogen is known to produce three necrotrophic effectors (NEs), Ptr ToxA, Ptr ToxB and Ptr ToxC which interact with their corresponding host sensitivity genes *Tsn1*, *Tsc2* and *Tsc1*, respectively, to cause disease (reviewed in Ciuffetti et al. 2010; Faris et al. 2013). The interactions between NE and wheat sensitivity gene leads to susceptibility and disease, and thus

it has been referred to as an inverse gene-for-gene system (Ciuffetti et al. 2010; Faris et al. 2013). Because the three NEs are important virulence factors, the production of these NEs has been used to classify *Ptr* isolates into eight races with race 1 producing Ptr ToxA and Ptr ToxC, race 2 (Ptr ToxA), race 3 (Ptr ToxC), race 4 (none), race 5 (Ptr Tox B), race 6 (Ptr Tox B and Ptr ToxC), race 7 (Ptr ToxA and Ptr ToxB), and race 8 (Ptr ToxA, Ptr ToxB and Ptr ToxC) (Lamari and Strelkov 2010).

Both Ptr ToxA and Ptr ToxB are proteins, the fungal genes encoding them have been cloned and designated *ToxA* and *ToxB*, respectively. *ToxA* is a single copy gene only present in Ptr ToxA-producing isolates/races (Ciuffetti et al. 1997). The *ToxA* function has been confirmed by the transformation of the gene into an avirulent isolate and gene replacement in *ToxA*-containing isolates (Ciuffetti et al. 1997; Moffat et al. 2014; Manning and Ciuffetti 2015). A nearly identical *ToxA* gene and its surrounding region has been identified from two other wheat fungal pathogens including *Parastagonospora nodorum* and *Cochliobolus sativus* and the gene was shown to be important for their disease development (Friesen et al. 2006, 2018; McDonald et al. 2018).

Unlike *ToxA*, *ToxB* is a multiple copy gene present in races 5, 6, 7, and 8 (Martinez et al. 2001, 2004; Lamari et al. 2003; Strelkov et al. 2005). A single copy of *ToxB* was detected in race 3 but it is not expressed (Strelkov et al. 2005). The level of virulence in Ptr ToxB-producing isolates on sensitive wheat lines is highly correlated with the copy number present in those isolates (Orolaza et al. 1995; Strelkov 2002; Strelkov and Lamari 2003; Amaike et al. 2008; Ciuffetti et al. 2010). Several genomic loci containing the *ToxB* gene have been cloned from DW7 (a US race 5 isolate) and Alg3-24 (an Algerian race 5 isolate) (Strelkov et al. 2005). Sequence analysis showed all copies of the *ToxB* gene have an identical DNA sequence and are

commonly linked to truncated retrotransposon-like sequences (Martinaz et al. 2004; Strelkov et al. 2005). The *ToxB* loci are likely located to two different chromosomes in DW7 and other race 5 isolates (Martinaz et al. 2004; Aboukhaddour et al. 2009). A highly similar *ToxB* is also present in *Pyrenophora bromi,* the causal of brown spot of bromegrass (Andrie et al. 2008). In addition, a homologous gene *toxb*, about 86% similarly to *ToxB* is also present in race 4 isolates (Strelkov and Lamari 2003; Chapter 3).

Although much is known about Ptr ToxA and Ptr ToxB and their encoding genes, not much is known about Ptr ToxC. Furthermore, many studies have indicated that additional NE or other virulence factors are involved in the disease system. The identification and purification of two putative Ptr ToxDs have been reported in two conference papers (Meinhardt et al. 2003; Ciuffetti et al. 2003). Friesen et al. (2003) demonstrated that the race 2 isolate 86-124 still caused disease on the *tsn1* mutant of Kulm. Many *Ptr* isolates without the production of three NEs can cause disease on wheat (Simon et al. 2015; Guo et al. 2018; Kariyawasam et al. 2018; Liu et al. 2020; Chapter 3). All these suggested the production of new NEs by the fungal pathogen.

Because Ptr ToxA and Ptr ToxB are proteins, the cloning of *ToxA* and *ToxB* was done by using a biochemical approach which starts with protein purification and sequencing followed by the screening of cDNA fungal library with degenerate primers deduced from the amino acid sequence (Ciuffetti et al. 1997; Martinez et al. 2001). However, this approach is not feasible to identify and clone the genes of NEs which are not highly expressed in culture or are not a protein. Recently, our group has developed a genetic approach to map NE gene(s) (Ameen et al. 2017). In this approach, the homothallic fungus was converted into heterothallic strains followed by the development of segregating populations and genetic mapping. Using this approach,

Kariyawasam (2019) has successfully conducted the genetic mapping of gene(s) controlling the Ptr ToxC production.

Ptr genome structure and sequence has been studied in recent years, which provides important tools for genetic mapping and cloning fungal virulence genes. The *Ptr* genome was first investigated by using pulsed field gel electrophoresis (PFGE) on contour-clamped homogeneous electric field (CHEF) apparatus (Lichter et al. 2002; Aboukhaddour et al. 2009). These studies revealed abundant karyotype polymorphisms among different races/isolates, including the number and size of *Ptr* chromosomes. Manning et al. (2013) reported the first *Ptr* reference genome and its optical map based on the race 1 isolate Pt-1C-BFP from US. The Pt-1C-BFP genome was shown to be 40 Mb in size composed of 11 chromosomes that encode 12,141 predicted genes. A high-quality genome sequence has also been reported for two Australian race 1 isolates M4 and V1 (Moolhuijzen et al. 2018, 2019). Comparative genomic revealed large-scale sequence arrangements among three race 1 isolates (Moolhuijzen et al. 2018, 2019). Draft genome sequences of other isolates from races 1, 2, 4 and 5 as well as a new race were also obtained (Manning et al. 2013; Moolhuijzen et al. 2018, 2019).

To map a new virulence gene in 86-124, we developed a fungal bi-parental population between 86-124 and DW5 with the corresponding genetically modified heterothallic strains (Ameen et al. 2017). The fungal population was genotyped with SNP (single nucleotide polymorphism), SSR (simple sequence repeat) and several gene markers and phenotyped on the spring wheat cultivar CDC-Osler that is susceptible to 86-124 but resistant to DW5. The fungal population also segregates for the *ToxB* gene which has multiple copies in DW5 genome. Therefore, the fungal population was used to map individual *ToxB* loci.

2.3. Materials and Methods

2.3.1. Fungal population

The development of the fungal population from the cross between 86-124 and DW5 was described in Ameen et al. (2017). Briefly, two *P. tritici-repentis* isolates, 86-124 and DW5, were used in the development of the bi-parental population. The isolate 86-124 (race 2-Ptr ToxA producing) was collected in Manitoba, Canada in the 1980s and DW5 (race 5-Ptr ToxB producing) was collected from durum wheat in North Dakota, USA in 1998 (Friesen and Faris 2004). The heterothallic strains 86-124Δ*MAT1-2-1* and DW5Δ*MAT1-1-1* were obtained by the deletion of one of mating type genes. Conidial suspensions of each heterothallic strain were mixed with an equal volume and aliquots of mixed suspension were dropped onto dried and sterilized corn leaf fragments that were laid on water agar. After about one-month long incubation at 16 ºC, pseudothecia formed on the corn leaves became mature and discharged ascospores. The ascospores were picked and then transferred to V8-PDA for culturing. Single sporing was then performed to purify the culture and to form individual progeny isolates. A total of 117 genetic pure progeny isolates were collected for the linkage map development and genetic mapping of the new virulence genes and the *ToxB* loci.

2.3.2. Plant materials

Previously, our group has identified three spring wheat cultivars or breeding lines, including CDC-Osler, Lillian and SD4112 that were susceptible to 86-124, but resistant to DW5 (Liu et al. unpublished data). Very interestingly, these lines were all insensitive to Ptr ToxA strongly suggesting 86-124 carries new virulence factor other than Ptr ToxA toward them (Liu et al. unpublished data). The CDC-Osler was selected for this study because it had the most difference in reaction to the two isolates. This spring wheat was developed and released in 2004

by the Crop Development Centre, University of Saskatchewan, Saskatoon. SK, Canada (Hucl 2003). The original seeds were kindly provided by Dr. Mike Pumphrey, at Washington State University. The seeds for this line were increased by self-pollinating and maintained in our laboratory. All 117 progeny isolates from the population as well as the isolates 86-124 and DW5 were inoculated individually onto CDC-Osler for phenotyping. Tan spot differential lines: Salamouni (universal resistant), Glenlea (Ptr ToxA sensitive), 6B365 (Ptr ToxC sensitive), 6B662 (Ptr ToxB sensitive) were included in each inoculation.

The seeds of all genotypes were planted in plastic cones (Stuewe & Sons, Inc., Corvallis, OR) filled with Sunshine SB 100 soil (Sun Grow Horticulture, Bellevue, WA) with two cones for each line and three seeds for each cone. After planting, a small teaspoon of Osmocote Plus 15-19-12 fertilizer (Scotts Sierra Horticultural Product Company, Maysville, OH) was applied to each cone. For each inoculation, all cones were placed onto a small rack that were handed made from a RL98 rack by splitting into two halves. Hard red winter wheat cultivar "Jerry" was planted in cones along the borders to eliminate edge effects (Liu et al. 2015). The plants were grown in greenhouse room till three leaf stage (two weeks from seed sown) for inoculations. Disease phenotyping was conducted in NDSU Lord and Burnham greenhouse. The inoculation experiment was repeated three times and a randomized complete block design (RCBD) was used to arrange the plants.

2.3.3. Phenotyping

Due to the space limit in the misting and growth chambers, ten progeny isolates were phenotyped each week. Approximately 12 weeks were needed for the whole set of progeny isolates. The phenotyping for each progeny isolate was repeated at least three times.

Inoculum preparation was done as previously described (Liu et al. 2015). Prepared mycelial plugs from the margin of growing cultures were used to start the culture on V8-PDA Petri dish plate. After incubation in the dark under room temperature for five days, the mycelium was flattened with sterilized distilled water and excess water was decanted. Plates were then incubated at room temperature under fluorescent light overnight and then incubated at 15 ºC with no photoperiod.

To prepare inoculum, the plate was flooded with sterile distilled water, and conidia were dislodged and harvested using wire loops. The inoculum concentration was adjusted to 3,000 spores/ml. One drop of Tween 20 (polyoxyethylene sorbitan monolaurate) per 100 mL solution was added to the final conidial suspension. When plants reach seedlings at the two-three leaf stage were sprayed with prepared inoculum till runoff. The inoculated plants were placed in a humidity chamber with 100% humidity and incubated for 24 h. After that, the plants were moved to a growth chamber with a temperature set at 21 ºC and incubate for six days for disease to develop. Disease reaction on the secondary leaf was scored at the 7th day after inoculation using the scale described by Lamari and Bernier (1989) that was largely based on the lesion types of 1 to 5 with 1 being highly resistant and 5 being highly susceptible. If two lesion types were observed, the average was used.

2.3.4. Marker development and genotyping

Each progeny of the 86-124Δ*MAT1-2-1*×DW5Δ*MAT1-1-1* population was grown on V8-PDA (potato dextrose agar) in dark for 5 days and mycelial tissues was collected for DNA extraction following the instructions in Shjerve et al. (2014). All progenies were first genotyped for the presence of *ToxA*, *ToxB* and mating type genes using the corresponding published primers (Table 2.1).

The published primer pair TB71F and TB6R amplified the coding region of the *ToxB* region. However, DW5 has multiple *ToxB* copies located in different loci. Therefore, each *ToxB* locus was investigated. The sequence information of the *ToxB* loci in DW7, which is very close to DW5 genetically, was used as reference. DW7 has six *ToxB* copies cloned and sequenced, which have been designated *ToxB1* to *ToxB6* (AY425480-AY425485, Martinez et al. 2004). The primer set to amply each copy has been reported (Martinez et al. 2004). In my preliminary experiment, these primers did not work well for the most *ToxB* copies except *ToxB2* in DW5 under our laboratory conditions. Therefore, the sequences of the six copies were compared and a new common forward primer was designed (Table 2.1). The designed forward primer was used with the five published specific reverse primers to amply *ToxB1*(TBF/TB29), *ToxB3*(TBF/TB42), *ToxB4*(TBF/TB50), *ToxB5*(TBF/TB26) and *ToxB6*(TBF/TB28) while *ToxB2* was amplified using the published primer set (TB21/TB41) (Martinez et al. 2004, Table 2.1). Internal transcribed spacer (ITS) regions was amplified using the universal primers ITS4 and ITS5 (White et al. 1990, Table 2.1) as a control for DNA quality and quantity.

The genomic DNAs of DW5 and 86-124 were included as checks. PCR was conducted with 10 ng of DNA template in a 20 µl volume PCR reaction mix containing 1× buffer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.5 µM of each forward and reverse primer and 2U of Bullseye Taq DNA polymerase (MIDSCI, St. Louis). The PCR program was set as following: 95 ºC for 5 mins, 30 cycles at 95 ºC for 30s, 60 ºC for 30s, 72 ºC for 1 min, 72 ºC final elongation step for 5 mins and 4 ºC holding step. Products were visualized using 1% agarose gel electrophoresis. The ratios of the presence and absence of *ToxA*, mating type genes and different *ToxB* locus were calculated and χ^2- test was performed to test whether the observed ratio is significantly different from the expected ratio.

Table 2.1. List of primers used in chapter 2 study

Primers	Sequences (from 5' to 3')	Purpose	Reference
PtrPLP2	CAGAACAAAGGCAGGACTGTGAGC	Amplify a partial region of *MAT1-1-1* gene	Lepoint et al. (2010)
PtrPLP4	ATGCGCTCAGCAAGGAAGGTCG		
PtrPLP7	GCTTTACTACAACTTTCCTCTACC	Amplify a partial region of *MAT1-2-1* gene	Lepoint et al. (2010)
PtrPLP10	GTACGGGCCAGCATGACGTGC		
TA51F	GCGTTCTATCCTCGTACTTC	Amplify the *ToxA* gene	Andrie et al. (2008)
TA52R	GCATTCTCCAATTTTCACG		
TB71F	GCTACTTGCTGTGGCTATC	Amplify the *ToxB* gene	Andrie et al. (2008)
TB6R	ACGTCCTCCACTTTGCACACTCTC		
TBF	GAGAGAGAACAGATGTAAGAGAAGG	Common forward primer for *ToxB* copies	this work
TB21	ATGAATCCGGGCATGAGGTG	Forward primer for *ToxB2*	Martinez et al. (2004)
TB41	TCCGCAACAATCAATTAAGTG	Reverse specific primer for *ToxB2*	Martinez et al. (2004)
TB29	TAGACTCCGCAACAATCAATTC	Reverse specific primer for *ToxB1*	Martinez et al. (2004)
TB42	GAAGCTGAAGGGAAAGGG	Reverse primer specific for ToxB3	Martinez et al. (2004)
TB50	GCTTTGCTGTAGCGAGGT	Reverse primer specific for ToxB4	Martinez et al. (2004)
TB26	GAAGGGCAGAAGGATGGAGA	Reverse primer specific for ToxB5	Martinez et al. (2004)
TB28	GTGTGGTTGGGGTTGGGTTA	Reverse primer specific for ToxB6	Martinez et al. (2004)
ITS4	TCCTCCGCTTATTGATATGC	Amplify the ITS	White et al. (1990)
ITS5	GGAAGTAAAAGTCGTAACAAGG		

2.3.5. Simple sequence repeat (SSR) marker development

SSRs were searched from the reference genome (Pt-1C-BFP) (Manning et al. 2013) and primer pairs for SSR were designed as described in Zhong et al. (2009). A total of 313 pairs have been synthesized in our laboratory (Appendix A) and used by Kariyawasam (2019). These primers were screened on DW5 and 86-124 in my research. In addition, nine pairs of SSR primers were designed specifically for the supercontigs 19 and 20 after I knew the new virulence gene was mapped to that region. To visualize the PCR products in a LI-COR 4300 DNA analyzer (LI-COR Bioscience, Lincoln, NE, USA), M13 tail (5'-TGTAAAACGACGGCCAGT-3') was added to all forward primers. The PCR amplifications were performed with 5 ng of DNA template in a total volume of 10 µl containing 1× buffer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.05 µM of each forward and reverse primer, 0.1 µM DY682 fluorescently labeled M13 primer and 1U of Bullseye Taq DNA polymerase (MIDSCI, St. Louis). All PCRs were performed with the

followed cycling conditions: initial denaturation at 95 ºC for 5 mins, 8 cycles at 95 ºC for 20s, 50 ºC for 20s, 72 ºC for 30s, 38 cycles of 95 ºC for 30s, 52 ºC for 30s, 72 ºC for 30s. For marker scoring, the progeny was recorded as '1' if the PCR produced the DW5Δ*MAT1-1-1* allele type and '0' if it produces the 86-124Δ*MAT1-2-1* allele type. Missing data was recorded as "3". If primer sets produced two or more polymorphic bands, they were scored as separate markers as marker.1, marker.2, etc.

2.3.6. SNP identification and SNP marker development

Previously, a set of 256 STARP (semi-thermal asymmetric reverse PCR) primer sets were designed by Kariyawasam (2019) to map the *ToxC* gene in AR CrossB10 and 86-124 population. Among them, 163 sets produced polymorphism between 86-124 and DW5. To design SNP markers on the genomic regions not covered by Kariyawasam (2019), SNPs between DW7 and Pt-1C-BFP on the reference genome browser at the JGI website (https://mycocosm.jgi.doe.gov /Pyrtr1/Pyrtr1.home.html, Manning et al. 2013) were examined and selected along the regions with a distance between 100 to 200 kb. The 400 bp sequence spanning each selected SNP (200 bp upstream and 200 bp downstream) was download and compared to the DW5 and 86-124 draft genome sequences using local BLASTn search with Bioedit (Hall et al. 2011). The draft genome sequences of DW5 and 86-124 published by Moolhuijzen et al (2018) were download from the NCBI with the accession No. MUXC00000000 and NRDI00000000, respectively. If the SNP exists between DW5 and 86-124, the download sequence was then used to design STARP primer set. If it is not, nearby SNPs on the browser were examined one by one till one was identified. In total, 175 STARP primer sets were designed as the descriptions in Long et al. (2017).

The STARP PCR mix consisted of 10 µl reactions containing 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 PCR program was as follows: 94 ºC for 3 mins, 6 cycles at 94 ºC for 20s, 55 ºC for 90s with 1 ºC reduction per cycle, 37 cycles at 94 ºC for 30s and 60 ºC for 30s, followed by 12 ºC holding step. The PCR products were checked on polyacrylamide gel electrophoresis using a LI-COR 4300 DNA analyzer (LI-COR Bioscience, Lincoln, NE, USA).

The marker scoring method was the same as for SSR primers, the progeny was recorded as '1' if the PCR produced the DW5Δ*MAT1-1-1* allele type and '0' if it produces the 86-124Δ*MAT1-2-1* allele type. Missing data was recorded as "3". If primer sets produced two or more polymorphic bands, they were scored as separate markers as marker.1, marker.2, etc.

2.3.7. Linkage map construction

The linkage map in 86-124Δ*MAT1-2-1*×DW5Δ*MAT1-1-1* population was constructed by using MapDisto v2.0 beta (Heffelfinger et al. 2017). Initially, LOD value of 3.0 and an *r* value of 0.3 were used to find linkage groups (LGs). The big LGs were separated by increasing LOD value. The obtained LGs were then ordered and adjusted with "check inversion" and "ripple order" till there is no better order sequence available. Small LGs were connected according to information from the optical map of reference genome. For a comparison between my linkage map and optical map of Pt-1C-BFP, the "drop locus" function was not performed in order to keep all possible markers. Genetic distance was calculated using Kosambi function (Kosambi 1943). LGs were named based on their identity to individual chromosome of the Pt-1C-BFP optical map.

2.3.8. QTL mapping

The data of maps and marker was directly outputted from MapDisto as QGene 4.0 format. The disease means of individual progeny isolate on wheat line CDC-Osler were calculated from all the evaluations and used as phenotypic data. The QTL mapping was performed using QGene 4.0 (Joehanes and Nelson 2008). Composite multiple interval mapping function installed in the software was used for QTL identification. A permutation test with 1000 iterations was used to yield a LOD threshold at p=0.05 experiment-wise significance level. R^2 values for each QTL were given by the software.

2.4. Results

2.4.1. Marker development and fungal population genotyping

2.4.1.1. SSR marker developments

A total of 322 pairs of SSR primers (Appendix A) were screened between 86-124Δ*MAT1-2-1* and DW5Δ*MAT1-1-1* for polymorphisms. Among them, 105 pairs (32.6%) produced one or two clear polymorphic bands and were genotyped in the entire 86-124Δ*MAT1-2-1* and DW5Δ*MAT1-1-1* population.

2.4.1.2. SNP marker developments

A total of 338 STARP SNP primer sets (Appendix B) across the genome were tested between 86-124Δ*MAT1-2-1* and DW5Δ*MAT1-1-1*. Among them, 257 (76%) SNP-STARP primer sets generated polymorphic amplicons and were genotyped in the population. The remaining primers sets either did not produce any amplicon or produce mono-morphic amplicon.

2.4.1.3. Segregation of ToxA and mating type genes

Because the two parental isolates DW5Δ*MAT1-1-1* and 86-124Δ*MAT1-2-1* had a presence/absence polymorphism for *ToxA*, *MAT1-1-1* and *MAT1-2-1* genes, the population

segregates for those loci (Figure 2.1, Appendix C). The ratio for the presence: absence of the *ToxA* gene was 46: 71 (χ^2 =5.3, p=0.02), which significantly different from the 1:1 ratio at the p=0.05 level. All progeny isolates except C-100 had either the *MAT1-1-1* or the *MAT1-2-1* gene and the *MAT1-1-1* and *MAT1-2-1* loci segregated in a ratio of 59: 57(χ^2 =0.008, p=0.9) which fits a 1:1 ratio. The progeny C-100 had both *MAT1-1-1* and *MAT1-2-1* genes.

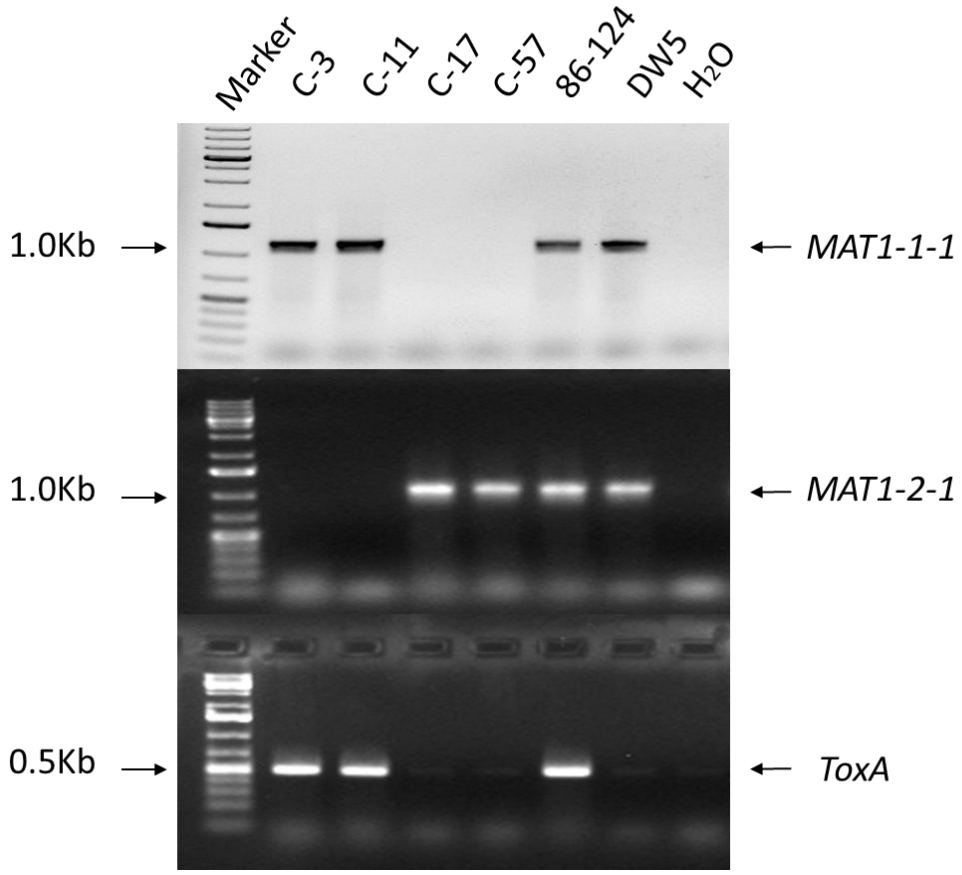

Figure 2.1. PCR amplification of the *ToxA*, *MAT1-1-1* and *MAT1-2-1* in 86-124, DW5 and the progeny isolates. The representative progenies with the presence or absence of *ToxA*, *MAT1-1-1* and *MAT1-2-1* loci were shown with C-3 and C-11 contain *MAT1-1-1* and *ToxA,* C-17 and C-57 contain *MAT1-2-1* only. Water control was included in the PCR.

2.4.1.4. Segregation of ToxB copies

When coding region primer pair was used, the segregation ratio of *ToxB* was 94:23 (presence: absence), which is not significantly deviated from the 3:1 ratio (χ^2 =1.7, p=0.09) strongly suggesting two *ToxB* loci.

The primer pairs amplifying individual *ToxB* loci were tested in the parental isolates and the entire population. All six pairs of primers corresponding to different *ToxB* copies produced PCR amplicon(s) in DW5, but not in 86-124 (Figure 2.2). Except *ToxB1*, all others produced a single amplicon. The whole population were genotyped with each *ToxB* copy primer. *ToxB1* to *ToxB5* were found to co-segregate without any recombination, which is designate as the *ToxB1-5* locus. However, the *ToxB6* locus segregated independently. Among 117 progeny isolates, 33 had only *ToxB6*, 37 had *ToxB1-5*, 24 had both, and 23 had none of them. The ratio fits 1:1:1:1 (χ^2 =0.17, p=0.68). For the 23 progeny isolates that had no amplification for six *ToxB* copies, there were no an amplification either with *ToxB* coding region primer pair (TB71F/TB6R).

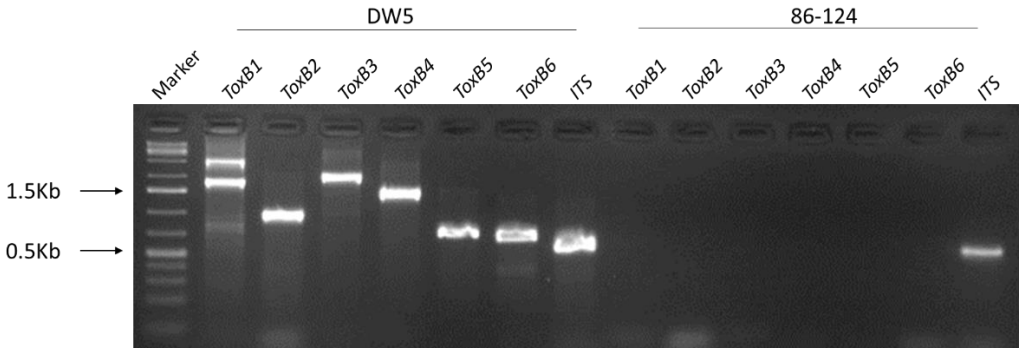

Figure 2.2. PCR amplification of six *ToxB* copies in the isolates DW5 and 86-124. ITS was included as DNA control. Marker was the DNA size standard with two brighter bands being 1.5 kb and 0.5 kb. Amplicons were shown for all six *ToxB* copies (*ToxB1* to *ToxB6*) in DW5, but not in 86-124. Two bands were obtained for *ToxB1* amplification, but the one close to 1.5 kb size standard is *ToxB1*.

2.4.2. Linkage map construction and comparison to optical map

In total, 367 markers were used to assemble the linkage map including 257 SNPs, 105 SSRs, *ToxA*, *MAT1-1-1*, *MAT 1-2-1*, and two *ToxB* loci. When the LOD=3.0 and r=0.3 was used, one huge LG and two small LGs were obtained. The big LG was broken by using LOD=6.0 and r=0.3 into smaller LGs. By using the information of optical map, some LGs were connected if the distances were lower than 50 cM.

The constructed genetic linkage map was composed of 11 linkage groups (LGs) with a total genetic distance of 4,780.22 cM (Table 2.2, Figure 2.3). The 11 LGs largely corresponded to the 11 chromosomes of Pt-1C-BFP optical map. Therefore, LGs were designated based on their identity to each chromosome of the reference genome. LG1 was the largest, spanning 1124.29 cM and containing 78 independent markers while the LG9 was the smallest, having twenty-one markers spanning 207.90 cM in genetic distance (Table 2.2). Most of two adjacent markers had a distance lower than 30 cM, but a few of them were more than 30 cM (on LGs 1, 3, 5 and 11, highlighted in gray in Figure 2.3) and one of them was 40.2 cM (on LG5 highlighted in red in Figure 2.3).

The reference genome had a total 47 assembled SCs (SC1 being largest and SC47 being smallest), 26 of which were anchored on the 11 chromosomes and 21 of which (small SCs) were not anchored (Table 2.2, Figure 2.3). Although the genetic linkage map is highly comparable to the optical map, there were markers mapped to the unexpected chromosome or location, in particularly for markers from the smaller size SCs (Figure 2.3, marked with orange bar). For example, markers on SC25 mapped to LG5 in my map but to Chr.2 in the optical map. There were a few markers of SC9 and SC22 mapped to LG2 in my map but to LG9 in the optical map.

A few small SCs that were not anchored in optical map were mapped in my linkage map, including SC19, 20, 21, 28, 29, 34, 36 and 42 (Table 2.2, Figure 2.3).

Table 2.2. Genetic linkage map developed for the cross between *Pyrenophora tritici-repentis* isolates 86-124Δ*MAT1-2-1* × DW5Δ*MAT1-1-1*. Linkage groups were named based on the chromosome names of the Pt-1C-BFP genome assembly.

Optical map (Pt-1C-BFP)[a]	Supercontigs in each Chr.	Linkage group	Supercontigs in each LG[b]	No. of markers	Genetic distance (cM)
Chr.1	10, 33, 18, 1, 17	LG1	10, (29),18, 1, 17	78	1124.29
Chr.2	16, 2, 25, 23	LG2	2, (19, 20, 22, 9), 16	48	487.25
Chr.3	12, 13, 14	LG3	34, 12, 14, 13, (42), 23, (29)	37	538.32
Ch.4	3	LG4	3, (17)	33	438.88
Chr.5	5	LG5	33, 25, 5, 1, 21	32	373.66
Chr.6	4	LG6	4, 36	25	358.24
Chr.7	11, 15, 30	LG7	11, 15, 26	21	333.24
Chr.8	8, 24, 26	LG8	28, 24, 3, (26)	30	335.11
Chr.9	9, 22, 27	LG9	9	21	207.90
Chr.10	6	LG10	23,6, (30)	13	245.42
Chr.11	7	LG11	32, 7	27	337.91
unanchored	19, 20, 21, 28, 29, 31, 32, 34-47	unlinked		2	
		Total		367	4780.22

[a] The optical map of Pt-1C-BFP, was published by Manning et al. (2013). The map has eleven chromosomes (Chr.), each of which consists of one or more supercontigs (SC) assembled from the genome sequencing. There were 21 small SCs that could not be anchored to any chromosome.

[b] The genetic map developed in 86-124Δ*MAT1-2-1* × DW5Δ*MAT1-1-1* population contains eleven linkage groups (LG), each of which contains mapped SCs of Pt-1C-BFP. The SC in bracket indicates that one or a few markers designed from this SC was mapped within another SC which was shown before the bracket.

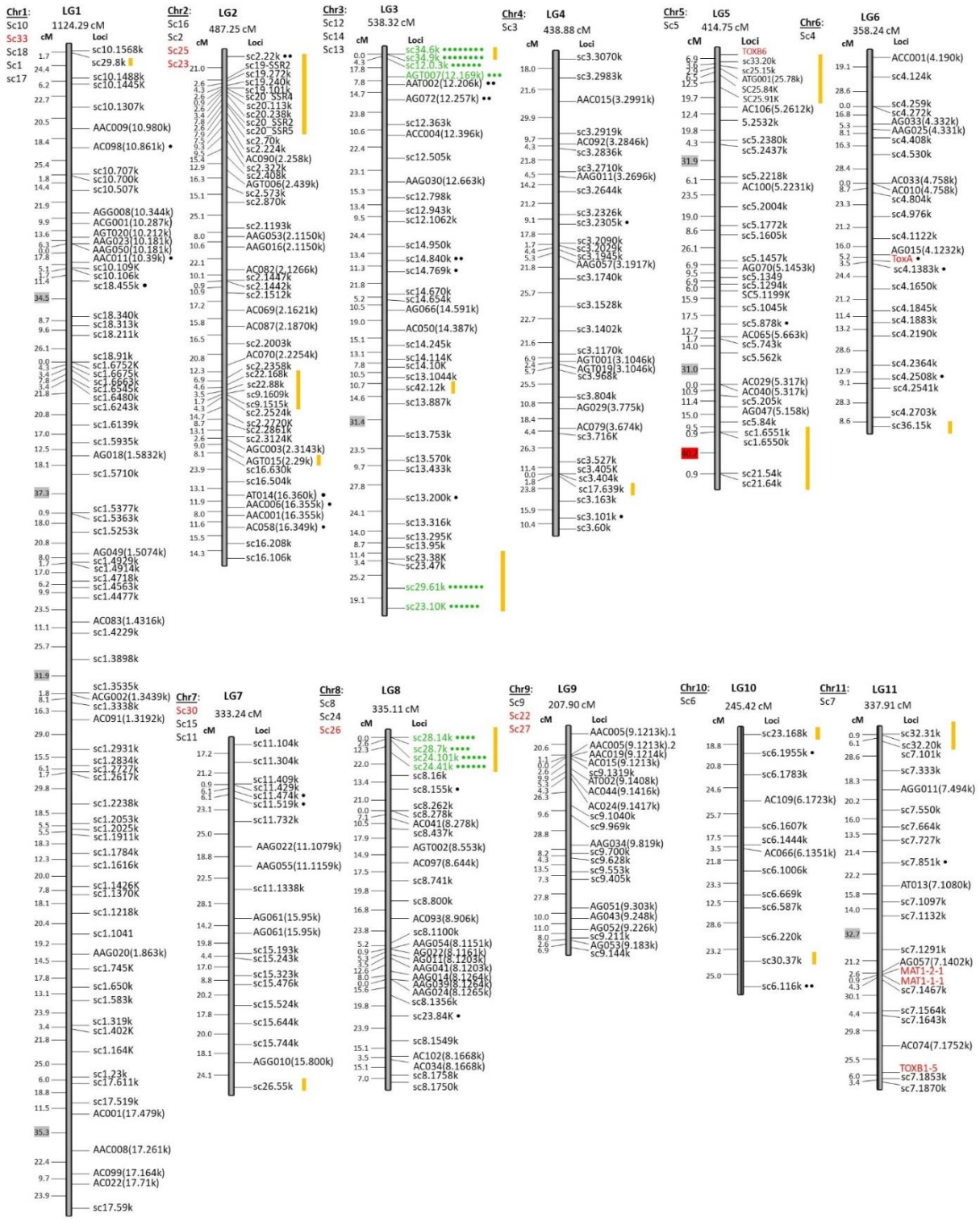

Figure 2.3. Genetic linkage map of *P. tritici-repentis* 86-124Δ*MAT1-2-1* × DW5Δ*MAT1-1-1* population. Linkage groups (LG) were named according to their identity to corresponding chromosome of the Pt-1C-BFP optical map. For a comparison purpose, the supercontigs in each chromosome were list on the upper left corner of each LG. The SC in red has no marker mapped

in the LG. The locus name and its physical position were shown to right of LG and the genetic distance in cM between adjacent markers were shown to left of LG. The markers in red are the loci of known genes, including the *ToxA*, *ToxB* (*ToxB1-5* and *ToxB6*) and *MAT* genes. The markers in green are highly significantly distorted from 1:1 segregation ratio with P<0.001 (three and more asterisks). The markers that mapped to unexpected places are marked with orange bars of different size. The distance between two markers were highlighted in red if higher than 40 cM and were highlighted in gray if higher than 30 and lower than 40 cM.

2.4.3. Genetic mapping of *ToxA, MAT1-1-1, MAT1-2-1* and *ToxB* loci

The *ToxA* gene was mapped to LG6 between SNP marker *sc4.1383k* and SSR marker *AG015* (sc4.1232k bp). The *ToxA* gene was physically located at the SC4.1448894 bp on the Pt-1C-BFP genome assembly. *MAT1-1-1* and *MAT1-2-1* were mapped between SNP marker *sc7.1467k* and SSR marker *AG057* (SC7.1402000 bp) in LG11 with 0.9 cM distance right in the expected physical location (SC7:1419217-1425508 bp). *ToxB1-5* which contains five *ToxB* copies was mapped to LG11 between SNP marker *sc7.1870k* and SSR marker *AC074* (SC7.1752000 bp) while *ToxB6* was mapped to the distal end of LG5, 6.9 cM away from *sc33.30k* marker (Figure 2.3).

2.4.4. Virulence segregation on differential lines

All the progeny isolates as well as the isolates 86-124 and DW5 were phenotyped on tan spot differential lines: Salamouni, Glenlea, 6B662 and 6B365 and the spring wheat line CDC-Osler. Salamouni, which is the universal resistant line in tan spot differential set was shown highly resistant to both isolates 86-124 and DW5 with the development of pin-size black dots (Figure 2.4). The reactions of Salamouni to all the progeny isolates were similar to that for the two isolates. On 6B365 which is differential for Ptr ToxC, both isolates (86-124 and DW5) caused small dark lesions with very little chlorosis with DW5 causing slightly higher disease. Small differences were also observed among the progeny isolates, but the data was not analyzed. Glenlea is a differential line for Ptr ToxA, thus it was highly susceptible to 86-124 but resistant to DW5. Virulence of the progeny isolate on Glenlea was highly correlated with the presence of the

ToxA gene. All the progeny isolates carrying *ToxA* caused large necrotic lesions on the Glenlea as 86-124 (Figure 2.4). 6B662 is the different line for Ptr ToxB. The line developed resistant reaction (pin-sized dark lesion with very little chlorosis) to 86-124 while it developed large chlorotic lesions to DW5 (Figure 2.4). Because two *ToxB* loci (*ToxB1-5* and *ToxB6*) segregated in the population, different reactions were observed (Figure 2.5). For progeny isolates with neither of loci (e.g. C30), the reaction was similar to that for 86-124 without the development of any chlorotic lesion. For progeny isolates carrying *ToxB6* only (e.g. C49), very small and weak chlorotic lesions were observed on the leaves. For the progeny isolates carrying *ToxB1-5* with *ToxB6* (e.g. C26) or without *ToxB6* (e.g. C31), they all caused large chlorotic lesions as DW5 and no obvious difference was observed among them.

CDC-Osler were highly susceptible to 86-124 with development of large areas of necrosis and chlorosis on the leaf. In contrast, this line developed tiny chlorotic dots when inoculated with DW5 (Figure 2.4). This line has been shown to be insensitive to Ptr ToxA suggesting that other NE(s) from 86-124 cause disease. The reaction of CDC-Osler to individual progeny isolates ranged from highly resistant as for DW5 to highly susceptible as 86-124 (Figure 2.6, Appendix D).

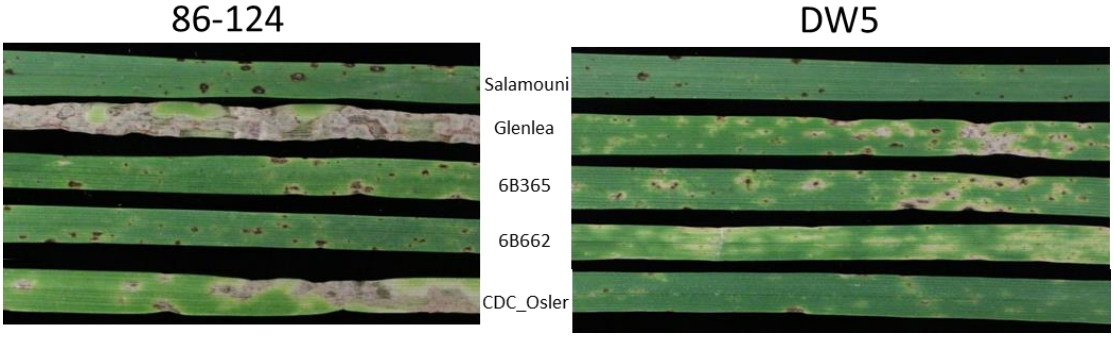

Figure 2.4. Reaction of tan spot differentials and CDC_osler to 86-124 and DW5. The names for different genotypes was indicated in the middle and reaction to 86-128 was shown to left and that to DW5 was shown to right.

Figure 2.5. Reaction of 6B662 to progeny isolates carrying different *ToxB* loci. The names of progeny isolates which carry different *ToxB* loci are indicated to the left.

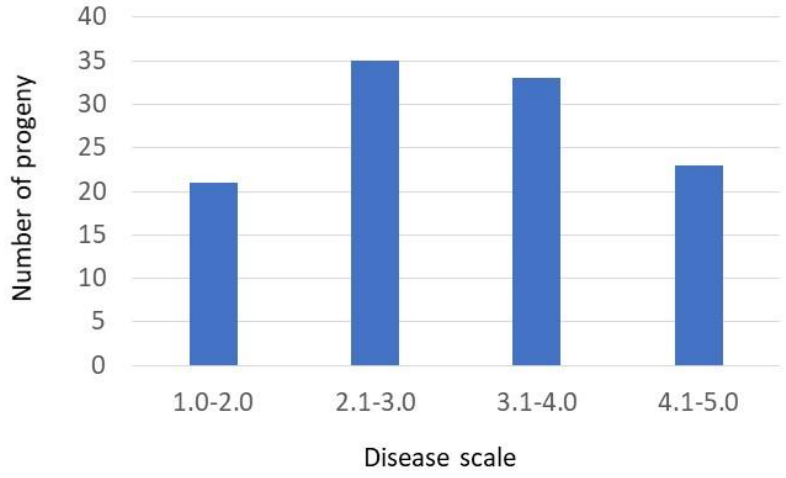

Figure 2.6. Histogram of reaction of progeny isolates from the population on spring wheat line CDC-Osler. The x-axis indicates disease scale of 1.0 to 5.0 (see methods) and y-axis indicates the number of progeny isolates in each disease category.

2.4.5. QTL mapping

A LOD threshold 3.2 was obtained at α=0.05 after 1,000-time permutation test. QTL analysis identified two QTLs on two different linkage groups (Table 2.3, Figure 2.7). The major QTL, designated as *VirOsler1*, was located on LG2 between the SSR marker *sc20_SSR4* (SC20.130000 bp) and SNP marker *sc20.238k.* The QTL had a LOD value at 32.7 and explained

74% of disease variations. A minor QTL was identified on LG7 between the SNP markers *sc15.744k* and *sc26.55k* with the SSR marker *AGG010* (SC15.800000 bp) underlying the peak of the QTL. This QTL had a LOD value at 3.9 accounting for 15% of disease variations.

Table 2.3. QTLs associated with *Ptr* virulence on CDC-Osler from composite interval mapping analysis

QTL	Chr.	Interval (cM)	Flanking markers	LOD[a]	R^{2b}
VirOsler1	2	32-38	*sc20_SSR4 - sc20.238k*	32.7	0.74
VirOsler2	7	300-318	*sc15.744k – sc26.55k*	3.9	0.15

[a] Permutation test with 1000 iterations yielded a LOD value of 3.2 and it was used as the cut-off to identify significant QTL.
[b] R^2 value indicates the amount of phenotypic variation explained by the individual QTL.

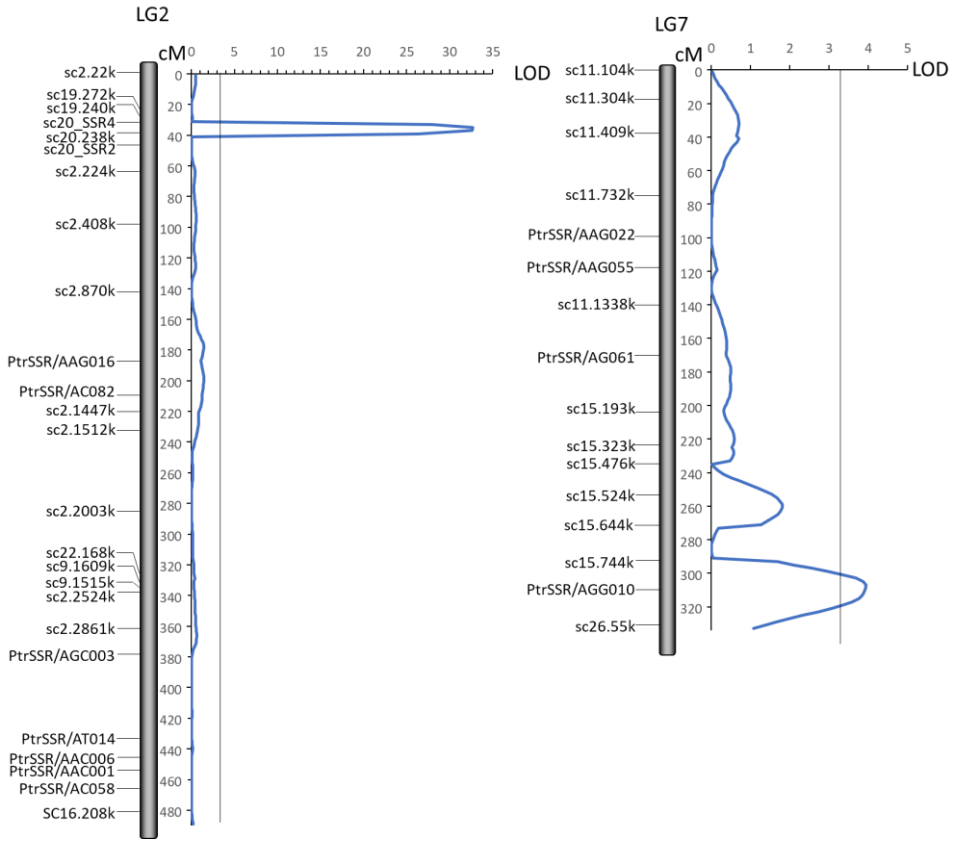

Figure 2.7. Composite interval regression maps of the LG2 and 7 containing QTLs significantly associated with virulence of 86-124 on CDC-Osler. The positions of marker loci are shown to the left of the linkage groups (LGs) and genetic scales in centimorgan (cM) are shown along the right of each LG. A solid line represents the logarithm of the odds (LOD) significance threshold of 3.2. The LOD and R^2 values for each QTL are presented in Table 2.3.

2.5. Discussion

Genetic linkage mapping has been a routine approach to identify and clone virulence or avirulence genes in many fungal pathogens of plants (Zhong et al. 2002; Kema et al. 2002; Lai et al. 2007; Lendenmann et al. 2014; Koladia et al. 2017; Zhong et al. 2017). This is very difficult to do in *Ptr* because it is a homothallic fungus which precludes the development of bi-parental fungal population. Our group has recently demonstrated the possibility of developing heterothallic *Ptr* strains and using them to develop segregating fungal population (Ameen et al. 2017). Using a developed bi-parental population, Kariyawasam (2019) have successfully mapped the gene (s) for Ptr ToxC production. In my research, I used another *Ptr* bi-parental population and successfully located QTLs conferring pathogen virulence. The research demonstrates genetic mapping is an effective and powerful approach to identify new virulence genes in *Ptr*. This approach is particularly effective for identifying NEs which is not a protein or is not produced in a large quantity *in vitro*. The identification of new virulence gene from this research is highly significant for the further understanding of pathogen virulence in wheat tan spot system because no progress has been made since 2001 in this area. Additionally, I have also successfully mapped the individual *ToxB* loci, which provides a better understanding of genome organization for the multiple copy gene of *ToxB*.

The virulence QTLs I mapped is derived from 86-124, a race 2 isolate which produces Ptr ToxA. The major QTL was identified on chromosome 2, not at the *ToxA* locus on chromosome 6 strongly indicating the presence of a new virulence gene rather than *ToxA* in 86-124. Another fact is that the wheat line CDC-Osler is insensitive to Ptr ToxA. Friesen et al. (2003) showed that 86-124 still caused as much disease on a *Tsn1* mutant of Kulm as on the wild type Kulm, which suggested 86-124 produce other virulence factor than Ptr ToxA. The major QTL identified in this

research could also confer virulence toward Kulm. Two putative Ptr ToxDs have been reported from *Ptr* (Meinhardt et al. 2003; Ciuffetti et al. 2003). It remains unknown if *VirOsler1* or *VirOsler2* encodes any of Ptr ToxD because the two Ptr ToxDs have not been formally published.

The major QTL *VirOsler1* was mapped to the physical region between 130 kb and 238 kb on SC20. The SC20 is a small supercontig in the reference genome without being anchored in any chromosome. The markers developed from this SC all mapped to the beginning of SC2 on LG2 indicating SC20 is likely connected with SC2. Based on the reference genome annotation, there were sixteen genes (PTRT11797 to 11812) predicted in the physical region of SC20:130,000-238,000 bp. Among them, most are hypothetical proteins, but three of them are of an interest with two being a small secreted protein gene and one being secondary metabolite synthetase gene. Currently, we don't know if the NE is a protein or secondary metabolite. Therefore, these three are strong candidate gene for *VirOsler1*. In addition, the draft genome of 86-124 is highly fragmented and there is no continuous contig covering the region. It is possible that the candidate gene encoding for *VirOsler1* is not present in Pt-1C-BFP. The high-quality genome sequence for 86-124 is needed to identify the final candidate gene. The minor QTL *VirOsler2* mapped to the distal end of LG7. Because the marker density is low and the QTL region likely involves two SC, it is difficult to pinpoint the candidate gene.

Kariyawasam (2019) developed the first linkage map in *Ptr* using a bi-parental population between AR CrossB10Δ*MAT1-2-1* (a new race) and 86-124Δ*MAT1-1-1*. The map contained consisted of 13 LGs with 303 markers spanning a total of genetic distance of 5075.83 cM. A total of 367 markers was developed in my research for the 86-124Δ*MAT1-2-1*×DW5Δ*MAT1-1-1* population. Because more markers were developed in this population, the map I constructed had 11 LGs with much less numbers of genetic gaps (greater than 30 cM). The

total genetic distance of this map was estimated at 4,780.22 cM. Both maps probably had overestimated a total genetic distance because "drop locus" was not performed. However, we can see the ratios of physical/genetic distance between any two adjacent markers are around 8 kb/cM in both maps suggesting that *Ptr* has a high level of recombination rate across the genome during meiosis. The high recombination rate is probably due to the high-level genetic similarity of two isolates even they are classified into two different races.

Overall, the linkage map I developed correlates well with the reference genome optical map. First, most of SCs, particularly the large ones, were mapped to the chromosomes they are supposed to be in. Second, orders for most markers agree with well their positions on the corresponding SCs. However, we did observe some disagreements, mainly related to small SCs between our linkage map and optical map (Figure 2.3, marked with orange bars). A major disagreement concerns SC25 where all SC25 markers were mapped to Ch.5 instead of Ch.2. This is also observed in the map developed in AR CrossB10Δ*MAT1-2-1* × 86-124Δ*MAT1-1-1* population. In the M4 high-quality genome sequence, SC25 sequence was located to Ch.5 (Moolhuijzen et al. 2018). Therefore, an explanation would be that either Pt-1C-BFP has a structure variation on Ch.2 or there was an error in mapping SC25. Another disagreement is that a few markers from SC9 and SC22 mapped in the middle of Chr.2. The M4 sequence assemblies did not provide support for my mapping results, but this was observed in linkage map of AR CrossB10Δ*MAT1-2-1* × 86-124Δ*MAT1-1-1* population. A big advantage for my linkage map is that it has placed several SCs unmapped in the optical map to one of chromosomes. These included SC19, SC20, SC21, SC24 and SC34. The M4 assemblies were checked and their chromosomal locations were confirmed.

ToxB was known to have multiple copies in Ptr ToxB-producing isolates (Martinez et al. 2001; Strelkov and Lamari 2003). *ToxB* copies in different fungal isolates/races have been estimated from 2 to 10 (Strelkov et al. 2002, 2005). By using genomic DNA cloning and Southern analysis with CHEF gel blotting, Martinez et al. (2004) determined that six copies of *ToxB* are in the North Dakota race 5 isolate DW7 with most of them on a 2.7 Mb chromosome and the rest on a larger chromosome (3.1 Mb). Aboukhaddour et al. (2009) used the same techniques to analyze the chromosomal locations of *ToxA* and *ToxB* in diverse isolates/races. *ToxB* loci were either on two separated chromosomes or a single chromosome. However, it remained unclear which two chromosomes harbor *ToxB* loci and how *ToxB* loci are arranged in each chromosome. Using genetic linkage mapping, I showed that five of six loci are likely clustered as one locus (*ToxB1-5*) on the end of Ch. 11 and the sixth locus (*ToxB6*) is at the end of Ch.5. The two loci showed an independent segregation in the population. Although *ToxB6* caused small amount of chlorosis, the *ToxB1-5* locus containing five copies of *ToxB*, contributes the nearly same level of virulence as all six copies.

ToxA was mapped to the expected position determined by the reference genome sequence (Manning et al. 2013). However, it is interesting to note the segregation of *ToxA* didn't fit a 1:1 ratio, which is not expected for a single copy gene. In contrast, Kariyawasam (2019) did not observe the distorted segregation of *ToxA* in AR CrossB10Δ*MAT1-2-1* × 86-124Δ*MAT1-1-1* population. Therefore, this is likely due to the specific genetic background of isolates of DW5 which allows non-ToxA allele to be preferentially transmitted. It is also possible that ascospore picking during the population construction was not random. We also notice the marker mostly close to *ToxA* also had a slight distorted segregation. Highly distorted segregation was observed for the markers in both ends of Ch.3 and one end of Ch.8. The reason for this is unknown.

In summary, I developed a bi-parental population in *Ptr* and used it to successfully map individual *ToxB* locus and two new QTLs conferring pathogen virulence. My research is a significant step in further understanding of pathogen virulence and host pathogen interaction in wheat tan spot system.

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CHAPTER 3. CHARACTERIZING VIRULENCE OF THE *PYRENOPHORA TRITICI-REPENTIS* ISOLATES LACKING BOTH TOXA AND TOXB GENES

3.1. Abstract

The fungus *Pyrenophora tritici-repentis* (*Ptr*) causes tan spot of wheat crops, including common wheat (*Triticum aestivum* L.) and durum (*T. turgidum* ssp. *durum* L.). The disease is very common and economically important in many wheat growing regions worldwide. Based on the production of the three known necrotrophic effectors (NEs), the fungal isolates are classified into eight races with race 4 producing no known NEs. From a laboratory cross between 86-124 (*ToxA* only) and DW5 (*ToxB* only), we have obtained some *Ptr* isolates lacking both *ToxA* and *ToxB* genes (Chapter 2) and producing no Ptr ToxC, which, by definition, should be classified as race 4. In this work, I characterized virulence of eight of these isolates. Two of the eight isolates were first evaluated by inoculating them onto various spring wheat and durum cultivars. The results showed that the two isolates still caused disease on some of the cultivars tested. Disease evaluations for eight isolates were conducted in a recombinant inbred line population derived from two hard red winter wheat cultivars: Harry and Wesley. QTL mapping revealed a total of six genomic regions that are significantly associated with disease caused by different isolates. These QTL are located to different genomic places other than the three known NE sensitivity loci. The result further indicates the existence of additional NE-host sensitivity gene interactions in the wheat tan spot disease system.

3.2. Introduction

Tan spot is a devastating foliar disease on both common wheat (*Triticum aestivum* L.) and durum (*T. turgidum* ssp. *durum* L.) (Faris et al. 2013). This disease is caused by the fungal pathogen *Pyrenophora tritici-repentis* (*Ptr*), which belongs to the family of dothideomycetes in

ascomycete. The typical symptoms incited by this pathogen on susceptible cultivars include tan-colored, elliptical-shaped necrotic lesions, which are often surrounded by chlorotic halos (Friskop and Liu 2016). Under favorable conditions, the lesions can coalesce forming a large area of dead leaf tissue. These symptoms can be indicative of the necrotrophic nature of lifestyle where the fungus may produce necrotrophic effectors to cause the death of plant cells.

Since the 1980s, several studies have revealed that the symptoms of necrosis and chlorosis induced in the host by *Ptr* were genetically distinct (Lamari and Bernier 1989, 1991; Lamari et al. 2003). These studies also led to the development of a wheat differential set for tan spot, a lesion-type based disease rating scale, and a basic race classification system, which have been widely adopted today. Later, the fungal pathogen was known to produce host selective toxins, now called necrotrophic effector (NEs), to induce necrosis or chlorosis symptoms by interacting with their corresponding host sensitivity genes. Up to date, three fungal-produced NEs have been identified, namely Ptr ToxA (NCBI accession ID: AAB61464.1), Ptr ToxB (NCBI accession ID: AAO73337.1), and Ptr ToxC, which interact with the wheat genes *Tsn1*, *Tsc2* and *Tsc1*, respectively (Ciuffetti et al. 1998, 2010). Therefore, eight races have been designated according to their ability to produce single or the combination of the three necrotrophic effectors (NEs). Races 2, 3 and 5 produce one NE, Ptr ToxA, Ptr ToxC and Ptr ToxB, respectively, causing disease on differential lines Glenlea (necrosis), 6B365 (chlorosis) and 6B662 (chlorosis), respectively. Races 1, 6, and 7 produce a combination of two NEs with Ptr ToxA and Ptr ToxC for race 1, Ptr ToxB and Ptr ToxC for race 6, and Ptr ToxA and Ptr ToxB for race 7. Race 8 produces all three NEs while race 4 produces no known NEs (Strelkov and Lamari 2003; reviewed in Faris et al. 2013). However, the current race classification in *Ptr* has been challenged by the identification of isolates that do not conform to any of the eight races (Ali

and Francl 2003; Ali et al. 2010). It has been reported that *Ptr* likely produces additional NEs in addition to the three previously described (Meinhardt et al. 2003; Ciuffetti et al. 2003; reviewed in Faris et al. 2013).

Ptr is a homothallic fungus, which means it can be sexually reproduced by self-crossing. This precludes the possibility to further identify fungal virulence factors through genetic analysis in *Ptr*. Recently, we have developed a new method for fungal cross and genetic mapping in *Ptr* (Ameen et al. 2017). The race 2 isolate 86-124 and race 5 isolate DW5 were only known to produce Ptr ToxA (encoded by the *ToxA* gene, ID: 5983599) and Ptr ToxB (encoded by the *ToxB* gene ID:AY243460), respectively, but they have been shown to produce other unknown NEs (Ciuffetti et al. 2010; reviewed in Faris et al. 2013). We have obtained some isolates from the cross between 86-124 and DW5 that do not produce any of the three NEs, and theses isolates should be classified as race 4 according to the current classification system. However, our hypothesis is that these isolates produce other unknown NEs and are still virulent. In this work, we tested the hypothesis by using pathogenicity test on various common and durum wheat genotypes and mapped the host factors that interact with the unknown NEs or virulence factors present in these isolates.

3.3. Materials and Methods

3.3.1. Fungal isolates and PCR testing

The *Ptr* isolates used in this study were derived from a fungal cross between 86-124 (race 2) and DW5 (race 5) that were modified for the mating type system (Ameen et al. 2017). We obtained a total of eighteen progenies that have neither *ToxA* nor *ToxB* gene and randomly selected eight of them including B-16, B-17, C-7, C-9, C-16, C-32, C-44 and C-55 for the characterization of fungal virulence outside of the three known NEs. To confirm the presence of

the *ToxA* and *ToxB* genes, the gene specific primers that have been published previously were used (Table 3.1). Since the eight isolates were derived from the mating type gene modified strains of 86-124 (race 2) and DW5 (race 5), they were also tested for the presence of the mating type genes using the corresponding primers (Table 3.1). The fungal isolates were grown on V8-PDA for 7 days and then the mycelium was collected for DNA extraction by gently scratching the surface of the cultures. Genomic DNA extraction was done after following the processes described by Shjerve et al. (2014). In PCR testing, the *ToxA* primers were multiplexed with those for the *MAT1-1-1* gene and primers for the *ToxB* gene were multiplexed with those for *MAT1-2-1*. The multiplex PCRs were performed according to the protocols by Ameen et al. (2017). 86-124 and DW5 were also used as controls in PCR and the pathogenicity tests.

Table 3.1. List of primers used in chapter 3 study.

Primers	Sequences (from 5′ to 3′)	Purpose	Reference
PtrPLP2	CAGAACAAAGGCAGGACTGTGAGC	Amplify the *MAT1-1-1*	Lepoint et al. (2010)
PtrPLP4	ATGCGCTCAGCAAGGAAGGTCG		
PtrPLP7	GCTTTACTACAACTTTCCTCTACC	Amplify the *MAT1-2-1*	Lepoint et al. (2010)
PtrPLP10	GTACGGGCCAGCATGACGTGC		
TA51F	GCGTTCTATCCTCGTACTTC	Amplify the *ToxA*	Andrie et al. (2007)
TA52R	GCATTCTCCAATTTTCACG		
TB71F	GCTACTTGCTGTGGCTATC	Amplify the *ToxB*	Andrie et al. (2007)
TB6R	ACGTCCTCCACTTTGCACACTCTC		

3.3.2. Plant materials, experimental designs, and disease evaluations

A total of 32 wheat lines including common wheat and durum wheat genotypes as well as the tan spot differential lines (Salamouni, Glenlea, 6B365, and 6B662) and susceptible check ND495 were used in pathogenicity tests. To map host genetic factors interacting with the new virulence factor, we used a bi-parental wheat population derived from the cross between the two Nebraska hard red winter wheat cultivars Harry and Wesley. The population, referred to as the HW population, consisted of 178 recombinant inbred line (RILs) and was kindly provided by Dr.

Stephen Baenziger at the University of Nebraska, Lincoln, NE, USA. All the inoculation experiments were carried out in the greenhouse room with a temperature ranging from 23 to 28 ºC and a growth chamber with a setting at 21 ºC and a 12 h photoperiod. All the wheat lines and the RILs were planted in small cones with three seeds per cone (Stuewe and Sons, Inc., Corvallis, OR, USA) filled with Sunshine SB100 soil (Sun Grro Horticulture, Bellevue, WA, USA) supplied with Osmocote Plus 15-19-12 fertilizer (Scotts Sierra Horticultural Product Company, Maysville, OH, USA). All the cones were arranged in RL98 trays (Stuewe and Sons, Inc., Corvallis, OR, USA). The winter wheat cultivar Jerry was planted as the border to reduce the edge effect (Liu et al. 2015). The plants were grown in the greenhouse room and were inoculated at the two to three leaf stages, which were approximately two weeks from the planting. Fungal inoculum preparation, fungal inoculation, and post inoculation incubation of plants followed the method described in Lamari and Bernier (1989). For each line, six seeds were planted in two cones with six plants in each experiment. The inoculation experiment was repeated three times and, within each experiment, a randomized complete block design (RCBD) was used to arrange the plants. Disease ratings were conducted using the 1–5 scale with 1 being highly resistant and 5 being highly susceptible, which was described by Lamari and Bernier (1989). We added 0 to the scale when no obvious reaction was observed.

3.3.3. Statistical analysis and QTL mapping

In the pathogenicity tests, the NPAR1WAY procedure was performed to compute exact p-values for the simple linear rank statistics based on Wilcoxon scores with the disease readings that were collected from different replications and experiments. The ordinary disease data was transformed into parametric data using PROC RANK followed by ANOVA and t tests of the least significant difference (LSD) in SAS 9.4 Software (SAS Institute, 2016) to detect the

significance of the difference among different wheat genotypes. Normal distribution of the disease data for each isolate was evaluated with the Shapiro-Wilk test using t PROC UNIVARIATE in SAS 9.4 (SAS Institute, 2016). Levene's test was used to test the homogeneity among different replicates (Levene 1960).

Disease means for each RIL from three replications were used for QTL mapping. The genetic linkage maps of the HW population have been published containing 3,641 SNP markers and covering all 21 wheat chromosomes and has been successfully applied to the QTL mapping of flag leaf related traits (Hussain et al. 2017). The same map has been used in the mapping of QTL associated with reaction to five common *Ptr* races (Kariyawasam et al. 2018). Here, the genetic map was used in this study to locate QTLs for the reaction to these isolates that do not produce any known NEs. The QTL mapping was performed using PC-based software QGene 4.0 (Joehanes and Nelson 2008). The single trait multiple interval mapping (SIM) function installed in the software was used for QTL analysis. A permutation test with 1,000 iterations yielded an LOD threshold of 3.2 at a p=0.05 experiment-wise significance level.

3.4. Results

3.4.1. Pathogenicity test

In our previous study, we used genetically modified mating type isolates 86-124Δ*MAT1-2-1* (only carry *MAT1-1-1*) and DW5Δ*MAT1-1-1* (only carry *MAT1-2-1*) to develop a segregating population and obtained several isolates that had neither *ToxA* nor *ToxB* gene (Chapter 2). From these isolates, we randomly selected eight of them: B-16, B-17, C-7, C-9, C-16, C-32, C-44 and C-55 for this study. Using gene specific primers (Table 3.1), we confirmed that the eight isolates lacked both *ToxA* and *ToxB* genes (Figure 3.1). As expected, the *ToxA* and *ToxB* genes were amplified from 86-124 and DW5, respectively. Regarding the eight isolates from the cross of 86-

124Δ*MAT1-2-1* and DW5Δ*MAT1-1-1*, they should harbor only one mating type gene (Appendix C).

Two of eight isolates, B-16 and B-17 were first tested on the tan spot differential lines including Salamouni, Glenlea, 6B365, and 6B662 as well as the highly susceptible line ND495. To compare the difference in virulence, 86-124 and DW5 were also inoculated onto these lines side-by-side with B-16 and B-17. As shown in Figure 3.2, 86-124 and DW5 caused high levels of disease on ND495 and their differential lines Glenlea (necrotic lesions) and 6B662 (chlorotic lesions), respectively. Compared to 86-124 and DW5, B-16 and B-17 caused less disease due to the development of smaller sizes of necrotic lesions on Glenlea and ND495 (Figure 3.2). This is likely due to the fact they do not produce Ptr ToxA and Ptr ToxB.

B-16 and B-17 were further tested on a set of wheat lines including common wheat (*Triticum aestivum* L.) and durum genotypes (*T. turgidum* ssp. *durum* L.) (Table 3.2). The majority of these lines are hard red spring wheat and durum wheat cultivars in the Northern Great Plains of the United States. Disease means from three replications for all the line were listed in Table 3.2 and the least significant difference (LSD) were calculated among those lines.

Significant differences were observed in disease means among some lines, according to the LSD values. The most common wheat lines had a disease mean equal or lower than 2.00, which indicates a resistance reaction. A few common wheat lines such as Mott, Steele-ND, Harry and Barlow had a disease mean greater than 4.00, which is highly susceptible. All the durum wheat lines had disease means greater than 3.00, which indicates that they are moderately or highly susceptible to tan spot caused by B-16 and B-17 (Table 3.2).

Table 3.2. Reaction of wheat genotypes to tan spot caused by the isolates B-16 and B-17.

Genotypes	Wheat Type	Agent or Origin[b]	Year Released	B-16 [a]		B-17 [a]	
				Disease Mean	Rank Mean	Disease Mean	Rank Mean
Rollag	Common wheat	MN	2011	1.2	6.17O	1.2	9.83JK
Velva	Common wheat	ND	2011	1.2	6.17O	1.5	17.50JK
Jenna	Common wheat	AgriPro	2009	1.5	11.50NO	1.5	17.50JK
RB07	Common wheat	MN	2007	1.5	14.50MNO	1.5	17.50JK
Brennan	Common wheat	AgriPro	2009	1.7	17.17MNO	1.7	21.33IJK
Glenn	Common wheat	ND	2005	1.8	25.33KLMN	1.5	18.33IJK
Briggs	Common wheat	SD	2002	2.0	28.50JKLM	2.3	38.17GH
Chinese Spring	Common wheat	China	-	2.0	28.50JKLM	2.5	23.00FG
Prosper	Common wheat	ND	2011	2.0	28.50JKLM	1.3	13.67JK
Vantage	Common wheat	WestBred	2007	2.0	28.50JKLM	2.2	33.67GHI
Wesley	Common wheat	USDA-ARS	2001	2.0	28.50JKLM	2.0	29.33HIJ
Howard	Common wheat	ND	2000	2.2	33.67IJKL	2.2	33.67GHI
Kelby	Common wheat	AgriPro	2006	2.5	42.83HIJ	2.5	43.00FG
Select	Common wheat	SD	2010	2.5	42.83HIJ	2.5	43.00FG
SY Soren	Common wheat	Syngenta/AgriPro	2011	2.7	46.83GHI	2.7	47.50EFG
SY Tyra	Common wheat	Syngenta/AgriPro	2011	3.0	56.00FGH	3.5	67.50CD
Mott	Common wheat	ND	2007	4.0	81.50BCD	4.0	80.00BC
Steele-ND	Common wheat	ND	2004	4.0	81.50BCD	4.0	80.00BC
Harry	Common wheat	Nebraska	2004	4.0	81.50BCD	4.5	96.00A
Barlow	Common wheat	ND	2009	4.1	86.67ABC	4.3	90.67AB
Alkabo	Durum wheat	ND	2005	2.2	33.67IJK	2.7	47.50EFG
Lebsock	Durum wheat	ND	1999	3.0	56.00FGH	3.5	66.00CD
Grenora	Durum wheat	ND	2006	3.0	56.00FGH	3.2	59.67DE
Carpio	Durum wheat	ND	2012	3.1	60.00EFG	3.2	59.67DE
Tioga	Durum wheat	ND	2010	3.5	68.50DEF	3.5	66.00CD
Ben	Durum wheat	ND	1996	3.8	77.00BCD	4.0	80.00BC

Table 3.2. Reaction of wheat genotypes to tan spot caused by the isolates B-16 and B-17 (continued).

Genotypes	Wheat Type	Agent or Origin[b]	Year Released	B-16 [a]		B-17 [a]	
				Disease Mean	Rank Mean	Disease Mean	Rank Mean
Dilse	Durum wheat	ND	2002	4.0	81.50BCD	4.0	80.00BC
Divide	Durum wheat	ND	1994	4.0	81.50BCD	4.0	80.00BC
Langdon	Durum wheat	ND	1956	4.3	91.83AB	4.5	96.00A
Rusty	Durum wheat	ND	2004	4.3	91.83AB	4.3	90.67AB
Mountrail	Durum wheat	ND	1998	4.5	97.00A	4.5	96.00A
Pierce	Durum wheat	ND	2001	4.5	97.00A	4.5	96.00A
Least Significant Difference					15		15.7

[a] Disease was rated using a 1–5 scale with 1 being highly resistant and 5 being highly susceptible (Lamari and Bernier 1989). Data represented mean values of 12 individuals. Disease means were transformed into rank means using PROC RANK in the SAS program. In the rank mean column, means with the same letters are not significantly different.

[b] Indicates private companies or public breeding programs which developed the cultivars, MN = University of Minnesota; ND=North Dakota State University; SD=South Dakota State University, or the place the genotype came from.

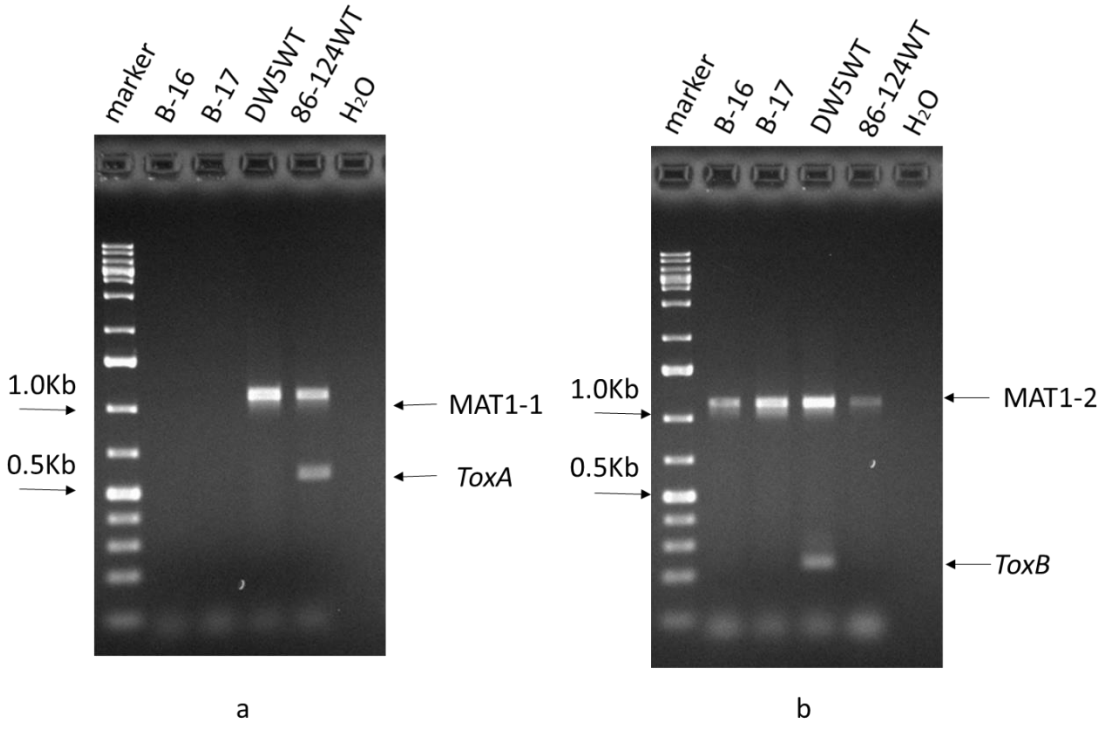

Figure 3.1. PCR testing of the presence of the *ToxA* and *ToxB* genes in *Pyrenophora tritici-repentis* isolates B-16 and B-17. Parental isolates 86-124 and DW5, which were used to obtain B-16 and B-17 as well as water control (H_2O) were also included in the PCRs (**a**) *ToxA* was multiplexed with the *MAT1-1-1* gene. (**b**) *ToxB* was multiplexed with the *MAT1-2-1* gene.

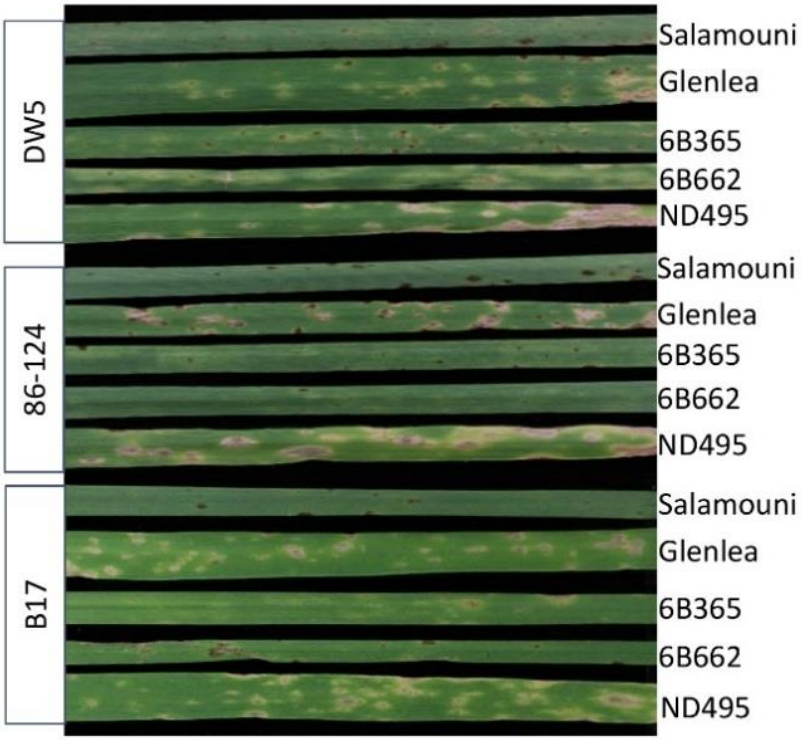

Figure 3.2. Disease reactions of wheat tan spot differential lines to fungal inoculation with B17, DW5, and 86-124. Tan spot differentials included Salamouni (universal resistant), Glenlea (sensitive to Ptr ToxA), 6B365 (sensitive to Ptr ToxC), and 6B662 (sensitive to Ptr ToxB). The hard spring wheat line ND495 was included as a susceptible control. The secondary leaves of the lines were photographed 7 days after inoculations.

3.4.2. QTL mapping

Because Harry and Wesley differed in reaction to B-16 and B-17 with Wesley being resistant and Harry being highly susceptible (Figure 3.3), the HW population was used in the mapping of QTL associated with reaction to these eight isolates. The constructed genetic linkage maps contained 3,641 SNP markers covering all 21 wheat chromosomes and spanning 1,959 cM in the genetic distance with the marker density at 1.8 cM per marker.

Disease evaluations showed the population segregated from highly resistant to high susceptible in the reaction to all eight isolates (Figure 3.3). Shapiro-Wilk tests indicated an acceptance of a normal distribution hypothesis for a disease reaction to B-17 (p=0.1011), but a rejection of a normal distribution hypothesis for the other seven isolates (p <0.0001-0.0012).

Homogeneity analysis with Leven's test indicated that the variances among the replicates were not significant (p=0.06-0.63). The disease means of the population varied for different isolates inoculation with 3.4 for B-16, 3.4 for B-17, 1.9 for C-7, 3.1 for C-9, 2.6 for C-16, 2.3 for C-32, 2.5 for C-44, 3.3 for C-55.

Single trait multiple interval mapping revealed six genomic regions designated as *QTs-1DS*, *QTs-3AS*, *QTs-6BS.1*, *QTs-6BS.2, QTs-7AL,* and *QTs-7DS*, respectively, which are significantly associated with the reaction to tan spot caused by these eight isolates and the resistance alleles for these QTL all come from Wesley (Table 3.3, Figure 3.4). The major QTL, *QTs-7AL,* has been identified on chromosome 7AL between markers *HWGBS5451* and *HWGBS5422*. This QTL was associated with the reaction to six of eight isolates, including B-16, B-17, C-9, C-16, C-44 and C-55. The LOD values of this QTL for different isolates ranged from 3.2 to 6.0 and disease variations they explained ranged from 7 to 13%. The QTL *QTs-3AS* was identified for C-7 and C-16 and was located on the short chromosome of 3A between markers *HWGBS2040* and *HWGBS2066*. The QTL explained 10-11% of the disease variation for C-7 and C-16 with LOD values of 4.3 and 5.1, respectively. The QTL on 7DS (*QTs-7DS*) was identified for B-17, C-9 and C-55 and was flanked by markers *HWGBS6078* and *HWGBS6066.* The last three QTLs were identified for only one isolate, including *QTs-1DS* for C-44, *QTs-6BS.1* for C-7 and *QTs -6BS.2* for C-16. *QTs-1DS* was located on the distal end of 1DS between markers *HWGBS1003* and *HWGBS983* with a LOD value at 4.3 accounting for 10% of disease variations. *QTs-6BS.1* was flanked by *HWGBS4490* and *HWGBS4477* explaining 7% of disease variations and *QTs.zhl-6BS.2* was located between *HWGBS4551*and *HWGBS4556* explaining 9% of disease variations. No significant QTL was detected for C-32 even the population segregated in reaction to this isolate.

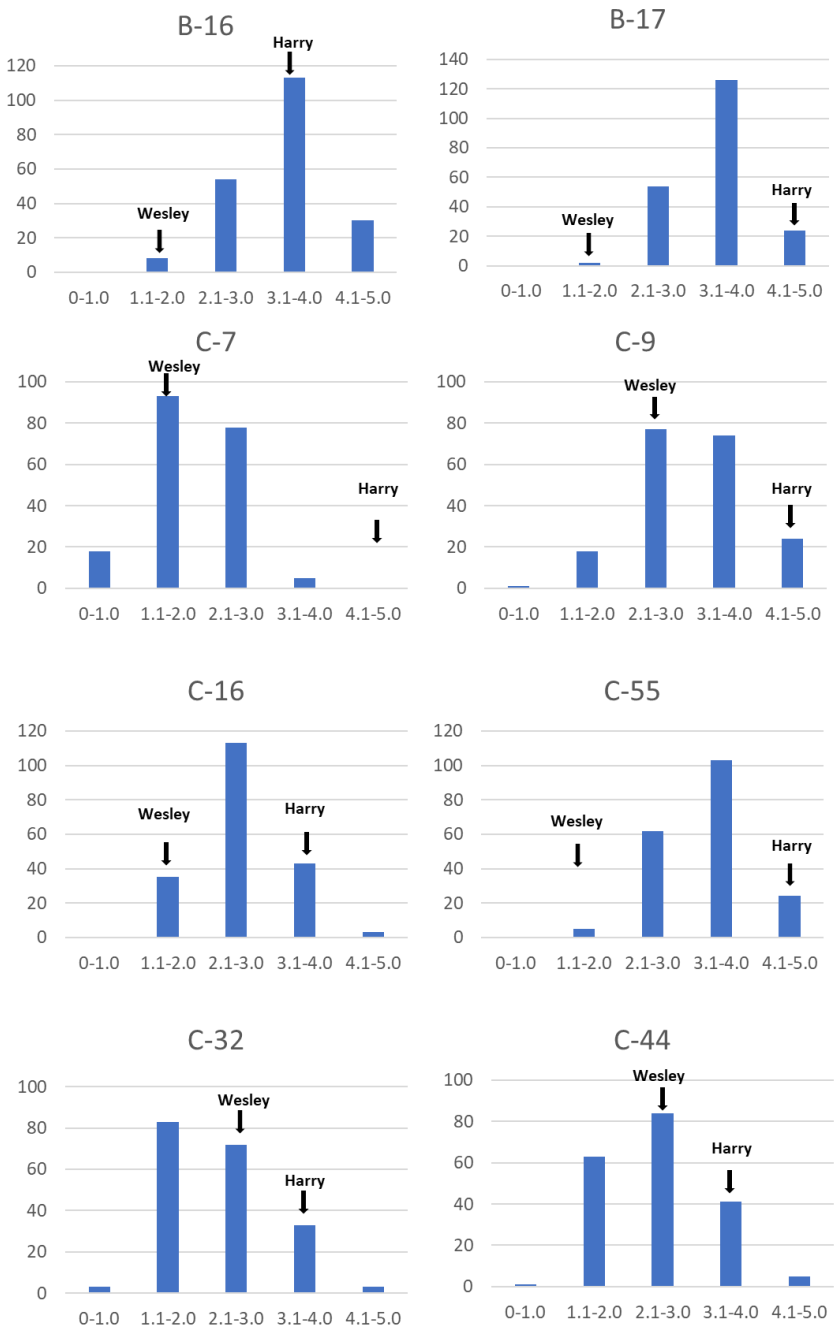

Figure 3.3. Histograms of disease reaction of the Harry × Wesley population to individual *Pyrenophora tritici-repentis* isolate. Disease was rated using a 1-5 scale with 1 highly resistant and 5 highly susceptible. We added 0 to the scale when no obvious reaction was observed. The x-axis is the disease scale, and y-axis is the number of recombinant inbred lines.

Table 3.3. Summary of the QTLs detected in HW population associated with reaction to tan spot caused by *Pyrenophora tritici-repentis* isolates that do not produce all three known necrotrophic effectors.

QTL	Interval (cM)	Position (Mbp)	Flanking markers	LOD[a](R^{2b})						
				B-16	B-17	C-7	C-9	C-16	C-44	C-55
QTs-1DS	0-4	41-104	*HWGBS1003-HWGBS983*						4.3 (0.1)	
QTs-3AS	24-38	61-159	*HWGBS2040-HWGBS2066*			4.3 (0.1)		5.1 (0.11)		
QTs-6BS.1	0-2	139-146	*HWGBS4490-HWGBS4477*			3.2 (0.07)				
QTs-6BS.2	28-32	177-183	*HWGBS4551-HWGBS4556*					3.9 (0.09)		
QTs-7AL	120-128	142-166	*HWGBS5451-HWGBS5422*	3.5 (0.08)	5.4 (0.12)		3.2 (0.07)	4.2 (0.09)	4.4 (0.1)	6.0 (0.13)
QTs-7DS	176-180	201-213	*HWGBS6078-HWGBS6066*		5.2 (0.12)		3.7 (0.08)			3.2 (0.07)

[a] Permutation test with 1,000 iterations yielded a LOD value of 3.2 and it was used as the cut-off to identify significant QTL

[b] R^2 value accounts for the amount of phenotypic variation explained by the QTL.

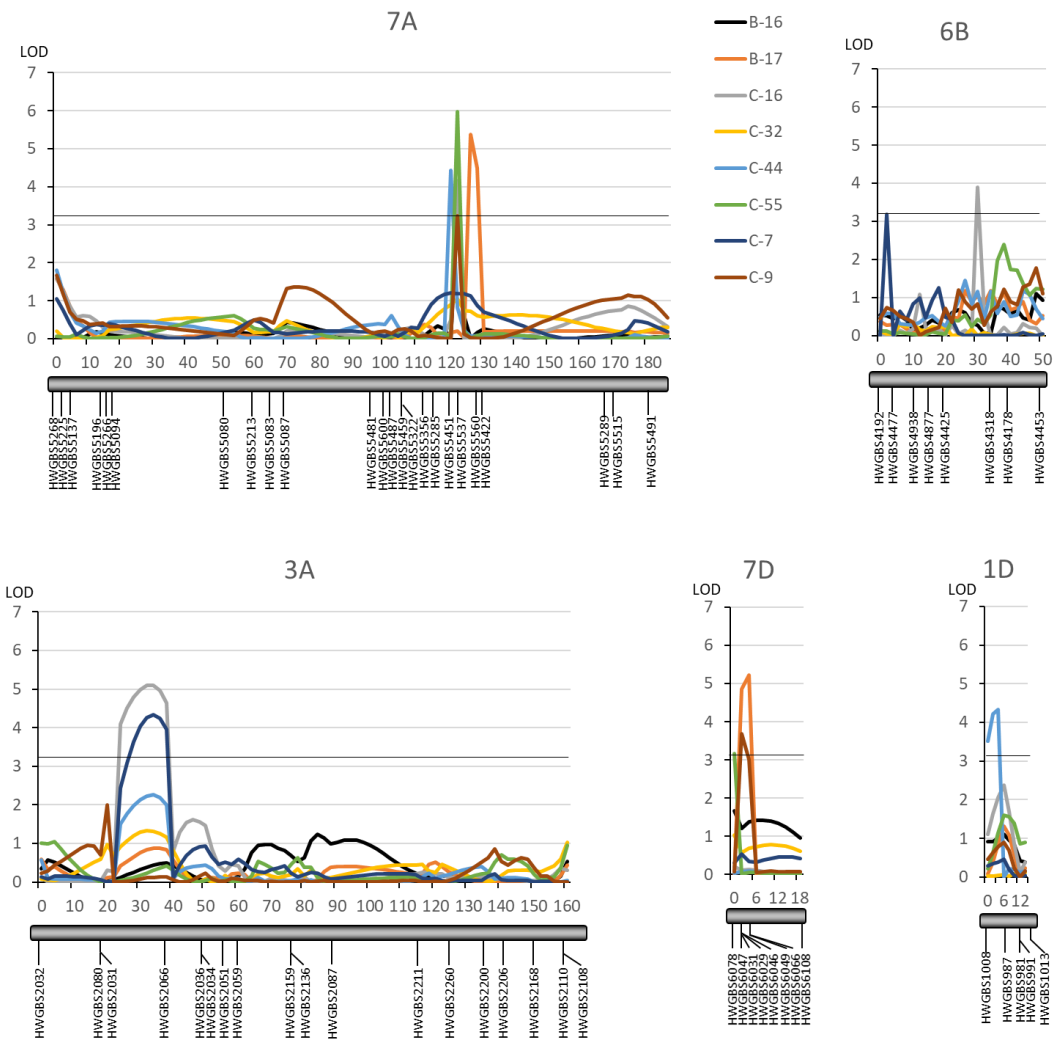

Figure 3.4. Single trait multiple interval mapping of QTLs on chromosomes 1D, 3A, 6B, 7A and 7D in the HW population that are associated with a reaction to the *Ptr* isoaltes that do not produce any of three known necrotrophic effectors. QTL lines were drawn for B-16 (black), B-17 (orange), C-7 (dark blue), C-9 (red), C-16 (grey), C-32 (yellow), C-44 (light blue) and C-55 (green). The black bars represent individual linkage groups with marker loci shown on the bottom and a genetic position in cM is shown on the top. A dash line represents the logarithm of the odds (LOD) significance threshold of 3.2. The LOD and R^2 value for each QTL is presented in Table 3.3.

3.5. Discussion

The tan spot disease is well known to involve three fungal-produced NE-host sensitivity gene interactions including Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2*, and Ptr ToxC-*Tsc1* (reviewed in Ciuffetti et al. 2010). Many studies have shown that all three interactions can play a significant

role in the development of tan spot in diverse wheat germplasm and populations (Faris et al. 2013; Virdi et al. 2016; Kariyawasam et al. 2016; Liu et al. 2017). However, several lines of evidence have suggested that the disease system is not only based on these three NE-sensitivity gene interactions. Friesen et al. (2003) developed a mutant at the *Tsn1* gene using ethyl methanesulfonate (EMS) treatment and found that even though the *tsn1* mutant is insensitive to Ptr ToxA, it was still highly susceptible to 86-124, which is only known to produce Ptr ToxA (Friesen et al. 2003). Even though some isolates of races 3 and 5 are known to produce only chlorosis-inducing NE Ptr ToxB, they were found to induce necrosis on specific common and durum wheat genotypes (Gamba et al. 1998; Singh et al. 2008, 2010). In a study to compare the difference in virulence between a race 1 isolate and its *ToxA* mutant, See et al. (2018) did not observe any significant disease reduction on some Australian wheat varieties. Many QTL mapping studies have identified genomic regions other than three known sensitivity loci (*Tsn1*, *Tsc1*, and *Tsc2*) (reviewed in Faris et al. 2013; Virdi et al. 2016; Liu et al. 2017). All these strongly suggested that the disease system involves additional NE-sensitivity gene interactions or other types of interactions. In this study, we used eight fungal isolates that produce no known NEs and showed that they still caused disease on some durum and common wheat lines. We also identified host genetic factors that interact with the unknown NEs in those isolates using host QTL mapping. Our work further demonstrates that the fungal pathogen produces additional NEs or other types of virulence factors besides the three known ones that interact with the host factor.

Our work also demonstrates that the current race classification system is not sufficient. In the current system, race 4 does not produce any known NEs, which means it is avirulent to all the wheat lines in the differential set. All the eight isolates do not produce any of the known NEs. Therefore, by definition, it should be classified as race 4. However, our pathogenicity tests

showed they still caused disease on Glenlea as well as ND495 even though they had a lower level of virulence compared to 86-124 and DW5. A few studies have also identified new isolates that cannot be classified using the current race system (Ali et al. 2010; Moreno et al. 2015; Abdullah et al. 2017a, b). To solve the problem, the new NEs or virulence factors and their corresponding host genetic factors need to be identified. The tan spot differential set should be expanded to include the lines harboring the host genetic factor(s) that interact with the new NE(s) or virulence factor(s). Using QTL mapping, we identified six wheat genomic regions that are associated with a reaction to these race 4 isolates. These genomic regions must harbor the host genes that could directly or indirectly interact with the new NE(s) or virulence factor(s) in those isolates. After these QTLs are confirmed in diverse genetic backgrounds, the RILs from the HW population, which harbor the individual QTL, could be used in a differential set to detect the new NE.

A total of six genomic regions were identified harboring QTLs and some of them are shared for at least two isolates but others are unique to specific isolate. For example, *QTs-7AL* was identified for six of the eight isolates, while *QTs-1DS* was detected for only C-44. We observed the recombination and segregation of the *ToxA* and *ToxB* genes in the progeny derived from the cross between 86-124 and DW5 (Ameen et al. 2017, Chapter 2). Likewise, the other unidentified NEs or virulence factors would also segregate in the population if they were polymorphic between 86-124 and DW5. Although those isolates lack both *ToxA* and *ToxB* genes, they could differ in the presence of other NEs, which leads to the identification of different set QTL in the HW population. A total of eighteen isolates were obtained from the cross, which do not have neither the *ToxA* nor *ToxB* gene. It would be interesting to test all these isolates onto the

HW population or other segregating populations. By doing that, we can potentially catalog all the new NEs that are segregating in the progeny from the 86-124 × DW5 cross.

In addition to the NE sensitivity loci *Tsn1* on 5BL and *Tsc2* on 2BS, QTL associated with tan spot disease have been mapped to other wheat chromosomes or regions for both 86-124 and DW5 using bi-parental or association mapping (Chu et al. 2010; Gurung et al. 2011; Faris et al. 2012; Liu et al. 2015, 2017; Virdi et al. 2016; Kariyawasam et al. 2016). By using the HW population, Kariyawasam et al. (2018) identified two QTLs for 86-124Δ*ToxA* (producing no known NE), one being on 7AL and the other on 7DL. However, these QTLs were not identified for 86-124. This is may be due to epistasis of Ptr ToxA-*Tsn1* interaction over NE-sensitivity interaction. Six of eight isolates had a QTL on 7AL and three of them had a QTL on 7DL which are likely same as the one identified for 86-124Δ*ToxA*. These QTLs interact with new NEs that are probably presented in 86-124, but not in DW5 because these QTLs were not identified for DW5 (Kariyawasam et al. 2018).

We identified a QTL on 3A for two isolates which was located between 24-38 Mbp. Many studies have already identified genes or QTLs on 3A. Singh et al. (2008) reported a QTL (*QTs.ksu.3AS*) around 54 Mbp position conferring resistance to *Ptr* race 1 using a hexaploid wheat population. Chu et al. (2010) identified a QTL(*QTs.fcu-3A*) around the 11-16 Mbp region in a tetraploid population. A recessive resistance gene *Tsr4* was located on 3A around 60Mbp based on the closely linked marker *Xgwm2* (Tadesse et al. 2006; 2010). Liu et al. (2020) published three overlapped QTLs on 3A from tetraploid wheat using 86-124 and 86-124Δ*ToxA*, which are located between 7.4-26.2 Mbp. The 3A QTL we identified is likely same as the one reported by Liu et al. (2020), because the same isolates were used, and positions were close. However, Kariyawasam et al. (2018) didn't detect any QTL on 3A from the HW population

when using the 86-124 and 86-124Δ*ToxA*. It is possible that the effect of this 3A QTL was masked by others like the case of epistasis of Ptr ToxA-*Tsn1* interaction. In C-7 and C-16 isolates where the QTL is detected, the epistasis is probably not present due to the segregation in the population.

In this work, two QTLs with minor effect were identified on 6BS. There were some QTLs previously reported on 6B (Dinglasan et al. 2019; Singh et al. 2008, Zou et al. 2017). Early in 2008, Singh et al. reported a QTL (*QTs.ksu-6BS*) in spring wheat. Based on the closed linked marker *Xbarc198*, the QTL was around 279 Mbp. From spring wheat, Zou et al. (2017) identified another QTL (*Qts.dms-6B*) in the 55-61 Mbp region to race 1 isolate. Recently, Dinglasan et al. (2019) detected tan spot resistance QTLs on 6B in the Vavilov wheat collection through GWAS, which located around 705 Mbp. Our QTLs should be different from theirs because the positions are quite far away. *QTs-6BS.1* and *QTs-6BS.2* could be the same QTLs due to in a proximate region. The *QTs-1DS* for C-44 is likely same as the one reported by Gurung et al. (2011) because they are located in the same region.

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CHAPTER 4. PYRENOPHORA TRITICI-REPENTIS RACE 4 ISOLATES CAUSE DISEASE ON TETRAPLOID WHEAT

4.1. Abstract

The ascomycete fungus *Pyrenophora tritici-repentis* is the causal agent of tan spot of wheat. The disease can occur on both common wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* ssp. *durum*) and has potential to cause significant yield and quality losses. The fungal pathogen is known to produce necrotrophic effectors (NEs) that act as important virulence factors. Based on the NE production and virulence on a set of four differentials, *P. tritici-repentis* isolates have been classified into eight races. Race 4 produces no known NEs and is avirulent on the differentials. From a fungal collection in North Dakota, we identified several isolates that were classified as race 4. These isolates caused no or little disease on all common wheat lines including the differentials; however, they were virulent on some durum cultivars and tetraploid wheat accessions. Using two segregating tetraploid wheat populations and QTL mapping, we identified several genomic regions significantly associated with disease caused by two of these race 4 isolates. Some QTL have not been previously reported. This is the first report that race 4 is virulent on tetraploid wheat, likely utilizing unidentified NEs. Our findings further highlight the insufficiency of the current race classification system for *P. tritici-repentis*.

4.2. Introduction

Tan spot is caused by the necrotrophic fungal pathogen *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoem.). This disease was first reported in the United States in the 1940s (Barrus 1942), but it was rarely observed until the 1970s when no-tillage practices were widely adopted (Hosford 1982; Rees and Platz 1992; reviewed in Faris et al. 2013). The typical symptoms on susceptible cultivars include tan-colored

necrotic lesions, which are often surrounded by chlorotic halos or extensive chlorosis. These symptoms are associated with fungal-produced necrotrophic effectors (NEs), previously known as host-selective toxins (HSTs) (Wolpert et al. 2002; reviewed in Faris et al. 2013). In the last several decades, the disease has become one of the economically important foliar diseases in all major wheat growing regions of the world (reviewed in Faris et al. 2013). The fungal infection damages leaf tissues reducing photosynthesis activity, which can result in significant yield losses. Yield losses of nearly 50% have been reported in Australia (Rees et al. 1982). The disease can also cause red smudge on wheat kernels, particularly in durum wheat, which can lead to downgrading of wheat grains (Fernandez et al. 1996).

Outbreaks of tan spot were thought to be attributed by the adoption of conservation agriculture practices to reduce erosion and maintain soil fertility. Crop rotation, residue management, and fungicide application can be integrated to manage tan spot of wheat. However, cultural practices and fungicide applications are not always effective. For example, it has been documented that *P. tritici-repentis* isolates have developed resistance to commonly used fungicides (Murray and Brennan 2009). The most cost effective and environmentally friendly way is to use resistant cultivars (Faris et al. 2013; Friskop and Liu 2016).

The wheat-*P. tritici-repentis* pathosystem follows an inverse gene-for-gene model where NEs produced by the pathogen interact with the products of the host sensitivity genes to induce necrosis or chlorosis leading to disease. Therefore, these NEs act as important virulence factors. To date, three NEs have been identified from *P. tritici-repentis*, namely Ptr ToxA, Ptr ToxB, Ptr ToxC, and the wheat genes interacting with them are *Tsn1*, *Tsc2* and *Tsc1*, respectively (reviewed in Ciuffetti et al. 2010; Faris et al. 2013). Ptr ToxA is a small protein (~13.2 kDa) that induces necrosis, and it is encoded by the single copy gene *ToxA* (Balance et al. 1996; Ciuffetti et al.

1997; Manning et al. 2013). Very interestingly, this NE is also produced by other two wheat fungal pathogens, *Parastagonospora nodorum* and *Bipolaris sorokiniana,* to cause disease on wheat upon recognition by the product of the *Tsn1* gene (Friesen et al. 2006, 2018; Liu et al. 2006; McDonald et al. 2018; Navathe et al. 2020). It was shown that a genomic segment containing the *ToxA* gene was transferred from one fungal pathogen to another through horizontal gene transfer events (Friesen et al. 2006; McDonald et al. 2018). Ptr ToxB is also a small protein, but different from Ptr ToxA. It induces chlorosis and has a smaller molecular size (6.61 kDa). The fungal gene encoding Ptr ToxB, *ToxB,* has been cloned and was shown to be present in multiple copies (Martinez et al. 2001, 2004; Amaike et al. 2008; Strelkov et al. 2002). The *ToxB* gene is also present in *P. bromi*, a fungus causing leaf disease on grasses (Andrie et al. 2008). Some races of *P. tritici-repentis* also carry a nonfunctional form of *ToxB*, designated *toxb* (Amaike et al. 2008; see below). Ptr ToxC is a chlorosis-inducing NE and was initially characterized as a nonionic, polar, low molecular weight secondary metabolite (Effertz et al. 2002). The chemical structure of Ptr ToxC and fungal gene responsible for its production remains unknown.

The current classification system defines eight races for *P. tritici-repentis*, which is largely based on their ability to produce individual or a combination of the three NEs. This correlates with their virulence on a set of four common wheat lines including Salamouni (universally resistant), Glenlea (sensitive to Ptr ToxA), 6B662 (sensitive to Ptr ToxB) and 6B365 (sensitive to Ptr ToxC) (Lamari et al. 2003). Two durum lines, Coulter (sensitive to Ptr ToxA) and 4B1149 (insensitive to all NEs), are sometimes included in the differential set (Lamari et al. 1995), but they do not increase the power to describe additional races. Races 2, 3 and 5 produce only one known NE, Ptr ToxA, Ptr ToxC and Ptr ToxB, and are virulent only on the individual

differentials Glenlea, 6B365 and 6B662, respectively. Race 1 combines virulence of races 2 and 3, race 6 combines virulence of race 3 and 5, and race 7 combines virulence of races 2 and 5. Race 8 produces all three known NEs. In contrast, race 4 produces no known NE and is avirulent to all the differentials. Using this classification system, the *P. tritici-repentis* populations from different geographic locations have been characterized (Sarova et al. 2005; Gamba et al. 2012; Aboukhaddour et al. 2013; reviewed in Ciuffetti et al. 2014; Abdullah et al. 2017a; Bertagnolli et al. 2019). Races 1 and 2 were found to be predominant in North and South America, and Australia, and relatively complicated race structures were found in the Middle East, North Africa and Caucasus regions (Kamel et al. 2019). In addition, there were isolates identified in several studies that did not conform to the current race classification system (Ali et al. 2002, 2010; Benslimane et al. 2018; Kamel et al. 2019).

Race 4 was first described as pathotype 4 (nec- chl-), which did not produce any necrosis and chlorosis symptom on wheat differentials (Lamari and Bernier 1989b; Lamari et al. 1991, 1995). However, a cytological investigation showed that the isolates of this race can penetrate wheat epidermal cells and reach mesophyll cells where it halts growth (Lamari et al. 1991). This race has been shown to be rare among many *P. tritici-repentis* collections from wheat (Lamari and Bernier 1989b; Lamari et al. 1998; Ali and Francl 2003; Sarova et al. 2005; Benslimane et al. 2011; Kamal et al. 2019). However, collections from noncereal grasses had a large portion of race 4 isolates (Ali and Francl 2003). Although race 4 does not produce any known NEs and is avirulent to the differentials, it carries *toxb*, which is 86% similar to *ToxB* and can be transcribed at a very low level (Amaike et al. 2008; Martinez et al. 2004).

A collection of *P. tritici-repentis* isolates was obtained from wheat fields in North Dakota and further characterized the race structure in the state. Race typing on differential lines and

molecular characterization have shown that a few isolates likely belong to race 4 (Z. Liu, *unpublished data*). These isolates couldn't produce any necrosis or chlorosis on all differentials. However, they can cause disease on some tetraploid wheat lines in our preliminary evaluations. The objectives of this study were to confirm their race 4 identity, determine their virulence on a set of common wheat and durum cultivars, and map genomic regions conferring resistance/susceptibility to two of these race 4 isolates.

4.3. Materials and Methods

4.3.1. Fungal isolates

Five isolates from previous field survey were initially characterized as race 4 based on the phenotypes they exhibited on tan spot differential lines (Z. Liu, *unpublished data*). Those isolates were designated as Ls13-14, Ls13-78, Ls13-86, Ls13-192 and Ls13-198. The first three were collected in the North Dakota counties Mountrail, Kidder and Wells, respectively, and the last two were collected in Barnes county. Those isolates were subjected to molecular characterization, pathogenicity tests and genetic mapping of host factors conditioning resistance or susceptibility. Three isolates with known race identity including Pti2 (race 1), 86-124 (race 2) and DW5 (race 5) were also included as references in the experiments.

4.3.2. Molecular characterization

Genomic DNA of the above mentioned five isolates was extracted according to the protocol described by Shjerve et al. (2014). These isolates were first checked for their species identity by sequencing the internal transcribed spacer (ITS) regions. The ITS regions were amplified with the universal primers ITS4 and ITS5 (Table 4.1, White et al. 1990) which could amplify the partial 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and partial 28S rRNA gene. The PCR amplifications were performed with 10 ng of DNA template in a total volume of 20 µL

containing 1× buffer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 0.5 µM of each forward and reverse primer, and 2U of Bullseye Taq DNA polymerase (MIDSCI, St. Louis, MO). PCRs were performed with the followed cycling conditions: initial denaturation at 95 ºC for 5 min followed by 35 cycles of 95 ºC for 30s, 60 ºC for 30s, and 72 ºC for 1 min. A final elongation step was performed at 72 ºC for 5 min. PCR products were separated on 1% agarose gels in 1 × TAE buffer (0.04 M Tris-acetate, pH 8.0 and 0.001 M EDTA) and purified with PureLink™ PCR Quick PCR Purification Kit (Invitrogen) following the supplier's recommendation. The purified products were sequenced with the primers IT4 and ITS5 at the facility of Eurofins Genomics USA Inc. (Louisville, KY). The obtained sequences were compared to each other and blasted against the NCBI nonredundant database using BLASTn.

After these isolates were confirmed to be *P. tritici-repentis*, they were tested for presence of the *ToxA* and *ToxB* genes with published primer pairs (Table 4.1). Mating type gene *MAT1-1-1* was also included as a control for DNA quality and quantity (Ameen et al. 2017). In the PCR amplification, 86-124 (race 2, carrying *ToxA*) and DW5 (race 5, carrying *ToxB*) were used as positive controls for the *ToxA* and *ToxB* genes, respectively. The obtained PCR products were purified and sequenced as described above. Upon learning that these isolates contain *toxb*, we downloaded the sequence of a genomic region spanning *toxb* from GenBank (Accession: AY083456.2, Martinez et al. 2004) and designed a new pair of primers (toxbF/toxbR, Table 4.1) to amplify the full length *toxb* open reading frame (ORF) from these isolates. The *toxb* sequences were aligned with each other as well as the reference *toxb* sequence using MultAlin program (http://multalin.toulouse.inra.fr/multalin/).

Table 4.1. List of primers used in chapter 4 study

Primers	Sequences (from 5' to 3')	Purpose	Reference
PtrPLP2	CAGAACAAAGGCAGGACTGTGAGC	Amplify the *MAT1-1-1*	Lepoint et al. 2010
PtrPLP4	ATGCGCTCAGCAAGGAAGGTCG		
TA51F	GCGTTCTATCCTCGTACTTC	Amplify the *ToxA*	Andrie et al. 2007
TA52R	GCATTCTCCAATTTTCACG		
TB71F	GCTACTTGCTGTGGCTATC	Amplify the *ToxB*	Andrie et al. 2007
TB6R	ACGTCCTCCACTTTGCACACTCTC		
toxbF	TGTTTAATCTTATACTAATCCCTTG	Amplify the *toxb*	
toxbR	TCTTGTAGCGCCTAAACTCT		
ITS4	TCCTCCGCTTATTGATATGC	Amplify the ITS	White et al. 1990
ITS5	GGAAGTAAAAGTCGTAACAAGG		

4.3.3. Plant materials and disease evaluation

The current tan spot differential lines including Salamouni, Glenlea, 6B365 and 6B662 were initially used for race typing. Pathogenicity tests of these isolates were then conducted on a set of thirty wheat lines, the majority of which were hard red spring (HRS) wheat and durum wheat cultivars currently planted in North Dakota (Table 4.2). Disease evaluations were carried out in the greenhouse and growth chambers under controlled conditions. Seeds of these lines were planted in Super cell cones (Stuewe and Sons, Inc., Corvallis, OR) with three seeds per cone. The four differential lines mentioned above were also included in the evaluations. Plants were grown in Sunshine SB100 soil (Sun Gro Horticulture, Bellevue, WA) with a half teaspoon of the fertilizer Osmocote Plus 15-9-12 (Scotts Sierra Horticultural Product Company, Maysville, OH) applied to each cone after planting. The cones were placed onto RL98 racks. The border cones of each rack were planted with the highly susceptible winter wheat cultivar 'Jerry' to reduce the edge effects (Liu et al. 2015). Pathogenicity tests were conducted for four isolates (Ls13-14, Ls13-86, Ls13-192 and Ls13-198) along with Pit2, DW5 and 86-124 used as controls. Ls13-78 did not produce enough spores for pathogenicity test. For this experiment, three biological replications were carried out with a randomized complete block design (RCBD).

To identify genomic regions associated with resistance/susceptibility to tan spot caused by the race 4 isolates, we conducted disease evaluations of two isolates (Ls13-14 and Ls13-192) on two tetraploid populations. The first population was derived from the cross between Ben and PI 41025 and referred as BP025, which consisted of 200 recombinant inbred lines (RILs) (Zhang et al. 2014). Ben is a North Dakota hard amber durum variety released in 1998 (Elias and Miller 1998), and PI 41025 is a cultivated emmer wheat (*T. turgidium* ssp. *dicoccum*) accession collected near Samara, Russia. The second population, referred as RP336, was derived from a cross between the durum line Rusty and the tetraploid wheat *T. turgidum* ssp. *turgidum* accession PI 387336, which consisted of 186 RILs (Liu Y. et al. 2020a). In this experiment, disease evaluations included all the RILs and their parental lines from each population as well as the four differential lines. Three biological replications were included with a RCBD arrangement. The planting and growing plants followed the same procedures as described above.

The fungal culturing, inoculum preparation and inoculation methods mainly followed standard protocols described in Liu et al. (2015). Briefly, the fungus was grown on V8-PDA medium for five days under dark followed by mycelium flattening and a light-dark incubation cycle. Conidia were washed from the culture plates to make spore suspension. The spore concentration was adjusted to approximately 3000 spores/ml and two drops per 100 ml of the surfactant reagent Tween-20 were added before inoculation. Plants at the two to three-leaf stage were inoculated using an air-pressured spray gun. After inoculation, plants were kept in a mist chamber with 100% humidity for 24h. Then, the plants were transferred to a growth chamber with 12-hour photoperiod at 21 °C. Disease reactions were scored on the 7th day post inoculation using a 1-5 rating scale described by Lamari and Bernier (1989a). We added 0 to the scale when

no obvious reaction was observed. Disease scores lower than 2.0 were considered as being resistant.

Table 4.2. Reaction of wheat genotypes to tan spot caused by different *Pyrenophora tritici-repentis* race/isolate.

Wheat type	Genotypes	Agent or Origin[a]	Year Released	Race 4[b] Ls13-14	Ls13-192	Ls13-86	Ls13-198	Race 5[b] DW5	Race 1[b] Pti2	Race 2[b] 86-124
Common wheat	Barlow	ND	2009	0.3	0.3	0.8	0.0	3.8	4.5	2.0
Common wheat	Brennan	AgriPro	2009	0.3	0.4	0.5	0.5	1.5	3.0	2.0
Common wheat	Glenn	ND	2005	0.3	0.9	0.5	0.0	4.0	3.0	2.5
Common wheat	Howard	ND	2000	0.3	0.7	0.3	0.0	3.5	4.0	3.0
Common wheat	Jenna	AgriPro	2009	0.2	0.4	0.0	0.0	1.0	2.5	1.5
Common wheat	Kelby	AgriPro	2006	0.2	0.8	0.5	0.0	2.5	2.5	2.0
Common wheat	Mott	ND	2007	0.8	1.3	0.8	1.0	3.0	3.5	3.5
Common wheat	Prosper	ND	2011	0.5	0.6	0.3	0.0	4.0	4.0	3.0
Common wheat	RB07	MN	2007	0.2	0.5	0.3	0.0	2.0	2.5	1.5
Common wheat	Rollag	MN	2011	0.0	0.5	0.8	0.0	4.5	2.8	3.0
Common wheat	Select	SD	2010	0.2	0.9	0.8	0.3	4.3	4.5	2.5
Common wheat	Steele-ND	ND	2004	0.5	1.0	0.5	0.3	4.3	3.5	3.5
Common wheat	SY Soren	Syngenta/AgriPro	2011	0.2	0.6	0.5	0.5	2.5	1.8	1.0
Common wheat	SY Tyra	Syngenta/AgriPro	2011	0.0	0.7	0.3	0.0	1.8	3.5	1.0
Common wheat	Velva	ND	2011	0.2	0.8	0.0	0.0	3.3	2.3	1.0
Common wheat	Chinese spring	China	-	0.3	0.3	0.5	0.0	1.8	2.0	5.0
Common wheat	Vantage	WestBred	2007	0.2	1.0	1.3	0.3	2.0	3.5	2.0
Common wheat	Briggs	SD	2002	0.2	0.6	0.5	0.0	2.0	3.5	3.0
Means of disease reaction for common wheat				0.3	0.7	0.5	0.2	2.9	3.2	2.4
Durum wheat	Alkabo	ND	2005	0.5	1.5	1.0	1.8	2.5	1.8	2.0
Durum wheat	Ben	ND	1996	3.8	3.4	3.3	2.8	4.5	3.0	4.0
Durum wheat	Carpio	ND	2012	2.5	2.2	0.5	2.0	3.5	2.8	2.5
Durum wheat	Dilse	ND	2002	3.5	3.3	3.3	3.0	3.8	2.5	3.5
Durum wheat	Divide	ND	1994	3.0	3.1	2.3	2.8	4.5	2.0	3.5
Durum wheat	Grenora	ND	2006	3.2	3.2	2.5	2.8	4.0	3.5	3.0
Durum wheat	Lebsock	ND	1999	3.3	3.3	2.8	3.0	4.5	2.0	2.5
Durum wheat	Langdon	ND	1956	1.7	2.4	0.8	1.8	4.5	4.5	4.0
Durum wheat	Mountrail	ND	1998	2.5	3.0	1.5	2.0	4.5	1.5	3.0
Durum wheat	Pierce	ND	2001	3.2	3.1	2.5	2.8	4.5	2.0	4.0
Durum wheat	Rusty	ND	2004	1.5	1.7	1.0	0.0	4.5	1.5	2.5
Durum wheat	Tioga	ND	2010	3.3	2.6	2.5	3.0	4.0	2.0	2.5
Emmer wheat	PI 41025	Russia	-	1.2	0.5	-	-	-	-	-
Poulard wheat	PI 387336	Ethiopia	-	3.3	3.2	-	-	-	-	-
Means of disease reaction for tetraploid wheat				2.6	2.6	2	2.3	4.1	2.4	3.1
[c] Mean difference between Common wheat and durum groups Pr > |t|				<.0001	<.0001	<.0001	<.0001	<.0001	0.0163	0.001

[a] Indicates private companies or public breeding programs which developed the cultivars, MN=University of Minnesota; ND=North Dakota State University; SD=South Dakota State University, or the place the genotype came from.

[b] Disease was scored using 1 to 5 scale where 1 being resistant and 5 being susceptible. Average of three replicates were given as the final score.

[c] One way ANOVA was used to analyze the difference in disease means of common wheat and durum wheat. Difference is significant at value of Pr > |t| <0.05.

4.3.4. Statistical analysis

In pathogenicity tests, the difference between durum and common wheat groups was analyzed using the Wilcoxon test in PROC NON-PARAMETRIC in SAS 9.4 Software (SAS Institute, 2016). After the disease data was obtained for the two populations, normality of the disease data for each isolate/replication was evaluated using the Shapiro-Wilk test in PROC UNIVARIATE in SAS 9.4 Software (SAS Institute, 2016). If the data fit a normal distribution, it was tested for homogeneity using Bartlett's chi-square test (Snedecor and Cochran 1989). If the data did not fit a normal distribution, Levene's test was used to test the homogeneity among different replicates (Levene 1960). The data from homogeneous replications were combined and the means were used for QTL analysis.

4.3.5. QTL analysis

QTL analysis deployed the genetic linkage maps of the two populations that have been published previously (Liu Y. et al. 2020a, b). BP025 was genotyped several times through different methods including SSR analysis, Illumina iSelect 9K SNPs array and genotyping-by-sequencing (GBS). The genetic linkage map for this population contained a total of 4,883 markers (96 SSRs, 878 9k and 3,909 GBS) with a total length of 4,749.6 cM covering all 14 chromosomes. The genetic map of the RP336 population contained 2,894 GBS markers and had a total length of 2,858 cM covering all 14 chromosomes. QTL mapping was conducted using QGene 4.4.0 (Joehanes and Nelson 2008). Composite interval mapping (CIM) performed with forward cofactors selection was used to identify the genomic region associated with tan spot reaction and to quantify the disease variations explained by the QTL (R^2). A permutation test with 1,000 iterations resulted in a LOD threshold of 4.2 for an experiment-wise significance level of 0.05. To find out whether the QTLs detected in this study possibly represent known

genes or genetic loci, the physical positions and the markers linked were compared. The physical locations of markers were obtained using marker sequences as queries in BLASTn searches of the IWGSC Chinese Spring reference genome (v1.0).

4.4. Results

4.4.1. Species and race confirmation

Inoculation of all four isolates (Ls13-14, Ls13-86, Ls13-192 and Ls13-198) on four differentials (Salamouni, 6B662, 6B365 and Glenlea) produced no or little disease. Most of reactions were observed as pin-sized black dots either alone or surrounded by small amount of chlorosis (Figure 4.1). In particularly, nearly no reaction could be seen on the universal resistant line Salamouni. However, a large amount of necrosis or chlorosis was induced by three control isolates Pti2 (race 1), DW5 (race 5) and 86-124 (race 2) on their differential lines. These results indicated that these four Ls13 isolates belonged to race 4.

Partial ITS sequence (436 bp) was amplified and sequenced from all five isolates. 436 bp sequence was obtained after removal of low-quality base readings and they were all identical in five isolates. BLASTn searches showed that these sequences were completely identical to the published ITS sequences of *P. tritici-repentis* strains 90-2T (AM887495) (Lepoint et al. 2010), 90-2 (EF452479) (Andrie et al. 2008) and SY3-122 (AY739819) (Friesen et al. 2005), all of which were characterized as *P. tritici-repentis* race 4. ITS sequence of these five isolates has been deposited into GenBank with accession numbers MN855580 to MN855584.

All all isolates lacked the *ToxA* gene, but they produced a weak amplification (~250 bp) using the *toxb* primer set TB71F/TB6R (Figure 4.2). These weakly amplified fragments were sequenced and confirmed to be the *toxb* sequence. Using a new pair of primers (toxbF and toxbR, Table 4.1), we amplified the full-length fragment of the *toxb* gene from all five isolates.

Sequence analysis indicated that the *toxb* ORF (267 bp) was identical among all five isolates (Supporting file) and have been deposited in NCBI GenBank (MN864562-864566). BLASTn analysis using the full-length sequence indicated that the *toxb* sequence of these five isolates was 100% identical to that of the race 4 isolate SD20 (AY083456.2) (Martinez et al. 2004), and 99% identical to that of the race 4 isolate 90-2 (AF483832) (Strelkov and Lamari 2003). The sequence was 87% similar to the *ToxB* gene sequence from the race 5 isolate DW7 (AY425484) (Martinez et al. 2004). Therefore, these results provided further evidence that these isolates are race 4.

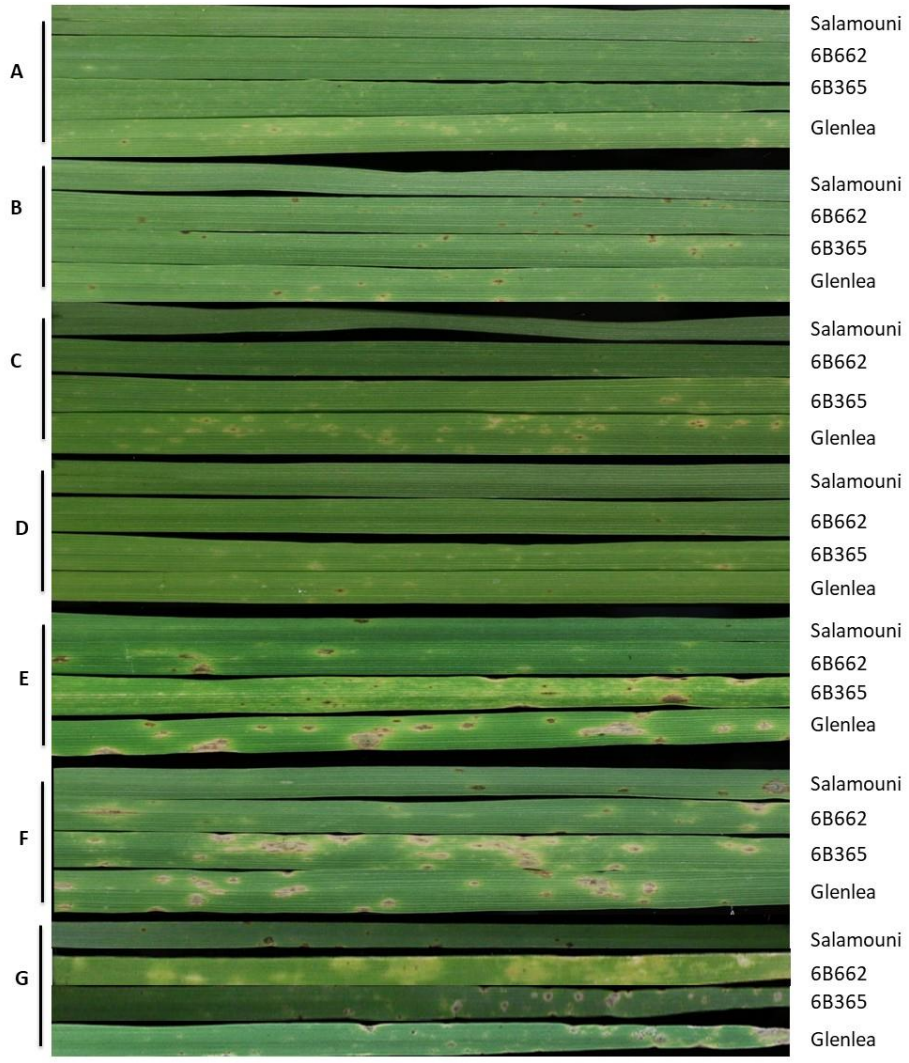

Figure 4.1. Reaction of wheat differential lines to tan spot caused by *Pyrenophora tritici-repentis* race 4 isolates and other known races. A: Ls13-192; B: Ls13-86; C: Ls13-14; D: Ls13-198; E: Pti2 (race 1); F: 86-124 (race 2); G: DW5 (race 5).

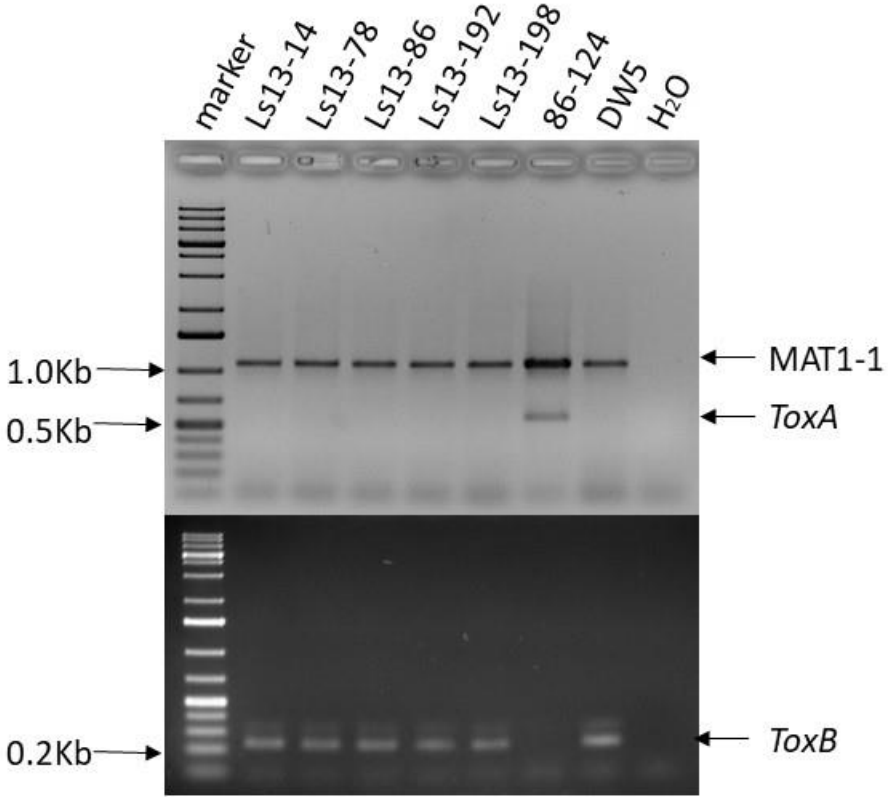

Figure 4.2. PCR testing of the presence of the *ToxA* and *ToxB* genes in race 4 isolates. *ToxA* was multiplexed with *MAT1-1*. 86-124, DW5 and H_2O were included in the PCR as controls. Arrows indicate size of DNA markers and the individual bands. (DNA ladder name is listed in material or here). It's better to use pictures with same background; Picture for ToxB amplification is not good enough, why there are so many faint bands in background.

4.4.2. Pathogenicity tests

The results of pathogenicity test of four isolates on thirty wheat lines were shown in Table 4.2. All HRS wheat cultivars had disease means less than 1.0 for all four isolates indicating nonpathogenic or avirulent reactions of these race 4 isolates on common wheat genotypes. The average disease rating was 0.26 for Ls13-14, 0.68 for Ls13-192, 0.49 for Ls13-86 and 0.15 for Ls13-198. Most HRS wheat cultivars, such as Jenna, Steele-ND and Vantage, developed no visible symptom or black pinpoint lesions on the leaves, whereas other cultivars such as Mott and Prosper developed small and restricted necrotic lesions (Figure 4.3). For the reference

isolates Pti2, DW5 or 86-124, all HRS cultivars except Jenna and SY Soren showed highly susceptible or susceptible reactions.

For tetraploid wheat, the disease means ranged from 0.5 (e.g. Ls13-14 on Alkabo) to 3.8 (Ls13-14 on Ben) indicating variable reactions to these race 4 isolates (Table 4.2). Some of the reactions were characterized as susceptible because their disease means were greater than 3.0. The average disease ratings were 2.66 for Ls13-14, 2.73 for Ls13-192, 1.98 for Ls13-86 and 2.29 for Ls13-198. The disease reactions were observed as large necrotic lesions surrounded by yellow margin on highly susceptible lines (Figure 4.3). This indicates that these race 4 isolates can cause disease on some tetraploid genotypes. For reaction to the reference isolates, most of durum cultivars were highly susceptible to DW5 (race 5), but less susceptible to the race 1 and 2 isolates.

A Wilcoxon test conducted between tetraploid and hexaploid wheat groups indicated a significant difference ($P < 0.0001$) for all race 4 isolates as well as DW5 (race 5) between the two groups. Although the two groups were significantly different in their reactions to races 1 and 2, the *p* values were less significant (Table 4.2).

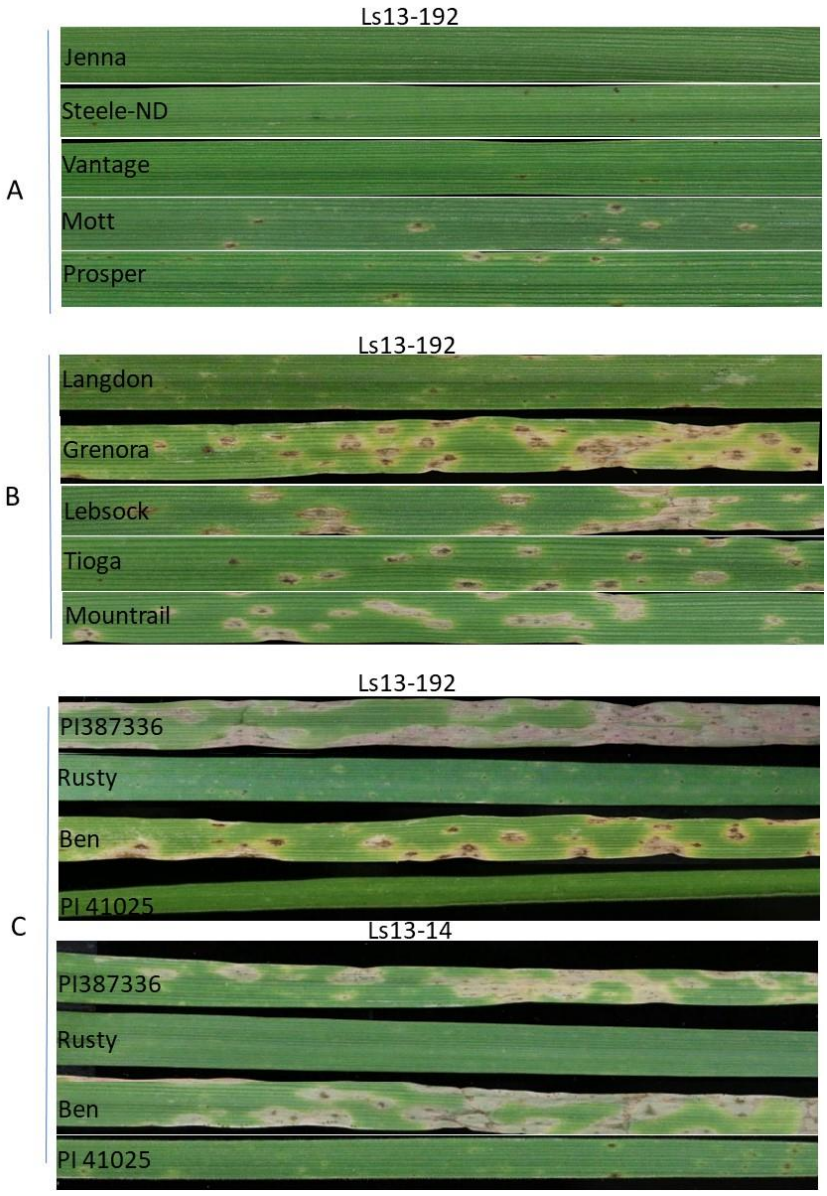

Figure 4.3. Reaction of representative wheat lines to fungal inoculation with race 4 isolates. A. Common wheat lines inoculated with Ls13-192; B. Durum wheat lines inoculated with LS13-192; C. The parental lines for two tetraploid population inoculated with Ls13-192 and Ls13-14. Disease scores for all wheat genotypes to different *Pyrenophora tritici-repentis* race/isolates are listed in Table 4.2.

4.4.3. QTL identification

Based on the results from pathogenicity test, Ben and PI 41025 as well as Rusty and PI 387336 showed great difference in reaction to the four race 4 isolates we identified (Table 4.2,

Figure 3.3). Therefore, the BP025 and RP336 populations were used to identify genomic regions associated with reaction to tan spot caused by the race 4 isolates Ls13-14 and Ls13-192 on each population. Normality tests rejected a normal distribution of the disease reaction to both isolates ($p < 0.05$). Homogeneity analysis with Levene's test indicated that the variance among the replicates for each isolate were not significant (p=0.12-0.44). Therefore, the means of three biological replicates for each isolate were used in subsequent data analysis and QTL identification. Both populations segregated for reaction to the two isolates exhibiting a range from highly resistant to highly susceptible reactions, and no obvious transgressive segregation was observed (Figure 4.4).

Six QTLs were identified in BP025 with three for each isolate (Table 4.3). The QTLs were distributed on chromosomes 1A (designated as *BP-ls192-1A* and *BP-ls14-1A*), 4B (*BP-ls192-4B* and *BP-ls14-4B*) and 5A (*BP-ls192-5A* and *BP-ls14-5A*) (Table 4.3, Figure 4.5). The resistance alleles for all the QTLs were contributed by PI 41025. The LOD values for these QTLs ranged from 4.8 to 13.0 and they explained from 11 to 26% of the variation in disease. The 5A QTL for two isolates (*BP-ls192-5A* and *BP-ls14-5A*) co-localized to the same physical interval (660 Mb to 672 Mb) whereas the 1A and 4B QTLs for the two isolates were located to different intervals. The 5A and 4B QTLs had slightly higher effect on disease compared to the 1A QTL (Table 4.3).

For the RP336 population, two QTLs (*RP-ls192-5A* and *RP-ls192-4B*) were identified for Ls13-192 and one (*RP-ls14-5A*) for Ls13-14 (Table 4.3, Figure 4.5). *RP-ls192-5A* and *RP-ls14-5A* were located at the same physical interval and played a major role in disease. This 5A QTL mostly likely is the same as the one identified in the BP025 population. However, the 4B QTL

was in a different physical interval compared to the ones identified in the ones identified in the BP025 population.

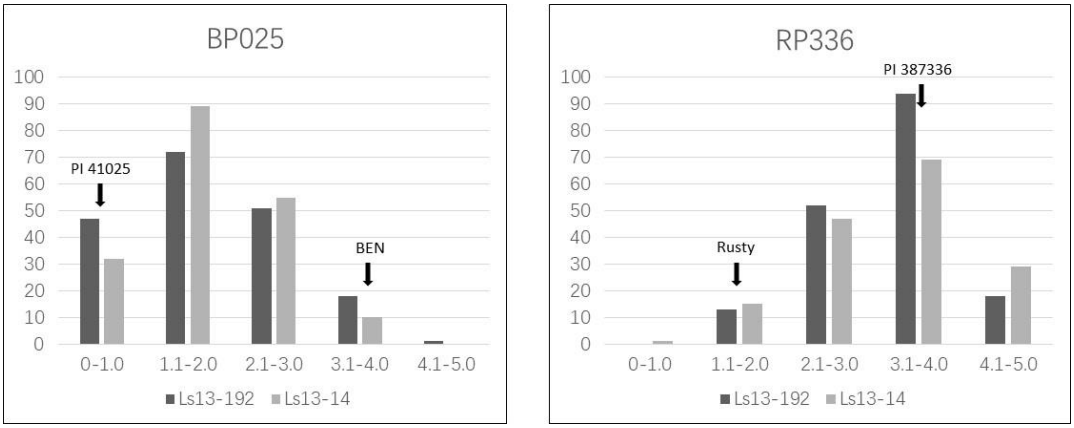

Figure 4.4. Histogram of disease reactions to tan spot caused by the race 4 isolates in BP025 and RP336 populations. The parental reactions were indicated by arrows.

Table 4.3. QTL associated with reaction to tan spot caused by Ls13-192 and Ls13-14 from composite interval mapping analysis in the BP025 and RP336 population.

Population	Isolate	QTL	Genetic interval (cM)	Physical Interval (Mbp)	LOD[a]	R^2 (%)[b]	Flanking markers
BP025	Ls13-192	*BP-ls192-1A*	92-96	51-56	4.8	0.11	*S1A_56880271 - S1A_51221934*
		BP-ls192-4B	58-60	53-106	13	0.26	*S4B_53863164 - S4B_106853468*
		BP-ls192-5A	408-410	668-672	7.6	0.17	*S5A_668189084 - S5A_672505022*
	Ls13-14	*BP-ls14-1A*	118-120	168-299	5.8	0.13	*S1A_168577107 - S1A_299657544*
		BP-ls14-4B	110-114	535-563	6.2	0.14	*S4B_535050204 - S4B_563205252*
		BP-ls14-5A	404-408	666-670	11.6	0.25	*S5A_666689644 - S5A_670630031*
RP336	Ls13-192	*RP-ls192-4B*	60-64	621-639	6.2	0.15	*4B_621667742 - 4B_639997885*
		RP-ls192-5A	236-238	666-674	19.8	0.41	*5A_666503690 - 5A_674124822*
	Ls13-14	*RP-ls14-5A*	236-238	666-674	17.2	0.39	*5A_666503690 - 5A_674124822*

[a] Permutation test with 1000 iterations yielded a LOD value of 4.2 and it was used as the cut-off to identify significant QTL.

[b] R^2 value indicates the amount of phenotypic variation explained by the individual QTL.

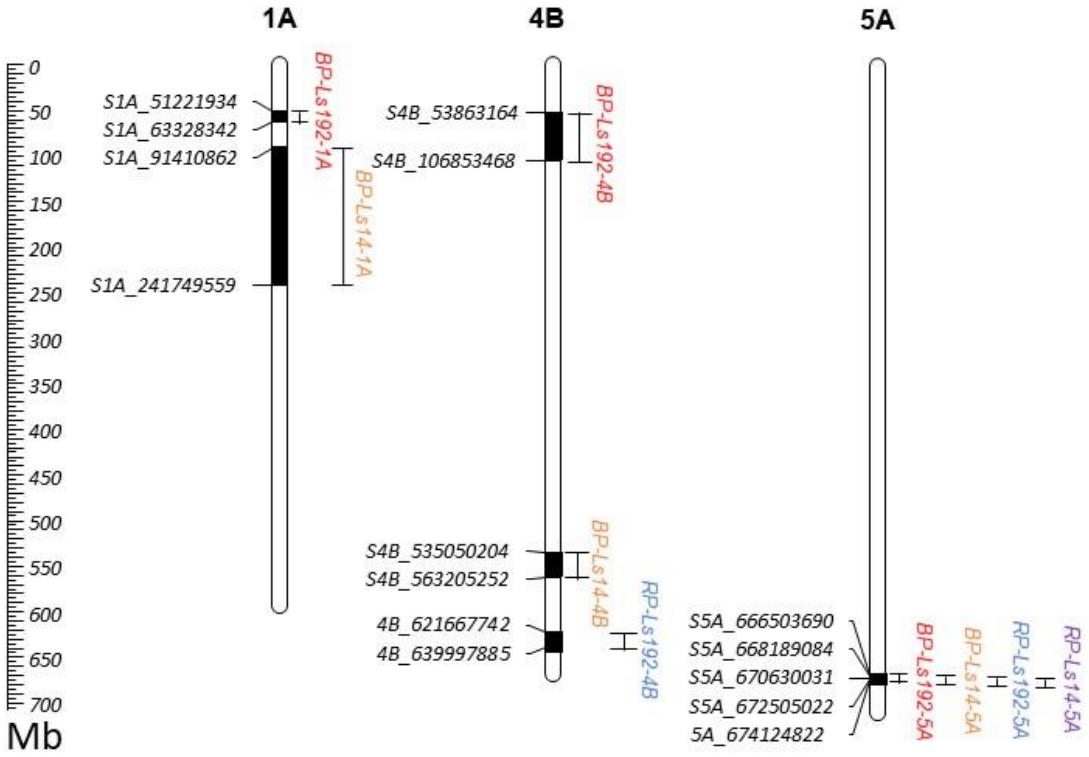

Figure 4.5. The genomic regions identified by QTL associated the development of tan spot disease caused by the race 4 isolates Ls13-192 and Ls13-14. A physical scale in Mbp for wheat chromosomes is shown to the left. The solid black box on each chromosome indicates the region significantly associated with tan spot on different population and caused by different isolate. Markers on the left of each chromosome define the physical region of the QTL. The QTLs are listed to the right of the chromosomes, with different colors for population and isolate combinations, including Red: BP025 with Ls13-192, Orange: BP025 with Ls13-14, Blue: RP336 with Ls13-192 and Purple: RP336 with Ls13-14. The LOD and R^2 values for each QTL are presented in Table 4.3.

4.5. Discussion

P. tritici-repentis isolates are classified into eight races according to their virulence on a set of wheat lines, including four common wheat and two durum lines, with race 4 being avirulent to all those lines (Lamari and Strelkov 2010; Aboukhaddour et al. 2013). So far, many studies have reported the identification of race 4 isolates from wheat and alternative hosts (Lamari et al. 1995, 1998, 2005; Ali and Francl 2003; Friesen et al. 2005; Singh et al. 2007;

Benslimane et al. 2011; Abdullah et al. 2017a, b; Kamal et al. 2019). Little attention has been paid to race 4 isolates because they were assumed to be avirulent to all wheat lines. It has been an intriguing question as to why race 4 exists in nature without causing disease. In this work, we confirmed the race 4 identity of five isolates collected from North Dakota through pathogenicity tests and molecular characterization. Although these isolates were avirulent to all tested common wheat lines, they could cause disease on some durum cultivars and other tetraploid wheat genotypes. Using two segregating tetraploid populations, we further showed that there are several genomic regions in wheat that govern reaction to these race 4 isolates. To our knowledge, this is the first study to show that race 4 isolates cause disease on durum cultivars and other tetraploids. This might provide a good explanation for why race 4 isolate can be consistently identified from infected wheat leaves and could be preserved for long in nature. Our finding also suggests that durum breeding programs should also include race 4 isolates in their disease screenings in future.

The five race 4 isolates used in this study were obtained from a collection of 352 isolates obtained from North Dakota wheat fields (Z. Liu, *unpublished data*). Unfortunately, we did not record the wheat type or cultivar they were collected from; thus, it remains unknown if they were collected from durum wheat. Previous studies have shown that race 4 can be isolated at low percentages (4-20%) from bread wheat (Lamari et al. 1991, 1995, 1998; Ali and Francl 2003; Abdullah et al. 2017b) and at high percentages (40-98%) from alternative hosts such as noncereal grasses and rye (Ali and Francl 2003; Abdullah et al. 2017a). There were also reports of race 4 being occasionally isolated from durum wheat (Benslimane et al. 2011; Kamel et al. 2019). It would be important to examine whether the previously reported race 4 isolates from other geographic regions and hosts are virulent on durum. Further research is needed to

systematically investigate the race structure of the fungal populations collected from durum and how prevalent race 4 is in durum wheat production areas. All such information will be valuable for durum wheat breeding programs. In addition, we observed that our race 4 isolates caused small necrotic lesions on some common wheat cultivars, and thus it is important to test these race 4 isolates on a wider range of common wheat cultivars.

Although *P. tritici-repentis* has eight races described, the genetic and evolutionary relationship among them remains unclear. According to Horsford (1982) and Maraite (1997), this fungus was first isolated from quack grass (*Agropyrum repens*, originally called *Triticum repens*) in 1903 and then from wheat in 1928. Before the 1940s, the fungus was described as a saprophyte or minor pathogen on wheat, but after that it became a highly virulent pathogen on wheat, probably through acquisition of the NE gene *ToxA* (Friesen et al. 2006). Our speculation is that the fungus first appeared on wheat as race 4 and later evolved into other virulent races by acquiring different virulence genes. Population genetic studies have revealed a clear genetic separation of race 4 from all other virulent isolates (Aung 2001; Aboukhaddour et al. 2011). This genetic separation was also supported by a recent whole genome phylogenetic analysis (Moolhuijzen et al. 2018). Considering that wheat evolved from tetraploid to hexaploid levels, it is possible that race 4 gained virulence first on tetraploid wheat by obtaining the *ToxB*, *ToxC* and other unknown virulence genes, and then on hexaploid wheat by acquiring the *ToxA* gene. This speculation can be supported by several lines of evidence: 1) highly similarly homologs of *ToxB* were identified from the related grass pathogen *P. bromi* and large percentage of race 4 could be isolated from the grasses (Andrie et al. 2008; Ali and Francl 2010; Andrie and Ciuffetti 2011); 2) *P. tritici-repentis* races 3 and 5, which produce the individual NEs Ptr ToxC and Ptr ToxB, respectively, were mostly isolated from durum wheat (Lamari and Bernier 1989b; Ali and Francl

2003; Lamari et al. 2005; Kamel et al. 2019); and 3) The Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1* interactions play important roles in disease on both tetraploid wheat and common wheat whereas Ptr ToxA-*Tsn1* interaction is only important for common wheat, i.e. the *ToxA* gene acts as a virulence factor on hexaploid but not tetraploid wheat (Faris et al. 2012 review, 2020; Virdi et al. 2016; Galagedara et al. 2020; Liu et al. 2020a, 2020b). In addition, there are numerous subspecies of tetraploid wheat, which have longer and more complex evolutionary and domestication histories than hexaploid wheat. It would be interesting to determine the pathogenicity or virulence of race 4 isolates on different tetraploid subspecies, which might provide a better understanding about the evolution of *P. tritici-repentis* races.

As mentioned, the current race classification system for *P. tritici-repentis* is largely based on the ability of the isolate to produce one or more of the three NEs, and the wheat lines with sensitivity to each of the three NEs serve as the differential set. Therefore, this system only allows the description of eight races (2^3) (Lamari et al. 2003). Two durum lines, Coulter (sensitive to Ptr ToxA) and 4B1149 (insensitive to all NEs), are sometimes included in the differential set (Lamari et al. 1995), but they do not increase the power to describe additional races. The limitation of the current race classification system was demonstrated by the fact either that the existing races produced additional virulence factors or that some isolates with no production of all three NEs caused disease on one of the differential lines (Lamari et al. 1995; Gamba and Lamari 1998; Friesen et al. 2003; Ali et al. 2010; Guo et al. 2018; Kamel et al. 2019). Our research further indicates there is a need to revise the current classification system. Here, we showed that at least some race 4 isolates can cause disease on tetraploid wheat; therefore, the susceptible tetraploid wheat lines, such as Ben and PI 387336, are suggested to be included in the differential set. The expansion of the race classification system should also rely on further

dissection of the genetics of host-pathogen interactions in both hexaploid and tetraploid wheats. The development of differential lines with individual susceptibility factors would be useful to describe and encompass all possible races.

Although race 4 is nonpathogenic, most race 4 isolates have been reported to carry *toxb*, which is 87% homologous to *ToxB* (Martinez et al. 2004; Strelkov et al. 2006). *ToxB* encodes Ptr ToxB, a chlorosis-inducing protein, and is present and highly expressed in isolates of races 5, 6, 7 and 8. The copy number, gene sequence and role in virulence for *ToxB* has been extensively investigated (Ciuffetti et al. 2010). However, there were only two *toxb* ORF sequences available in NCBI GenBank, SD20 and 90-2, which were collected in the USA (South Dakota) and Canada, respectively (Strelkov and Lamari 2003; Martinez et al. 2004). The *toxb* gene was also shown to be expressed in mycelium tissues and conidia of 90-2 (Martinez et al. 2004; Strelkov et al. 2006; Amaike et al. 2008). The race 4 isolates we collected in North Dakota all carry identical *toxb* sequences, which were identical to that of SD20 but slightly different (99% homology) from 90-2. We did not measure the expression of *toxb* for the ND isolates, but it would be an interesting to determine if *toxb* plays a role in conferring virulence on these tetraploid wheat genotypes.

To verify virulence of the race 4 isolates, we inoculated two of these isolates (Ls13-192 and Ls13-14) onto two segregating tetraploid wheat populations (BP025 and RP336) and identified wheat genomic regions conferring resistance or susceptibility to them using QTL mapping. In total, we identified six genomic regions distributed on chromosomes 1A, 4B and 5A (Table 4.3, Figure 4.5). Among them, the genomic region on chromosome 5A was identified for both isolates and populations indicating a common QTL in tetraploid wheat associated with reaction to race 4 isolates. Several regions on chromosome 5A have been previously identified to

harbor tan spot resistance or susceptibility QTL on both hexaploid and tetraploid wheat with virulent races (Chu et al. 2008, 2010; Virdi et al. 2016; Kariyawasam et al. 2016; Hu et al. 2019; Liu Y. et al. 2020a;). Our 5A QTL was physically located within 666.5-674.1 Mbp, which likely corresponds to the region for *QTs.fcu-5A.2*, *RP336_86-124_5A.2*, *RP696_86-124_5A.2* and *RP336_86-124 ΔToxA_5A* (Chu et al. 2010; Liu et al. 2020a). These QTLs were previously identified with races 1 and 2 or their *ToxA* knockout strains. This suggests that race 4 isolates might carry the same virulence factor as other virulent races which interact with the same 5A QTL to cause disease.

Three genomic regions on chromosome 4B were identified with one QTL (*BP-ls192-4B*) on the short arm and the other two (*BP-ls14-4B* and *RP-ls192-4B*) on the long arm, and they were specific to an individual population or isolate. No tan spot-associated QTL has previously been reported on chromosome 4B in hexaploid wheat, but Virdi et al. (2016) identified a QTL (*QTs.fcu-4B*) on chromosome 4B in a tetraploid wheat associated with disease caused by both L13-35 (race 2) and DW5 (race 5). This QTL might be in proximity of the QTL *BP-ls192-4B* identified in this research. However, the two QTL on chromosome 4B seem to be novel. Two closely linked regions on chromosome 1A were identified in the BP025 population, but not in RP336 suggesting this QTL might be host specific. Many QTLs have been identified on chromosome 1AS for tan spot resistance or susceptibility, but they are mainly associated with disease attributed to the Ptr ToxC-*Tsc1* interaction (Faris et al. 2013; Kariyawasam et al. 2016; Liu et al. 2017). However, race 4 does not produce Ptr ToxC and the physical location for *Tsc1* tends to be at distal end of chromosome 1A (Liu. et al. 2020a). Thus, the locations of these 1AS QTLs identified here unlikely correspond to the *Tsc1* locus. These two QTLs are also novel. The

identification of novel QTL suggests that these race 4 isolates carry unidentified and unique virulence factor(s) to cause disease on tetraploid wheat genotypes.

4.6. References

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GENERAL CONCLUSIONS

Tan spot of wheat is an economically important disease worldwide, which could pose a threat to food security. In last five decades, the disease system has been extensively studied and it has been known as a good example of inverse gene-for-gene model. In this model, the pathogen produces NEs to cause disease by interacting with corresponding sensitivity (susceptibility) genes. Therefore, removal of susceptibility genes can improve resistance in cultivars. Three such NE-host gene interactions have been identified in this system. However, many studies have shown that the disease system is much more complex invloving additional NE- host susceptibility interactions. To obtain complete resistance in cultivars, the additional NE-host interactions need to be identified and characterized. Thus, the goal of my PhD research was set to identify new virulence gene from the fungus and susceptibility genes in the host.

Using the newly developed genetic tools in our lab, I successfully identified two genomic regions containing the new virulence genes and mapped two loci for the *ToxB* genes in *Ptr*. This is the first identification of the *Ptr* new virulence effectors in the last two decades. This work provides strong evidence that there are addition virulence factors present in *Ptr*. The next step would be to identify the candidate genes in the regions, validate their functions and characterized the underlying mechanisms to cause disease. The mapping of two loci for *ToxB* genes advance our understanding of the genomic organization and evolution for this multiple copy gene. It is our plan to obtain a complete sequence of the two loci in the race 5 isolate DW5 for the further understanding of the loci. Using segregating wheat populations and QTL mapping, I identified novel genomic regions conferring resistance or susceptibility to some race 4 isolates that do not produce the three known NEs. My research was first to show natural race 4 isolates can cause disease on durum wheat and provide important message to durum breeders that breeding for tan

spot resistance should also consider these isolates. These further suggested the presence of additional virulence factors in Ptr and the need to revise the current race classification system. Future work would be to dissect these host susceptibility factors and provide tools for the development of a broader race classification system.

In summary, my PhD research not only greatly advance our understanding of the disease system but also provide important knowledge and tools for the development of resistant wheat and durum cultivars.

APPENDIX A. LIST OF SIMPLE SEQUENCE REPREAT PRIMERS USED IN GENETIC MAPPING OF THE 86-124Δ*MAT1-2-1* × DW5Δ*MAT1-1-1* POPULATION

Primer name	Polymorphism[b]	Primer sequence [a]
PtrSSR/AAC003F	M	CACGACGTTGTAAAACGACTTGTGGAGATGGGCGTTG
PtrSSR/AAC003R		GCTTCTTTGGTGTCTGCAGAA
PtrSSR/AAC004F	M	CACGACGTTGTAAAACGACTGGTGGATTCGTTGTTGTTGT
PtrSSR/AAC004R		CGCAATATCAAAACCAAGCC
PtrSSR/AAC008F	P	CACGACGTTGTAAAACGACTTCTGCTGTCTTCTTTGCCA
PtrSSR/AAC008R		TTGTATAGCCAGGTACGTCCG
PtrSSR/AAC010F	M	CACGACGTTGTAAAACGACAACGGACAAACGGTCCTTCA
PtrSSR/AAC010R		TTTGGACTTGCAGCAGTGAA
PtrSSR/AAC011F	P	CACGACGTTGTAAAACGACTACATGCACACGGTCATGTC
PtrSSR/AAC011R		TTCCGTACAATTGACCACGA
PtrSSR/AAC013F	M	CACGACGTTGTAAAACGACCGGCCTCGATTACTTCTTTT
PtrSSR/AAC013R		ACTGAACTACGGTCATGAGCA
PtrSSR/AAC015F	P	CACGACGTTGTAAAACGACTTGTGGTGGATTCGGGTGT
PtrSSR/AAC015R		TATCTGTACCACGGAAAGCGA
PtrSSR/AAC017F	M	CACGACGTTGTAAAACGACGCCACTGTGGTGGATTCTCTT
PtrSSR/AAC017R		TCGGTTCTCGTCTTGATGGAT
PtrSSR/AAG021F	M	CACGACGTTGTAAAACGACTCATCGCACTGTGGTGTATTC
PtrSSR/AAG021R		TTAGCCTCGGTGCCAAGAA
PtrSSR/AAG023F	P	CACGACGTTGTAAAACGACACTGCACTTTGACACGCAAT
PtrSSR/AAG023R		TGGTGCTGCCTCCACTGTT
PtrSSR/AAG026F	N	CACGACGTTGTAAAACGACCTTGGGGGTTGCGTTAAAAT
PtrSSR/AAG026R		AAGCGCATTCCTCACCTCTTT
PtrSSR/AAG040F	N	CACGACGTTGTAAAACGACTGGTCAATGTGGTGGATTCT
PtrSSR/AAG040R		AAGGGGGTCATGATGTATGGA
PtrSSR/AAG041F	P	CACGACGTTGTAAAACGACAGAGGCGTTCAAGTGGGATA
PtrSSR/AAG041R		TTAGCCTCGGTGCCAAGAA
PtrSSR/AAG045F	M	CACGACGTTGTAAAACGACTGGATTCCGCGATATGAAG
PtrSSR/AAG045R		ATACCGTAGCAGTCTCGCGTT
PtrSSR/AAG046F	M	CACGACGTTGTAAAACGACAGCTCATGTGGTGGATTCTGT
PtrSSR/AAG046R		AGTACACCAGCCATGCATGTT
PtrSSR/AAG048F	N	CACGACGTTGTAAAACGACACATTGTGGTGGATTCTCGTC
PtrSSR/AAG048R		TTATGCATGCTCGCCTTGAT
PtrSSR/AAG049F	N	CACGACGTTGTAAAACGACAAAGCATGATTCCCCCTGTT
PtrSSR/AAG049R		TCTTTGCTTGCTTGCTTGCT
PtrSSR/AAG050F	P	CACGACGTTGTAAAACGACCCCCTTGACTCATCACACTCT
PtrSSR/AAG050R		ATGTTCTCGAAGCATGTGCG
PtrSSR/AAG051F	M	CACGACGTTGTAAAACGACACGATGTGGTCGGTTATTAGG
PtrSSR/AAG051R		ACGGAAAAGCGTAGTTTGCA
PtrSSR/AAG054F	P	CACGACGTTGTAAAACGACCTGGGAGAACGAACGTATGAA
PtrSSR/AAG054R		GTTTAAAATCCCCAAAATCCA
PtrSSR/AAG055F	P	CACGACGTTGTAAAACGACCGCAAACATAAAAAACCGCC
PtrSSR/AAG055R		TCTGCTTTTGTGGTGCTTCA
PtrSSR/AAG056F	M	CACGACGTTGTAAAACGACATCGCCACTGTGGTGGATT
PtrSSR/AAG056R		ATCGTCATCGCGGGAAGA
PtrSSR/AAG057F	P	CACGACGTTGTAAAACGACCAATCAAAATTCCCACTCGG
PtrSSR/AAG057R		CCCACATCTGCGACAACAATA
PtrSSR/AAT001F	N	CACGACGTTGTAAAACGACCGATACATCTCAACAACGCGA
PtrSSR/AAT001R		TTCGGCCAAAGTCCTACAT
PtrSSR/AAT002F	P	CACGACGTTGTAAAACGACCCGAAGAAACCACCCATAGAA
PtrSSR/AAT002R		TTGCGCAGAGCTTAGGTGTA
PtrSSR/AAT003F	M	CACGACGTTGTAAAACGACTAGGCCGAAGTCTTGCATAGT
PtrSSR/AAT003R		GGCGTGGAGGCATTATGTG
PtrSSR/AAT004F	N	CACGACGTTGTAAAACGACTCTGGCTCGCTACTAATCAAA
PtrSSR/AAT004R		TACACCTAAGCTCTGCGCAA
PtrSSR/AAT006F	M	CACGACGTTGTAAAACGACTAAGCGGGAAGCTTGGTCTAA
PtrSSR/AAT006R		AAGTTGCAAAAGTTGGTGGG

Primer name	Polymorphism[b]	Primer sequence [a]
PtrSSR/AAT007F	M	CACGACGTTGTAAAACGACTATCGTGTCGAGTGTCTCCC
PtrSSR/AAT007R		AAGTCGGGCCGAAGTGTCAA
PtrSSR/AAT008F	N	CACGACGTTGTAAAACGACTGTGGTGGATTCTAAATGGAA
PtrSSR/AAT008R		CTGCTTCATTTGAAGGCACA
PtrSSR/AAT009F	N	CACGACGTTGTAAAACGACGTTGTTGAGATGCATCGCTT
PtrSSR/AAT009R		GCGGGAAGCTTGGTCTAACTA
PtrSSR/AAT011F	N	CACGACGTTGTAAAACGACTAGAGCCTGCCGAGATTGTTT
PtrSSR/AAT011R		GCCAAAGCCACAATTAGCAA
PtrSSR/AAT012F	N	CACGACGTTGTAAAACGACGCAAATCATCCCCCAAATTG
PtrSSR/AAT012R		CCTTCGTAGCAGCTATGTTCG
PtrSSR/AAT014F	M	CACGACGTTGTAAAACGACGCGAACCATACCAGAAAACCA
PtrSSR/AAT014R		CCCACTGTCTGCTTGTTATCA
PtrSSR/AAT015F	M	CACGACGTTGTAAAACGACCGAACCATACCAGAAAACCA
PtrSSR/AAT015R		CGATACATCTCAACAACGCGA
PtrSSR/AAT016F	M	CACGACGTTGTAAAACGACTCCTTCTGCTCGGGCTTACT
PtrSSR/AAT016R		ATGTAGCAGCGGGCTCTTTT
PtrSSR/AAT018F	N	CACGACGTTGTAAAACGACCGAACATAGCTGCTACGAAGG
PtrSSR/AAT018R		CCATCCAGCTCTTGCTCACTA
PtrSSR/AAT020F	N	CACGACGTTGTAAAACGACTCAGCACTAGCGCCTACTCTA
PtrSSR/AAT020R		ACCAATACCCAGTCACCAGAA
PtrSSR/AAT022F	N	CACGACGTTGTAAAACGACAAGGTGAGTAAAGTTGGTGGG
PtrSSR/AAT022R		TACAAGGCCTTCCAACAAGCT
PtrSSR/AAT025F	M	CACGACGTTGTAAAACGACCGATACATCTCAACAACGCGA
PtrSSR/AAT025R		TTAGCACCTGGCAACAAACA
PtrSSR/AC001F	P	CACGACGTTGTAAAACGACTGAACTGTGCCAACGCAAA
PtrSSR/AC001R		TTTCAGCGAGATAGCATGAGC
PtrSSR/AC003F	M	CACGACGTTGTAAAACGACAACCCCCCATGTCGCATAA
PtrSSR/AC003R		TCGCTAGATACGCCTTTTGTG
PtrSSR/AC004F	M	CACGACGTTGTAAAACGACATGAATGGCGTTCAGATGCA
PtrSSR/AC004R		TAACCTTGGAAACTCCGCAC
PtrSSR/AC005F	M	CACGACGTTGTAAAACGACTCTGTGGCTTGGTATTCGGT
PtrSSR/AC005R		CACCCATTTTTTAGGCCCTT
PtrSSR/AC006F	N	CACGACGTTGTAAAACGACATTGCCTGCGGGTCGATCTA
PtrSSR/AC006R		GTTTGACGGGCCTTTTGTTA
PtrSSR/AC007F	N	CACGACGTTGTAAAACGACTTTTCTTGTGACCGCGAAAG
PtrSSR/AC007R		AAAGGCGGACTAGGGGCTA
PtrSSR/AC008F	M	CACGACGTTGTAAAACGACATGCTTGCTGACCGACATAT
PtrSSR/AC008R		CCGTTGGAGAAGCTTGAAA
PtrSSR/AC009F	M	CACGACGTTGTAAAACGACAGCCACGGAGACAGACGTCAT
PtrSSR/AC009R		TCGCTGATCGATGAGGTAAGT
PtrSSR/AC010F	P	CACGACGTTGTAAAACGACTGGTGCATTCTGCATCTTCA
PtrSSR/AC010R		TGAACAACTGTGTGTGCGTT
PtrSSR/AC011F	N	CACGACGTTGTAAAACGACGTTTTCCGCGGTTCAACTTT
PtrSSR/AC011R		CATTAGGCGGGTTAAATTCC
PtrSSR/AC012F	N	CACGACGTTGTAAAACGACTCGCCCATCCATGTCCACT
PtrSSR/AC012R		CGCTCTCGAAGCATTTGTG
PtrSSR/AC013F	N	CACGACGTTGTAAAACGACTGTTGGCGTAAGTAGAGGGGT
PtrSSR/AC013R		CGGTCTCGTCTGATGGATAGT
PtrSSR/AC014F	M	CACGACGTTGTAAAACGACAAATCTCGGCGTGAACTCTT
PtrSSR/AC014R		AGGCAGTCTTGTCAACGCA
PtrSSR/AC015F	P	CACGACGTTGTAAAACGACCGAAGATAGCCGGAGATGTAT
PtrSSR/AC015R		ATGCAAGAATGGGAGGGTG
PtrSSR/AC016F	M	CACGACGTTGTAAAACGACTACTTACAGAGCTGCAGACGC
PtrSSR/AC016R		GCAACAGTGTGGAGAAGTGAA
PtrSSR/AC017F	M	CACGACGTTGTAAAACGACTGTCATGAGTCATGTGGTGGA
PtrSSR/AC017R		CAACGGTCTTTGTTCTTTGC
PtrSSR/AC019F	M	CACGACGTTGTAAAACGACTGGACCTTCCGAATAAGGTCA
PtrSSR/AC019R		CAGTCCGGTCTCGGACTTT
PtrSSR/AC020F	M	CACGACGTTGTAAAACGACTGCATAACGCATTCTGTCTTG
PtrSSR/AC020R		AGGGATCCGGGATGATGTTTA
PtrSSR/AC021F	N	CACGACGTTGTAAAACGACTTCTCCTTTTCGGCCTTCTTC

Primer name	Polymorphism[b]	Primer sequence [a]
PtrSSR/AC021R		AAAGACGGGGAAGGACAAACA
PtrSSR/AC022F	P	CACGACGTTGTAAAACGACTCACATTCCCATCTCAACCCT
PtrSSR/AC022R		AAACACGTATAGAGCGGGTGC
PtrSSR/AC024F	P	CACGACGTTGTAAAACGACAAACAAAACCGACAGCTGCA
PtrSSR/AC024R		CCCGGTCTGGACAATCATG
PtrSSR/AC025F	M	CACGACGTTGTAAAACGACTCCATCATGAGCTCTTTCCA
PtrSSR/AC025R		GCCTGCTGGTGATATCGTGTA
PtrSSR/AC026F	M	CACGACGTTGTAAAACGACTGGAAATCTGCTGCTAAGCA
PtrSSR/AC026R		TCGCTAGATACGCCTTTTGTG
PtrSSR/AC027F	N	CACGACGTTGTAAAACGACTTCGCGGAGTGTCAGTTGAGT
PtrSSR/AC027R		CGGGCCCATTTTTAAGCATT
PtrSSR/AC028F	N	CACGACGTTGTAAAACGACTACTTGTCTTGTGACCGCGA
PtrSSR/AC028R		AAAGGCGGACTAGGGGCTA
PtrSSR/AC029F	P	CACGACGTTGTAAAACGACATGGGAGTGAGGATACATGGG
PtrSSR/AC029R		GGTCTGGCTTGCATAGCGA
PtrSSR/AC031F	M	CACGACGTTGTAAAACGACACCACTGCCTGGCAATTATT
PtrSSR/AC031R		ACTCCAGCCACACAGACCTAT
PtrSSR/AC032F	N	CACGACGTTGTAAAACGACTGGAAATCTGCTGCTAAGCA
PtrSSR/AC032R		TCTTTACTCTCTCCCACGTCC
PtrSSR/AC033F	P	CACGACGTTGTAAAACGACATACCACACACGCACGCAATT
PtrSSR/AC033R		TGATGTGGCAAAGGAGATGA
PtrSSR/AC034F	P	CACGACGTTGTAAAACGACACCTCCCATCACCAGACAGA
PtrSSR/AC034R		GGACTTTTTGGCTTGGTGTT
PtrSSR/AC035F	M	CACGACGTTGTAAAACGACCATTGTGGTGGAATTCGAGA
PtrSSR/AC035R		TTTTATCGCCCTTCTTCCCT
PtrSSR/AC036F	M	CACGACGTTGTAAAACGACACATTCCCGTCTGTCCCTTTT
PtrSSR/AC036R		ACATTCCCGTCTGTCCCTTTT
PtrSSR/AC038F	N	CACGACGTTGTAAAACGACTTTGGAGTACATTGTGGTGGA
PtrSSR/AC038R		TTTTTCTTGTAGGCAGCGCA
PtrSSR/AC039F	N	CACGACGTTGTAAAACGACCACCACCTTAACGCCCAA
PtrSSR/AC039R		GTCGGTTCCGAAATCGATAGT
PtrSSR/AC040F	P	CACGACGTTGTAAAACGACGGAGTGAGGATACATGGGGTA
PtrSSR/AC040R		TTGCATAGCGATGGCGTTT
PtrSSR/AC041F	P	CACGACGTTGTAAAACGACGCTGTTTGAATACGGTTGATG
PtrSSR/AC041R		TAATGACGTGTCTGGCGACTA
PtrSSR/AC043F	M	CACGACGTTGTAAAACGACTTGATACATCGCTCCCCATT
PtrSSR/AC043R		AAATCCCGCTTACAAACCCA
PtrSSR/AC044F	P	CACGACGTTGTAAAACGACAGGGTCCTGTCTTGCACTGA
PtrSSR/AC044R		AACAACACCACTGGCGGTGTA
PtrSSR/AC045F	M	CACGACGTTGTAAAACGACATGCCTCCTTACTGACTTTGG
PtrSSR/AC045R		TGCTGATATCGACACCAACA
PtrSSR/AC047F	M	CACGACGTTGTAAAACGACACCTCCCACCCTCAAACATAT
PtrSSR/AC047R		GGGGAGAACAAGCAAACTTAA
PtrSSR/AC048F	N	CACGACGTTGTAAAACGACCAATCCCACCCCATTACAAA
PtrSSR/AC048R		CTGCGGTTTTTTCTCCTTCT
PtrSSR/AC050F	P	CACGACGTTGTAAAACGACACATCGGACGTCTGCTCACA
PtrSSR/AC050R		TCTTTTTAGGAGCAGGTGCTG
PtrSSR/AC051F	M	CACGACGTTGTAAAACGACCAAGCATAGCGGTGACGACT
PtrSSR/AC051R		TTGCATTGGCATTTTGTCCC
PtrSSR/AC053F	M	CACGACGTTGTAAAACGACATCATCGCACTGGGTGGATT
PtrSSR/AC053R		ATGCCCATTACACCCTAATGC
PtrSSR/AC055F	N	CACGACGTTGTAAAACGACATATGATGGGTGTGATGGGGA
PtrSSR/AC055R		TACGTCGTTCATCACGTTCTG
PtrSSR/AC056F	M	CACGACGTTGTAAAACGACTACGCGATTGGACTGCTGAA
PtrSSR/AC056R		TTTTTGCGTGCGCGTGTA
PtrSSR/AC057F	M	CACGACGTTGTAAAACGACTCATCGCACTGTGGTGGATT
PtrSSR/AC057R		CGGTCTTGGAAAAGTCACAA
PtrSSR/AC058F	P	CACGACGTTGTAAAACGACTCATTCAGTCCCAACCCAAAC
PtrSSR/AC058R		ATGGATCGCGGTTGTTCTG
PtrSSR/AC059F	M	CACGACGTTGTAAAACGACCCAGAAACTAGGTCCAAAGGA
PtrSSR/AC059R		TGAATGAATCGAGGACGACA

Primer name	Polymorphism[b]	Primer sequence [a]
PtrSSR/AC060F	N	CACGACGTTGTAAAACGACTAAATAGCCTAATGGGCCCCT
PtrSSR/AC060R		AACCACGTAACCCCACACTTT
PtrSSR/AC061F	N	CACGACGTTGTAAAACGACTCGCCTCAATACTGCCTGTAA
PtrSSR/AC061R		TGGAGGAGGTCGCTTCTGTT
PtrSSR/AC062F	N	CACGACGTTGTAAAACGACGAGGGTCAAGTTGTGCAGAGT
PtrSSR/AC062R		TCGGATATGCTCCGTACAACA
PtrSSR/AC063F	M	CACGACGTTGTAAAACGACATCATCGCACTGTGTGTGTGT
PtrSSR/AC063R		CTCTGGAAATTGCGGTTGAA
PtrSSR/AC065F	P	CACGACGTTGTAAAACGACATAACTTGCCACAGCCCCTAT
PtrSSR/AC065R		ATTCCTATCCCGTCCAAACA
PtrSSR/AC066F	P	CACGACGTTGTAAAACGACTCCGAATGACATATCGTGGTG
PtrSSR/AC066R		ATAGCCTAGCATCTTCCCTCA
PtrSSR/AC067F	M	CACGACGTTGTAAAACGACGTTTGAGGAGGACGAGGAGGT
PtrSSR/AC067R		AAGCCAGGTGTGTGTGTGTGT
PtrSSR/AC063F	M	CACGACGTTGTAAAACGACATCATCGCACTGTGTGTGTGT
PtrSSR/AC063R		CTCTGGAAATTGCGGTTGAA
PtrSSR/AC065F	M	CACGACGTTGTAAAACGACATAACTTGCCACAGCCCCTAT
PtrSSR/AC065R		ATTCCTATCCCGTCCAAACA
PtrSSR/AC066F	N	CACGACGTTGTAAAACGACTCCGAATGACATATCGTGGTG
PtrSSR/AC066R		ATAGCCTAGCATCTTCCCTCA
PtrSSR/AC067F	N	CACGACGTTGTAAAACGACGTTTGAGGAGGACGAGGAGGT
PtrSSR/AC067R		AAGCCAGGTGTGTGTGTGTGT
PtrSSR/AC069F	P	CACGACGTTGTAAAACGACGCAAGGGTGTTAAGATCGACA
PtrSSR/AC069R		TTATCCCGAGCCGGTCTTTA
PtrSSR/AC070F	P	CACGACGTTGTAAAACGACTTACAAAGTTCGAGCGAGAGC
PtrSSR/AC070R		GCACCGCCCTCTGAATCTT
PtrSSR/AC071F	N	CACGACGTTGTAAAACGACTTGTTTGGTACGATATCGGC
PtrSSR/AC071R		TTTGCACATGATCCGACCTT
PtrSSR/AC072F	M	CACGACGTTGTAAAACGACTATTCATCGCACTGTGGTGGA
PtrSSR/AC072R		AAGGACGCGTTTTTCGTGTA
PtrSSR/AC073F	M	CACGACGTTGTAAAACGACATTCAACGCGGCGTAATAGA
PtrSSR/AC073R		GGCATCCACATCCAGACGT
PtrSSR/AC075F	M	CACGACGTTGTAAAACGACTGGTGAGAAGTGAATGCATGG
PtrSSR/AC075R		TTGCCAAGGCACTAGCTACG
PtrSSR/AC076F	M	CACGACGTTGTAAAACGACAGGGATGGGAGTGTGAGTGTG
PtrSSR/AC076R		TGGGTTGATGGGGAGAATA
PtrSSR/AC077F	N	CACGACGTTGTAAAACGACAGAGAAGAAGCGTGCCAAGAT
PtrSSR/AC077R		TCAGAAGGGTCTGCTTTGTCA
PtrSSR/AC078F	M	CACGACGTTGTAAAACGACCAAAGGCAGTCTTTCCGAAA
PtrSSR/AC078R		ACTGTGAGCGGGGGTTGTT
PtrSSR/AC079F	P	CACGACGTTGTAAAACGACATTTGGGCATAGGAAACGGA
PtrSSR/AC079R		CGTAGGATTCAGTCGGTACCT
PtrSSR/AC080F	M	CACGACGTTGTAAAACGACTGTTCAGAACCATCGGAAAAG
PtrSSR/AC080R		AAGGAGACCAAGACGTAGCAT
PtrSSR/AC081F	M	CACGACGTTGTAAAACGACTGGATTCTTGGTTTATGCGG
PtrSSR/AC081R		AACAACCAACCTCCTAAACCC
PtrSSR/AC082F	P	CACGACGTTGTAAAACGACTGGATTCAACACGCTGTAGTC
PtrSSR/AC082R		TACACTTTCCTTGGACGGGAT
PtrSSR/AC083F	P	CACGACGTTGTAAAACGACCCAAGTGTATCCGCAGCAA
PtrSSR/AC083R		TCGGTGTAGAGGGTAATGTGG
PtrSSR/AG001F	M	CACGACGTTGTAAAACGACAGCGACGAACAGCTAGGAAAT
PtrSSR/AG001R		CTCAACGATAACCACCAGGAT
PtrSSR/AG002F	M	CACGACGTTGTAAAACGACCCGAAATCCCATGTTTGGTA
PtrSSR/AG002R		TGCATGGAACAAGGCCAGTA
PtrSSR/AG003F	M	CACGACGTTGTAAAACGACCCGAAATCCCATGTTTGGTA
PtrSSR/AG003R		AAAGGCATGCTAGGAACACA
PtrSSR/AG004F	N	CACGACGTTGTAAAACGACTAGGACTGAAAGACCCATGGA
PtrSSR/AG004R		GAAGGGCATAAGCAAAAGGA
PtrSSR/AG006F	M	CACGACGTTGTAAAACGACGGGATCCACATGACTGACTCA
PtrSSR/AG006R		ATAAGATGGTCGGAGGCACA

Primer name	Polymorphism[b]	Primer sequence [a]
PtrSSR/AG007F	N	CACGACGTTGTAAAACGACGGGATCCACATGACTGACTCA
PtrSSR/AG007R		TGCTCGGAAACGATTCAAGA
PtrSSR/AG008F	M	CACGACGTTGTAAAACGACGTTTGTTTCATTTGGGCCAG
PtrSSR/AG008R		AATCATGCCATCTCCTTCCC
PtrSSR/AG009F	N	CACGACGTTGTAAAACGACTGATGGATCCGATCTGATGGT
PtrSSR/AG009R		TGCAGACACTTCCATCCATCT
PtrSSR/AG010F	N	CACGACGTTGTAAAACGACATCCCTTTTTCTCGCCTTCT
PtrSSR/AG010R		TTTATGCATTAGCTGGCCTG
PtrSSR/AG011F	P	CACGACGTTGTAAAACGACAACTTGCTCATGTCATGCACC
PtrSSR/AG011R		AGCGCCATGTATTTGTCGAT
PtrSSR/AG012F	M	CACGACGTTGTAAAACGACTTTTGGCGGTACCTTCGGA
PtrSSR/AG012R		TATGGCACTGGAACTGGGATT
PtrSSR/AG015F	P	CACGACGTTGTAAAACGACTCGTAAGCTGTTCGGTAACG
PtrSSR/AG015R		TCATTCTCCTGCCCTTCTACT
PtrSSR/AG021F	M	CACGACGTTGTAAAACGACATCCCAACTGCTGCTCTACA
PtrSSR/AG021R		AGCATTCGGATAACATCAGCC
PtrSSR/AG022F	P	CACGACGTTGTAAAACGACTGACCTCATTCAGCCCGTT
PtrSSR/AG022R		ACTAGGCTCCCCAGAAGTGA
PtrSSR/AG027F	N	CACGACGTTGTAAAACGACTAACTAACGCCAGTCGAGTGG
PtrSSR/AG027R		AATGACGGGGTGGTGATAACA
PtrSSR/AG028F	N	CACGACGTTGTAAAACGACTGACATAGCTACGATGGTGGG
PtrSSR/AG028R		ATAGACGAACGGGGTTTTGGT
PtrSSR/AG030F	M	CACGACGTTGTAAAACGACGACATGGGAAGCAGAAATGA
PtrSSR/AG030R		ATTGAGGGGGTTATCGAGAA
PtrSSR/AG031F	M	CACGACGTTGTAAAACGACATGCACGGCAACACAACAA
PtrSSR/AG031R		ATCCTCTCAGCCAACACCG
PtrSSR/AG032F	M	CACGACGTTGTAAAACGACGGACCACGGGAATAGGAAGA
PtrSSR/AG032R		AGGGACGATTGCACTGAGAAA
PtrSSR/AG033F	P	CACGACGTTGTAAAACGACTACCTCGAGCGACGCTAAACA
PtrSSR/AG033R		CCGCGCTATGATATACCCTCT
PtrSSR/AG034F	N	CACGACGTTGTAAAACGACGCGTTTTTTTTGTGCTGTGC
PtrSSR/AG034R		AAACTTACCATGTCATGCCCC
PtrSSR/AG036F	P	CACGACGTTGTAAAACGACTCACACCAACAATCATGCCA
PtrSSR/AG036R		GTTTGTTTCATTTGGGCCAG
PtrSSR/AG038F	M	CACGACGTTGTAAAACGACATCGCGAGCAGGTACACCTT
PtrSSR/AG038R		CAAGTCGCTTGACCCACAA
PtrSSR/AG040F	M	CACGACGTTGTAAAACGACCCTGAGGAACGGTCTTGATAA
PtrSSR/AG040R		ATTCTTTTCCTGGAGTAGCGG
PtrSSR/AG041F	M	CACGACGTTGTAAAACGACTTGCATGGTCCGAAGGGGT
PtrSSR/AG041R		ATCGCAGCCATCCAAGCA
PtrSSR/AG042F	M	CACGACGTTGTAAAACGACTTCAAGGACCACGATTGGAT
PtrSSR/AG042R		TGGGGAGGAAGAATAGGAGAA
PtrSSR/AG043F	P	CACGACGTTGTAAAACGACAACGCGCTGGACCTCAGATTT
PtrSSR/AG043R		TGGGCCGCTTATTTATGCTA
PtrSSR/AG045F	M	CACGACGTTGTAAAACGACTCGTCTTTGTACGGCAAAAG
PtrSSR/AG045R		AACAGGACCCCACAAATTCA
PtrSSR/AG046F	N	CACGACGTTGTAAAACGACAGGTGTTCAGCGGGGAATTA
PtrSSR/AG046R		TCCTCTCTCTCCTCCTCCTCT
PtrSSR/AG047F	P	CACGACGTTGTAAAACGACCAGGCTCGAGGGAATCAAA
PtrSSR/AG047R		TTCGAGAAGCCTTTCACTCCA
PtrSSR/AG048F	M	CACGACGTTGTAAAACGACTTGACTCATGTGGTGGATTCA
PtrSSR/AG048R		TTTCTTTGCCTGTGCCATCT
PtrSSR/AG050F	M	CACGACGTTGTAAAACGACGCTGCGTAAAACGGTCGTATA
PtrSSR/AG050R		TCATTTTCTTTCCCTCCCTC
PtrSSR/AG052F	P	CACGACGTTGTAAAACGACGAACGAGCAGTCATAACGTGA
PtrSSR/AG052R		AAGACAAAGGTTGGGCCGA
PtrSSR/AG053F	P	CACGACGTTGTAAAACGACTGAACCGAACCGAACCGTA
PtrSSR/AG053R		TAATGTATAGCCGAGGCGAA
PtrSSR/AG054F	M	CACGACGTTGTAAAACGACATGAGCTCTGGGAGCTTTCTG
PtrSSR/AG054R		CCATCTCGCTTTTGAGATACC
PtrSSR/AG055F	M	CACGACGTTGTAAAACGACGTTCCTTGCTAGGGGGTCAAT

Primer name	Polymorphism[b]	Primer sequence [a]
PtrSSR/AG055R		TTTGGCCGGATTCCATGAT
PtrSSR/AG056F	M	CACGACGTTGTAAAACGACAGGGATGGGAGGAAAAGAA
PtrSSR/AG056R		CTGCGATGGTGATTTATCAAA
PtrSSR/AG057F	P	CACGACGTTGTAAAACGACACCATGTTGCACGGCTGATA
PtrSSR/AG057R		GGAGTGGACGAAGTCTGTTCT
PtrSSR/AG059F	N	CACGACGTTGTAAAACGACTTGTATAGGGCGGTGGTAGAT
PtrSSR/AG059R		GGCGTTTTAAAATGGGATGG
PtrSSR/AG060F	M	CACGACGTTGTAAAACGACTCGGAGTAACCATTGTGGATT
PtrSSR/AG060R		AAAGCTTAGGGTCGGGCTTAA
PtrSSR/AG062F	M	CACGACGTTGTAAAACGACTACCGTCGTTCCCCCATAGTT
PtrSSR/AG062R		GCTGGGTCAGTCACAGTCAT
PtrSSR/AG063F	M	CACGACGTTGTAAAACGACTTGCTTGGAAGGTCGTGAGA
PtrSSR/AG063R		AACCTTGAAAACGCCTTGCT
PtrSSR/AG064F	M	CACGACGTTGTAAAACGACCCGTCCGACACTAGATCATCA
PtrSSR/AG064R		CCGTGGTGATTGCTTGGT
PtrSSR/AG065F	N	CACGACGTTGTAAAACGACATCCAACCCCACGTTGAT
PtrSSR/AG065R		TGCGTGGAGTAGCGTCAAGT
PtrSSR/AG066F	P	CACGACGTTGTAAAACGACCCATGTAACAACAATACGCCC
PtrSSR/AG066R		CGTTTCGTCGCGAGAGAGA
PtrSSR/AG067F	M	CACGACGTTGTAAAACGACTTGTGGTGGATTCCATCAGA
PtrSSR/AG067R		GCAGCTTTTGGCGAAGTTTT
PtrSSR/AG069F	M	CACGACGTTGTAAAACGACATGCTTCTTGGCCGAATGTT
PtrSSR/AG069R		GCCTCTATGTGCCGAGAGAGA
PtrSSR/AG070F	P	CACGACGTTGTAAAACGACATAGCCAGCTCAAACGTTCC
PtrSSR/AG070R		TTGTCACATGGTTCAGTCGG
PtrSSR/AG072F	P	CACGACGTTGTAAAACGACTTGACGAGCGGGTTCTAGAAA
PtrSSR/AG072R		AAAATACCAGCGCTACCATCC
PtrSSR/AGC002F	N	CACGACGTTGTAAAACGACACATCTCAAGCTCGACGCCT
PtrSSR/AGC002R		TGACAGCTGGTCCGAACTGTA
PtrSSR/AGC003F	P	CACGACGTTGTAAAACGACTCAGAAAGTCTTTTGGGGCT
PtrSSR/AGC003R		TCATATCCCGCACCCGTT
PtrSSR/AGG002F	M	CACGACGTTGTAAAACGACTCGCCACTGTGGTGGATTATT
PtrSSR/AGG002R		CAAGTCCCCATTCTACAGCAT
PtrSSR/AGG003F	M	CACGACGTTGTAAAACGACTCGCTAGATCTATCGCTTGGG
PtrSSR/AGG003R		ACAAAGCAGATGACGAGGAGA
PtrSSR/AGG004F	N	CACGACGTTGTAAAACGACAGACGACAAAGCAGATGACGA
PtrSSR/AGG004R		CGGCAGGTATCTCGCTAGAT
PtrSSR/AGG005F	M	CACGACGTTGTAAAACGACTCGCTAGATCTATCGCTTGGG
PtrSSR/AGG005R		ACAAAGCAGATGACGAGGAGA
PtrSSR/AGG007F	M	CACGACGTTGTAAAACGACAATGTGGTGGAATTCCCTCCT
PtrSSR/AGG007R		CAACACTTACCTCACCGGTCT
PtrSSR/AGG010F	P	CACGACGTTGTAAAACGACACTACGTTGAGAGCACTGCTG
PtrSSR/AGG010R		AAGAGAAAGCTAGGGAGGGAA
PtrSSR/AGG011F	P	CACGACGTTGTAAAACGACTCAGCTTCAAGAATGGTGGT
PtrSSR/AGG011R		ACAGTCTCGTCCTGTTCGG
PtrSSR/AGT008F	M	CACGACGTTGTAAAACGACTGATGCGCAACATGTCGAGTA
PtrSSR/AGT008R		TAAGGGGCAGGACTTGGAAA
PtrSSR/AGT008F	M	CACGACGTTGTAAAACGACTGATGCGCAACATGTCGAGTA
PtrSSR/AGT008R		TAAGGGGCAGGACTTGGAAA
PtrSSR/AGT009F	M	CACGACGTTGTAAAACGACGGGGATCCACACTAGTCAACG
PtrSSR/AGT009R		GCGAACGAACTCGAGATTGA
PtrSSR/AGT014F	M	CACGACGTTGTAAAACGACTGCCGTAAGCAGTCCAAGC
PtrSSR/AGT014R		AAATCAGACGCCCAGTAGTCA
PtrSSR/AGT015F	P	CACGACGTTGTAAAACGACTGTCTTGAAGCCTGCTCTGAA
PtrSSR/AGT015R		CAGTTAAGCTAGCGGGGTAAA
PtrSSR/AGT017F	M	CACGACGTTGTAAAACGACATCCTGCATTCTGCTGCATA
PtrSSR/AGT017R		TCCTCGCAGCCTAGTGGTAGT
PtrSSR/AGT020F	P	CACGACGTTGTAAAACGACCCGTTAGGGTCGTGAGAAAAA
PtrSSR/AGT020R		TGCCATGAACTATACTCCGGT
PtrSSR/AGT021F	N	CACGACGTTGTAAAACGACTGTGGTGGATTCGGATTGATT
PtrSSR/AGT021R		TGGACATGATGCGCAACAT

Primer name	Polymorphism[b]	Primer sequence [a]
PtrSSR/AT004F	M	CACGACGTTGTAAAACGACTGCTTGGTGCCGTAGTACTTG
PtrSSR/AT004R		ACCCCCTATAGCCGAATATCT
PtrSSR/AT005F	M	CACGACGTTGTAAAACGACTACGGTCAATAGGGCTTCGAT
PtrSSR/AT005R		GACGCTCGCAAAGTCTACATA
PtrSSR/AT006F	M	CACGACGTTGTAAAACGACCGGATTTTTGGAGGGGTTAT
PtrSSR/AT006R		GCTCCATATGCCAGTATGGAT
PtrSSR/AT007F	N	CACGACGTTGTAAAACGACAAGGGTGTCCGTCACTGTAAA
PtrSSR/AT007R		ACATCCAGATCATCTCGACGA
PtrSSR/AT012F	M	CACGACGTTGTAAAACGACACGGCCTTCGGCAAGTCTA
PtrSSR/AT012R		CCTGTAGCGTGAGGCTGATAT
PtrSSR/AT014F	P	CACGACGTTGTAAAACGACGCAAGGGTCAGTAGCGTAAAA
PtrSSR/AT014R		ATGGTTTGATTATTCCCGCC
PtrSSR/AT016F	M	CACGACGTTGTAAAACGACGCACGTGACATGCGCAATA
PtrSSR/AT016R		AACAGCAATCAATCAGCGG
PtrSSR/AT017F	N	CACGACGTTGTAAAACGACAAACCAACACTGATCACCCCT
PtrSSR/AT017R		AACAGCAATCAATCAGCGG
PtrSSR/AT018F	N	CACGACGTTGTAAAACGACAGGATTGCAGCTGGTAGCTTT
PtrSSR/AT018R		TTTAGAAGGCTGCGAGGTGG
PtrSSR/AT019F	M	CACGACGTTGTAAAACGACAGCTGCACGCAACACGGAT
PtrSSR/AT019R		ACGCCATCCACACAAATCT
PtrSSR/AT021F	M	CACGACGTTGTAAAACGACTGAAGAAGAGTCGCGATGAA
PtrSSR/AT021R		ACCATCATGATTACGTCCTCC
PtrSSR/ATG002F	M	CACGACGTTGTAAAACGACCAGTGGCTTTGCCACTAAAA
PtrSSR/ATG002R		TAGGTCGCTCGACGACATGA
PtrSSR/ATG003F	M	CACGACGTTGTAAAACGACATGTTGCAGAGCCTCGACA
PtrSSR/ATG003R		AAGCGTCTTTGGGCGAAGA
PtrSSR/ATG004F	M	CACGACGTTGTAAAACGACAACTTTGCTCGACCGTCAA
PtrSSR/ATG004R		TTACCGTCGTCTGCTCGATAA
PtrSSR/ATG005F	N	CACGACGTTGTAAAACGACTCATCGCACTGTGGTGGATT
PtrSSR/ATG005R		TTGCTAGTCTTTTCCCCATCC
PtrSSR/AAC016F	M	CACGACGTTGTAAAACGACATAGTCTAGATGGGCGAGCGT
PtrSSR/AAC016R		ATAAGACGGTGGCGTAGCGT
PtrSSR/AAC018F	M	CACGACGTTGTAAAACGACTACTTAAGCATTCGAAGCCG
PtrSSR/AAC018R		AGCAGCGTTCTTGAGGAAGA
PtrSSR/AAG044F	N	CACGACGTTGTAAAACGACTGTGGTGGATTCCCATTTCA
PtrSSR/AAG044R		GCGAAGTAGAGTAGAGGGCCT
PtrSSR/AAT005F	P	CACGACGTTGTAAAACGACTAGAGCCTGCCGAGATTGTTT
PtrSSR/AAT005R		GCCTTCGGCCAAAGTCATA
PtrSSR/AAT023F	M	CACGACGTTGTAAAACGACCCCGCTGCTACATCTCTTTCT
PtrSSR/AAT023R		GGAGAACCTAGGCTTCTGAAA
PtrSSR/AC018F	N	CACGACGTTGTAAAACGACTGCAGTAAGCACAGTGAAGGC
PtrSSR/AC018R		ATCGGCGGAGAAGATCATTT
PtrSSR/AC023F	N	CACGACGTTGTAAAACGACCACAAAGGCCACCAAGGGA
PtrSSR/AC023R		AACCCGCTTTGTAGTGGCTT
PtrSSR/AC030F	M	CACGACGTTGTAAAACGACGGGAGACGACCATCCGTTATA
PtrSSR/AC030R		CAACCGCGGAAAATATCTTC
PtrSSR/AC037F	M	CACGACGTTGTAAAACGACTACCACTAACCGCCCCTTAA
PtrSSR/AC037R		TAAGCGAGGGAGGGATGTG
PtrSSR/AC042F	M	CACGACGTTGTAAAACGACAGCCACAGCATTACACACACA
PtrSSR/AC042R		TTTGCACTCTCTTTACACCCA
PtrSSR/AC046F	M	CACGACGTTGTAAAACGACGGGTAAATGAAAAACGCAGA
PtrSSR/AC046R		AACGATGGGCACAGAGCAGTA
PtrSSR/AC049F	M	CACGACGTTGTAAAACGACTGATTCATTCGCACTGTGGT
PtrSSR/AC049R		ATGTCCCACACTCAACACGT
PtrSSR/AC054F	N	CACGACGTTGTAAAACGACATTCCCGTCGTACCTGTATGA
PtrSSR/AC054R		ACGCACTACACGGCAATGA
PtrSSR/AC064F	N	CACGACGTTGTAAAACGACAAGGTCGTGGGTTTTTGCTT
PtrSSR/AC064R		AGCGGGGTGTATCAAAGTCAA
PtrSSR/AC068F	M	CACGACGTTGTAAAACGACGCATTCCAAAGTCCGGCT
PtrSSR/AC068R		TGCCACTGCACCTGTATTTCT
PtrSSR/AC074F	P	CACGACGTTGTAAAACGACCGTTGTCTTGTTTTGAGCGA

Primer name	Polymorphism[b]	Primer sequence [a]
PtrSSR/AC074R		GACACACCTCCAAACGCTATT
PtrSSR/AC085F	P	CACGACGTTGTAAAACGACTGTAGATGCGAGTGCGGGTA
PtrSSR/AC085R		GGAATCTTTTCATCCAATTGC
PtrSSR/AC089F	M	CACGACGTTGTAAAACGACTATCGGCGTTCTTGATGTGTC
PtrSSR/AC089R		ATTGCTTGTTGCTAGTCACCG
PtrSSR/AC109F	P	CACGACGTTGTAAAACGACGCGGCTGATAGAGTACATGTG
PtrSSR/AC109R		TGTGAAGGATTTGGCGAAGA
PtrSSR/AG025F	M	CACGACGTTGTAAAACGACTCTTGCGCTCGTTTGTTTTC
PtrSSR/AG025R		GAACGACACGTCTCCACGATA
PtrSSR/AG044F	M	CACGACGTTGTAAAACGACTTTTGTCTGAGTAACCACGGC
PtrSSR/AG044R		TCAACCATACGGCAGCCAT
PtrSSR/AGC001F	N	CACGACGTTGTAAAACGACAGAAGAGGAAGGAGCAGCAA
PtrSSR/AGC001R		TGTTGAAGACCTGGAGGAGTT
PtrSSR/AGG009F	N	CACGACGTTGTAAAACGACTGGATTCAAGCAGCCAAAGA
PtrSSR/AGG009R		TAACCCACGTCCAGTCCATCA
PtrSSR/AGT012F	M	CACGACGTTGTAAAACGACAACTGTGGTGGATTCAGTGGT
PtrSSR/AGT012R		AGCATAAATACGTTGACCCCC
PtrSSR/AT015F	M	CACGACGTTGTAAAACGACAAAGAGCAGGGTTATGGTGGA
PtrSSR/AT015R		GCAACCAGTCCAATCATATTG
PtrSSR/ATG006F	M	CACGACGTTGTAAAACGACGCACAGATATCTCCATTCCCA
PtrSSR/ATG006R		AAGCGACCATCGTGGCAAT
PtrSSR/AG035F	N	CACGACGTTGTAAAACGACTTGCAGGTCGACTCTAGAGGA
PtrSSR/AG035R		CGCACCCCAAACCTATACCTA
PtrSSR/AAT010F	M	CACGACGTTGTAAAACGACAGCGAACCATACCAGAAAACC
PtrSSR/AAT010R		CCCCTGAGTACCCTACTACGA
PtrSSR/AAG042F	N	CACGACGTTGTAAAACGACGGCTAAGAATGGAAATGACGA
PtrSSR/AAG042R		TGCGAGGTGAAAGAAAGAAGA
PtrSSR/AC052F	M	CACGACGTTGTAAAACGACATTCACCCATCGTATCGCAT
PtrSSR/AC052R		TTTGAGCTCTTCGAATTCGG
PtrSSR/AAG043F	N	CACGACGTTGTAAAACGACCGCCACTGTGGTGGATTCTAT
PtrSSR/AAG043R		TTGAGAGCACTGCTGCCCA
PtrSSR/AAT017F	N	CACGACGTTGTAAAACGACAGCCAAAAACCATATTAGCGA
PtrSSR/AAT017R		AATACAACGCCAGAGTCCCCT
PtrSSR/AAT021F	N	CACGACGTTGTAAAACGACTTACTCCACGAACCTCACCTC
PtrSSR/AAT021R		TAGTCTTCTGTTGGCGCAGGT
PtrSSR/AAT024F	M	CACGACGTTGTAAAACGACTCCCGGACTTCTGCTTCTTCT
PtrSSR/AAT024R		ACCACCCAAAATATGCCCAT
PtrSSR/AG020F	M	CACGACGTTGTAAAACGACTAACGTGTTGCCACATGTTG
PtrSSR/AG020R		TTGCGCTCCGACACTAAACTT
PtrSSR/AGT011F	M	CACGACGTTGTAAAACGACTATCGGGGGAAACGCAAA
PtrSSR/AGT011R		TACAAGATCTTCCGACGGGC
PtrSSR/AGT011F	M	CACGACGTTGTAAAACGACTATCGGGGGAAACGCAAA
PtrSSR/AGT011R		TACAAGATCTTCCGACGGGC
PtrSSR/AC002F	M	CACGACGTTGTAAAACGACGCCCTGGTATCTCACCAAGAA
PtrSSR/AC002R		TCAGCCAGGCATCCATTATT
PtrSSR/AAT019F	N	CACGACGTTGTAAAACGACACGGGGGACCCACATTATTAT
PtrSSR/AAT019R		TTCGGACGACCTTCACATCTT
PtrSSR/AAT013F	M	CACGACGTTGTAAAACGACAGCCAAACAAACACCCAAAC
PtrSSR/AAT013R		TGAGGCCGCACACAATAGAT
PtrSSR/AAC014F	M	CACGACGTTGTAAAACGACATTCATCGCTCGGGGGTTT
PtrSSR/AAC014R		TACTTCGACCGTCACGCAA
PtrSSR/AAC001F	P	CACGACGTTGTAAAACGACTATCGCGGTAGGATTGTGGTT
PtrSSR/AAC001R		TCAAGGCGGATCGGAAATTA
PtrSSR/AAC002F	M	CACGACGTTGTAAAACGACATCGCTTTCGTTGCGTCTGT
PtrSSR/AAC002R		ACGGCTGAACTGGCACAGTA
PtrSSR/AAC005F	P	CACGACGTTGTAAAACGACTGACTTGGGGGATCGTCTAT
PtrSSR/AAC005R		TGGCAGTCTAGGGGTTGTG
PtrSSR/AAC006F	P	CACGACGTTGTAAAACGACTGATCGTGCATTTGCGATG
PtrSSR/AAC006R		ATTCCAGGCCACAGTCAAGTA
PtrSSR/AAC007F	M	CACGACGTTGTAAAACGACTGTGGTGGATTCTTGTTGTTG
PtrSSR/AAC007R		AAATTACTTTGGCGCCCC

Primer name	Polymorphism[b]	Primer sequence [a]
PtrSSR/AAC009F	P	CACGACGTTGTAAAACGACTTTGGGAGATGGGGGAAA
PtrSSR/AAC009R		TTGCGTCTGTGCGACATG
PtrSSR/AAC012F	M	CACGACGTTGTAAAACGACACCATTTTGCATGGCCCT
PtrSSR/AAC012R		TCCCTTTCTAAGCCAGAAGCA
PtrSSR/AAC019F	P	CACGACGTTGTAAAACGACACGACCACTTTAGGGGAGAAT
PtrSSR/AAC019R		CGGCAAGGAGTATGATGAGTT
PtrSSR/AAG002F	M	CACGACGTTGTAAAACGACCGGGTACAATTGTGGTGGATT
PtrSSR/AAG002R		GGGTGTTTAAAAGTCAACGCA
PtrSSR/AAG003F	M	CACGACGTTGTAAAACGACTAGAGGACCCACGTGCAA
PtrSSR/AAG003R		AGCGTCAGGAAGAAGCCTTG
PtrSSR/AAG004F	M	CACGACGTTGTAAAACGACTTCGCCTGCAGATATGGGA
PtrSSR/AAG004R		TAACGTGTGACCTCGACACTT
PtrSSR/AAG005F	M	CACGACGTTGTAAAACGACATCAGTAGGTGCCGCAATCTT
PtrSSR/AAG005R		TTCATCGCGGTGAACTGAA
PtrSSR/AAG007F	M	CACGACGTTGTAAAACGACCCGTTCCCTATAAGCAAAAGC
PtrSSR/AAG007R		GCCAGAGAGATCTTCGAGTCA
PtrSSR/AAG008F	M	CACGACGTTGTAAAACGACCTGCGGCCAAAAAAAGATTG
PtrSSR/AAG008R		TATTCCCACAATAGCTCTGCC
PtrSSR/AAG009F	N	CACGACGTTGTAAAACGACAAATCATCGCACTGTGGTGG
PtrSSR/AAG009R		TATCCCAGGATTCCCGGAAA
PtrSSR/AAG010F	M	CACGACGTTGTAAAACGACGGTTCAATGTGGTGGATTCTG
PtrSSR/AAG010R		GAAGGAATGCCTGTATGCAA
PtrSSR/AAG011F	P	CACGACGTTGTAAAACGACCGGTACATGCATGACGTTGTT
PtrSSR/AAG011R		GCACGCTTATATGCGCTTTT
PtrSSR/AAG012F	M	CACGACGTTGTAAAACGACCGGCACAGTAAGGAGCTAGAT
PtrSSR/AAG012R		CCGTTAGGTCCTAGTGAATGC
PtrSSR/AAG013F	M	CACGACGTTGTAAAACGACTTGATGCTGTTCTGCGTGA
PtrSSR/AAG013R		GCCCTTATCCGATCACCTAGT
PtrSSR/AAG014F	P	CACGACGTTGTAAAACGACCGCCAACATAGTTGCGAATT
PtrSSR/AAG014R		AGGGTGTTTAAAAGTCAACGC
PtrSSR/AAG015F	N	CACGACGTTGTAAAACGACACTGTGGTGGATTCGTTGAA
PtrSSR/AAG015R		TTCTTAGCTCCGCCTAGGTG
PtrSSR/AAG016F	P	CACGACGTTGTAAAACGACGCGTGATCTCGCCACATATTA
PtrSSR/AAG016R		CGACGTCCTTCACAGGATTTA
PtrSSR/AAG017F	M	CACGACGTTGTAAAACGACATCATCGCACTGTGGTGGATT
PtrSSR/AAG017R		GGCGGCTTCATATTACTGAA
PtrSSR/AAG018F	M	CACGACGTTGTAAAACGACAGCGTCTGGAAGAAGCCTTG
PtrSSR/AAG018R		TCTCTGCATTTGTGGTGCAA
PtrSSR/AAG019F	M	CACGACGTTGTAAAACGACCTGCGGCCAAAAAAAGATTG
PtrSSR/AAG019R		TTTTCGGCCCACAGGGTTT
PtrSSR/AAG020F	P	CACGACGTTGTAAAACGACATTAGATATGAGTCCGGGCTG
PtrSSR/AAG020R		CGTAGGAGACCTGGTATCTCG
PtrSSR/AAG022F	P	CACGACGTTGTAAAACGACTCGTGGGTATAAAACGGCTCT
PtrSSR/AAG022R		TTCGGCGGCTTCATATTACT
PtrSSR/AAG024F	P	CACGACGTTGTAAAACGACCGCCAACATAGTTGCGAATT
PtrSSR/AAG024R		TCCGCCTCTGTAGGTGTTAAA
PtrSSR/AAG025F	P	CACGACGTTGTAAAACGACAAGAGGTGTCGACTAGCGTTT
PtrSSR/AAG025R		GGCTTAATTTTAAGCGCGTG
PtrSSR/AAG027F	M	CACGACGTTGTAAAACGACCAAATCATCGCCACTGTGGT
PtrSSR/AAG027R		TCGCCTTATCAATACGCCAT
PtrSSR/AAG028F	M	CACGACGTTGTAAAACGACTTAGCGAGATCACGCGTTAT
PtrSSR/AAG028R		GTGCATTCCAGAAGCCTAAA
PtrSSR/AAG029F	M	CACGACGTTGTAAAACGACCGGAGTACATTGTGGTGGATT
PtrSSR/AAG029R		CGGTACATGCATGACGTTGTT
PtrSSR/AAG030F	P	CACGACGTTGTAAAACGACCCATTAAAATCCAGTCCACCC
PtrSSR/AAG030R		AGCCAACTGCATCGTAGTTTG
PtrSSR/AAG031F	M	CACGACGTTGTAAAACGACTGGATTAGACGGGGACATTGT
PtrSSR/AAG031R		TAGCCACCTGCATCGTATTCT
PtrSSR/AAG032F	M	CACGACGTTGTAAAACGACTAGACGGGTACATTGTGGTGG
PtrSSR/AAG032R		ACCGTTGTGGGTGTATACCAA
PtrSSR/AAG033F	N	CACGACGTTGTAAAACGACACCCCACCCTCCCATTAAA

Primer name	Polymorphism[b]	Primer sequence [a]
PtrSSR/AAG033R		TTGCCCAGAAGAGGTTGAA
PtrSSR/AAG034F	P	CACGACGTTGTAAAACGACGCCCTTATCCGATCACCTAGT
PtrSSR/AAG034R		CGGCCAAGTCCATTGAATCTA
PtrSSR/AAG035F	M	CACGACGTTGTAAAACGACGCACGCTTATATGCGCTTTT
PtrSSR/AAG035R		ACACATGACACACGCTTGTTT
PtrSSR/AAG036F	M	CACGACGTTGTAAAACGACTTACGGATGTCGGAGGGTGTT
PtrSSR/AAG036R		TATACCAAGCTCTCCGCCTCT
PtrSSR/AAG037F	M	CACGACGTTGTAAAACGACATCGCCACTGTGGTGGATT
PtrSSR/AAG037R		ATTGCCGTGGGGTGAGAGT
PtrSSR/AAG038F	M	CACGACGTTGTAAAACGACTGCATTTGTGGTGCAAGATC
PtrSSR/AAG038R		AGAAGCCTTGGCCATTTTCA
PtrSSR/AAG039F	P	CACGACGTTGTAAAACGACCGCCAACATAGTTGCGAATT
PtrSSR/AAG039R		CGCCTCTGTAGGGTGTTTAAA
PtrSSR/AAG047F	M	CACGACGTTGTAAAACGACACATCGACACGTCGACCGT
PtrSSR/AAG047R		TATGGTGAGGGATCGAGGGTA
PtrSSR/AAG052F	M	CACGACGTTGTAAAACGACGCCGGAAATAACCAATCAGA
PtrSSR/AAG052R		ACGGATACGGTTCAAGCAAT
PtrSSR/AAG053F	P	CACGACGTTGTAAAACGACCAAGTGCATTCCAGAAGCCTA
PtrSSR/AAG053R		TCCTTCACAGGATTTAGCGA
PtrSSR/AAG058F	M	CACGACGTTGTAAAACGACGGTCCGTTAGGTCCTAGTGAA
PtrSSR/AAG058R		GGCTTAATTTTAAGCGCGTG
PtrSSR/AAG059F	M	CACGACGTTGTAAAACGACAGCTCATGTGGTGGATTCCTT
PtrSSR/AAG059R		TTTTTTTGGAAAGCCCCAGG
PtrSSR/AC110F	P	CACGACGTTGTAAAACGACCCTCTGCGCTCTTCTTCTTAG
PtrSSR/AC110R		CCAGAACATCAACAACACCGT
PtrSSR/AC112F	M	CACGACGTTGTAAAACGACAGCACAGCAGGCAAAGGAA
PtrSSR/AC112R		GCATTGCGGTAGCTGTGTCTA
PtrSSR/AG005F	M	CACGACGTTGTAAAACGACTCACCATGTCTGCTTCTGCAT
PtrSSR/AG005R		TAATCGCCCAAGTAGCATCGT
PtrSSR/AG013F	M	CACGACGTTGTAAAACGACTCTGCATTTGTGGTGCAAGA
PtrSSR/AG013R		TAACTTGATTTGAGCGCGTG
PtrSSR/AG014F	M	CACGACGTTGTAAAACGACAATCGTCACGTAGATCGAACC
PtrSSR/AG014R		TTAGCACCTAAGACGTCGATG
PtrSSR/AG016F	M	CACGACGTTGTAAAACGACTGTGTAGGGGACCGGAATG
PtrSSR/AG016R		TGAATGAGGAACGGCATTTG
PtrSSR/AG017F	M	CACGACGTTGTAAAACGACTTCGCCTGCAGATATGGGA
PtrSSR/AG017R		ACGGGTAGCTCTATGTGAGGA
PtrSSR/AG018F	P	CACGACGTTGTAAAACGACACGTCCCTGCTTACGGATGT
PtrSSR/AG018R		ATACCAAGCTTCTCCGCCTCT
PtrSSR/AG023F	M	CACGACGTTGTAAAACGACTCAAACTCAGAAATCCGCCT
PtrSSR/AG023R		TTTCCCACTCTCCTGCCCTAT
PtrSSR/AG024F	M	CACGACGTTGTAAAACGACTCAGGTTTTCGAATTCCCGT
PtrSSR/AG024R		GCGAGCATTTTTCAGGTTTAC
PtrSSR/AG026F	M	CACGACGTTGTAAAACGACAAAGATACCTCCACAGCAGCA
PtrSSR/AG026R		TGACGCTCAGACGTGGTTT
PtrSSR/AG029F	P	CACGACGTTGTAAAACGACAAGAGGGAGCCACTGCAAAT
PtrSSR/AG029R		AGGGAAGACGGAGTGCCTGTA
PtrSSR/AG037F	N	CACGACGTTGTAAAACGACTTGGAAGTGCATGGCAAACT
PtrSSR/AG037R		GGTGTGAGGGATTATTCTGCT
PtrSSR/AG039F	M	CACGACGTTGTAAAACGACTTTGTCCCGCACAACACAA
PtrSSR/AG039R		ACATGGAGAGTGAGTGCAGTG
PtrSSR/AG049F	P	CACGACGTTGTAAAACGACACCACCACTGACCCATCTTTT
PtrSSR/AG049R		TCACGGTGATGGCACATATA
PtrSSR/AG051F	P	CACGACGTTGTAAAACGACCCCGACCAGGTACTAAGAAGA
PtrSSR/AG051R		GGCTTGGTTCTTTTTTCGGA
PtrSSR/AG058F	M	CACGACGTTGTAAAACGACTGTCCGCCTTCATGTACCTA
PtrSSR/AG058R		TGGGGGTGAGTTTTACGTTT
PtrSSR/AG061F	P	CACGACGTTGTAAAACGACCAAGCTGTTCAACGCAAGAGT
PtrSSR/AG061R		ATGCGCGTAGAGACAGAAGG
PtrSSR/AG068F	N	CACGACGTTGTAAAACGACGCAGAGGAGGTTGTGATTCCA
PtrSSR/AG068R		TTATGCGTCGTCTTGGAGTT

Primer name	Polymorphism[b]	Primer sequence [a]
PtrSSR/AG071F	M	CACGACGTTGTAAAACGACTGAACCAGAGAGTGAGTGGAA
PtrSSR/AG071R		TCCGTTCGTGGACGTTGA
PtrSSR/AGG001F	M	CACGACGTTGTAAAACGACGGAGGAGAAGGAGGAGGAGAA
PtrSSR/AGG001R		ACGATCTGCTGTCTCATCTCA
PtrSSR/AGG006F	N	CACGACGTTGTAAAACGACTGGAGACCGATAGTTGAGGAT
PtrSSR/AGG006R		TCACTGTCCTCAACCACCG
PtrSSR/AGG008F	P	CACGACGTTGTAAAACGACGCGAGTTAGAAACGAGGCAA
PtrSSR/AGG008R		ATATGGTGGTTGCTTGCGCTA
PtrSSR/AGT001F	P	CACGACGTTGTAAAACGACCCACGCTCATCACTTTGTCTA
PtrSSR/AGT001R		CCACTTGATTGAGTTTTGCG
PtrSSR/AGT002F	P	CACGACGTTGTAAAACGACTTACGATGCGCTCACACTAGA
PtrSSR/AGT002R		ATGGCCCGCTTGTAGTCTTTA
PtrSSR/AGT003F	M	CACGACGTTGTAAAACGACATCCACCTCCGTTGCAGTT
PtrSSR/AGT003R		CTGATTTCACCGCGAAAACA
PtrSSR/AGT004F	M	CACGACGTTGTAAAACGACTTACGCCCCGTTCGTAGATAT
PtrSSR/AGT004R		TGTTCAATCTTGCGTCGATG
PtrSSR/AGT005F	M	CACGACGTTGTAAAACGACCCACTGGCTCATCACTTTGT
PtrSSR/AGT005R		ACCACTTGATTGAGTTTGCG
PtrSSR/AGT006F	P	CACGACGTTGTAAAACGACTTCGCTACCACACGACTGTAT
PtrSSR/AGT006R		CGAACGAGGCAGCCTAGTATT
PtrSSR/AGT007F	P	CACGACGTTGTAAAACGACCCATGAGGAGAAGTATTCCCA
PtrSSR/AGT007R		ACCGCGAAACATCTGTGAGA
PtrSSR/AGT010F	M	CACGACGTTGTAAAACGACTGGGGTGGTGTGATGTAAAT
PtrSSR/AGT010R		CCTGATTTCACGCGAAAACA
PtrSSR/AGT013F	N	CACGACGTTGTAAAACGACAGCAACCAATCAGCGTAGAGA
PtrSSR/AGT013R		AATGCAACGCAGTCCTCCT
PtrSSR/AGT016F	N	CACGACGTTGTAAAACGACATTGAGGCTCTGTCATCTTGC
PtrSSR/AGT016R		ACCCGGTGGTCGTAGTACTAG
PtrSSR/AGT018F	M	CACGACGTTGTAAAACGACTTGTATGCGAGTGAGTATGGG
PtrSSR/AGT018R		CCGCCGCTTAAGCTTGTATTA
PtrSSR/AGT019F	P	CACGACGTTGTAAAACGACAATCGCACAAGCACGTGTCA
PtrSSR/AGT019R		GTTTTGCGTTTTCCTTGTAGC
PtrSSR/AGT022F	M	CACGACGTTGTAAAACGACACTCCCATCCTTTTCTAGCGA
PtrSSR/AGT022R		TTCTGTATTCCCAACCACAGC
PtrSSR/AT001F	M	CACGACGTTGTAAAACGACTGATACCGGCCATGTGTATA
PtrSSR/AT001R		GGATGGGTGTAGACCCACATA
PtrSSR/AT002F	P	CACGACGTTGTAAAACGACCTAAGCGGGAAGCTTGGTCTA
PtrSSR/AT002R		GCATCGCTTAAGGTGCAAAA
PtrSSR/AT003F	M	CACGACGTTGTAAAACGACTTATATATATGGCGGTCCCCG
PtrSSR/AT003R		CACGAATAGTATATGTCGCGG
PtrSSR/AT008F	M	CACGACGTTGTAAAACGACTGTGGGTATTCGCATCTGAT
PtrSSR/AT008R		CGCTACGGATGCCTAGACATA
PtrSSR/AT009F	N	CACGACGTTGTAAAACGACGGGGTGGTGTAAAATGTTGA
PtrSSR/AT009R		ACACGGGTATGGATACGGTAT
PtrSSR/AT010F	M	CACGACGTTGTAAAACGACTCGTCACACGTTGTCTCGTTA
PtrSSR/AT010R		CCCCTCCTCACATATCGGTAT
PtrSSR/AT011F	M	CACGACGTTGTAAAACGACGGGACCCCGTGTGTATATAT
PtrSSR/AT011R		TGGAGATAGAGTCCTTTGTGG
PtrSSR/AT013F	P	CACGACGTTGTAAAACGACGGGGATATCATACATCCAGCA
PtrSSR/AT013R		GGTGCATGTACACGTGTGCAT
PtrSSR/AT020F	M	CACGACGTTGTAAAACGACAGGGGAAAGGGGTTTGGTTAT
PtrSSR/AT020R		TCACGAAAGCATTTCAGGGA
PtrSSR/AT022F	N	CACGACGTTGTAAAACGACTGCATCCAAGCTCGTCGA
PtrSSR/AT022R		CGCAGATCTTGCATGTGAGAT
PtrSSR/AT023F	N	CACGACGTTGTAAAACGACTCCTGAATGATGCACAGACGA
PtrSSR/AT023R		TCCCAATAACCGCCCATATA
PtrSSR/ATG001F	P	CACGACGTTGTAAAACGACTCCTGACCTTACCCAGAATCA
PtrSSR/ATG001R		GATCCGATACCACGGAGATT
SC20-SSR1F	M	ATACGGCAAAGGTGAGGTTG
SC20-SSR1R		GGTTTGAAGGGTGGGTAGGT
SC20-SSR2F	P	TCGATCATGGAGCAAAACCT

Primer name	Polymorphism[b]	Primer sequence [a]
SC20-SSR2R		CAGTGCGACGTTCTCGTCTA
SC20-SSR3F	M	CGGAAATTGGACTACGCAAC
SC20-SSR3R		TCTGCAATAGACGCTGCACT
SC20-SSR4F	P	AAATCAAACACCCGGAATCA
SC20-SSR4R		GCCGACGTCTCTACCGATAC
SC20-SSR5F	P	TAATCGAGCATGGTTGTGGA
SC20-SSR5R		TGCAGTTGTTGACGTCTTGTC
SC20-SSR6F	M	GCTCTTTAGCACCTGGCAAC
SC20-SSR6R		CGGTGCTGGTTTGGAATAGT
SC19-SSR1F	M	GAATGCCTTGGAATCAGGAA
SC19-SSR1R		TTCAGCACATTCGGCATTTA
SC19-SSR2F	P	CGGGTAGTTGAGACCACCAT
SC19-SSR2R		GCCTCAAAATGCAAGGAAAT
SC19-SSR3F	M	CGGACTTCTGCTTCTTCTGG
SC19-SSR3R		TCTGTTGTCCAACGCTGTTC

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

[b] "P" means primer set generated polymorphic amplicons between 86-124 and DW5; "M" means produce mono-morphic amplicon; "N" means no amplification.

APPENDIX B. LIST OF STARP PRIMERS USED IN GENETIC MAPPING OF THE 86-124Δ*MAT1-2-1* × DW5Δ*MAT1-1-1*

Primer name	Polymorphism[a]	Primer sequence
SC1.23kAMAS1	N	GCAACAGGAACCAGCTATGACAGCAACAAAGCTGGTATC
SC1.23kAMAS2		GACGCAAGTGAGCAGTATGACAGCAACAAAGCTGGCCTG
SC1.23kRev		ACTTCGGCGGATACAACAAC
SC1.164kAMAS1	P	GCAACAGGAACCAGCTATGACCGGTAGTGCGTCATCTGATAC
SC1.164kAMAS2		GACGCAAGTGAGCAGTATGACCGGTAGTGCGTCATCTGGCAT
SC1.164kRev		CGGCAACCCAGTTTGTAGTT
SC1.402kAMAS1	P	GCAACAGGAACCAGCTATGACAGCGGACGTTCAGGTTTC
SC1.402kAMAS2		GACGCAAGTGAGCAGTATGACAGCGGACGTTCAGGCCTT
SC1.402kRev		ATCGTTGCCCGTTAGACATC
SC1.477AMAS1	N	GCAACAGGAACCAGCTATGACGAAAGAAACGAGATACTGCATG
SC1.477AMAS2		GACGCAAGTGAGCAGTATGACGAAAGAAACGAGATACTGTGTA
SC1.477kRve		AGTTTACGACTTCGCATGAGC
SC1.745kAMAS1	P	GCAACAGGAACCAGCTATGACTGTCTCACTATCATGTGCTTCACC
SC1.745kAMAS2		GACGCAAGTGAGCAGTATGACTGTCTCACTATCATGTGCTTACCT
SC1.745kRev		CATTGGCGTTTTACCGACTT
SC1.823kAMAS1	N	GCAACAGGAACCAGCTATGACAGTTGAGCACCACCCCAG
SC1.823kAMAS2		GACGCAAGTGAGCAGTATGACAGTTGAGCACCACCTAAA
SC1.823kRev		CGAGATCGGGAGTTGATTTC
SC1.1041kAMAS1	P	GCAACAGGAACCAGCTATGACAGTCAGTCCGAAGAAAAAAGG
SC1.1041kAMAS2		GACGCAAGTGAGCAGTATGACAGTCAGTCCGAAGAAAAGCGT
SC1.1041kREV		TCACGCCATACAAATGGAAA
SC1.1266kAMAS1	M	GCAACAGGAACCAGCTATGACCACGCTCTCAAACTGGATCGAC
SC1.1266kAMAS2		GACGCAAGTGAGCAGTATGACCACGCTCTCAAACTGGATAAAT
SC1.1266kRev		ATCTCCGATCCATCTGCTTG
SC1.1370kAMAS1	P	GCAACAGGAACCAGCTATGACGCAATGAGAACCTTATACCTATC
SC1.1370kAMAS2		GACGCAAGTGAGCAGTATGACGCAATGAGAACCTTATACCCCTT
SC1.1370kRev		ATCCCGACAGTCCTGACATC
SC1.1426kAMAS1	P	GCAACAGGAACCAGCTATGACGCATCTAGGGGATTTGTCTG
SC1.1426kAMAS2		GACGCAAGTGAGCAGTATGACGCATCTAGGGGATTTGCTTA
SC1.1426kRev		TAGACAGCGCCAGCAAGTAA
SC1.1784kAMAS1	P	GCAACAGGAACCAGCTATGACAGTCAGGCTTTTAGCATAGGCCG
SC1.1784kAMAS2		GACGCAAGTGAGCAGTATGACAGTCAGGCTTTTAGCATAGAACA
SC1.1784kRev		AAGAAACTCCCGCCTTGGTC
SC1.1911kAMAS1	P	GCAACAGGAACCAGCTATGACAGTCAACCCACAATACAGATCCG
SC1.1911kAMAS2		GACGCAAGTGAGCAGTATGACAGTCAACCCACAATACAGACACA
SC1.1911kRev		GACGCCCAGGCTCGTATAAA
SC1.2053kAMAS1	P	GCAACAGGAACCAGCTATGACTCTGGTCTCACAGCATGG
SC1.2053kAMAS2		GACGCAAGTGAGCAGTATGACTCTGGTCTCACAGCCCGA
SC1.2053kRev		CAAGACCTCCACCGCACTAT

Primer name	Polymorphism[a]	Primer sequence
SC1.2834kAMAS1	P	GCAACAGGAACCAGCTATGACGTGACTATACATTGCGAGTCCGC
SC1.2834kAMAS2		GACGCAAGTGAGCAGTATGACGTGACTATACATTGCGAGTTTGG
SC1.2834kRev		TCAGTGCCGATGGGAGACTA
SC1.4229KAMAS1	P	GCAACAGGAACCAGCTATGACCAAGATGGACTGGTGTTCCC
SC1.4229KAMAS2		GACGCAAGTGAGCAGTATGACCAAGATGGACTGGTGTCTCA
SC1.4229KRev		CAATCAAAGGAAGTGCGTGA
SC1.4477KAMAS1	P	GCAACAGGAACCAGCTATGACGCCGGTCCATGAAATACAGC
SC1.4477KAMAS2		GACGCAAGTGAGCAGTATGACCGCGGTCCATGAAATATCGT
SC1.4477KRev		AGCGAAGAGTTGTCCGGTTA
SC1.4593KAMAS1	M	GCAACAGGAACCAGCTATGACGGTCCAGTTAGGAAGCAACGG
SC1.4593KAMAS2		GACGCAAGTGAGCAGTATGACGGTCCAGTTAGGAAGCACAGA
SC1.4593KRev		ACCAAGAGCACCAAGGTCAT
SC1.4929KAMAS1	P	GCAACAGGAACCAGCTATGACTCTCCACACCATCCTTATCC
SC1.4929KAMAS2		GACGCAAGTGAGCAGTATGACTCTCCACACCATCCTTGCCT
SC1.4929KRev		AGTGTGCGACTCCTGGAAAT
SC1.5363KAMAS1	P	GCAACAGGAACCAGCTATGACGCATTCCACAAAGTTCCTTAG
SC1.5363KAMAS2		GACGCAAGTGAGCAGTATGACGCATTCCACAAAGTTCCCCAA
SC1.5363KRev		TACACGAGCGAGAACAGTCG
SC1.5588KAMAS1	M	GCAACAGGAACCAGCTATGACCAAGTGTCTAGGCTGTCTCCTG
SC1.5588KAMAS2		GACGCAAGTGAGCAGTATGACCAAGTGTCTAGGCTGTCTTATA
SC1.5588KRev		GCCCCGATATAAGTGAGCTG
SC1.6480KAMAS1	P	GCAACAGGAACCAGCTATGACGAAGACGCTGACAATTTGCAC
SC1.6480KAMAS2		GACGCAAGTGAGCAGTATGACGAAGACGCTGACAATTTAAAA
SC1.6480KRev		AATGTTTCCCCAGCTTCCTT
SC1.6675KAMAS1	P	GCAACAGGAACCAGCTATGACTGTTGATGATTACCTTGACCCG
SC1.6675KAMAS2		GACGCAAGTGAGCAGTATGACTGTTGATGATTACCTTGATACA
SC1.6675KRev		CTACCTGGACACGGAGAACC
SC2.70KAMAS1	P	GCAACAGGAACCAGCTATGACCGCTTCTTTTGAAATGAAC
SC2.70KAMAS2		GACGCAAGTGAGCAGTATGACCGCTTCTTTTGAAATAGAA
SC2.70KRev		TAGTTTGGTGCGCTCTGTTG
SC2.322KAMAS1	P	GCAACAGGAACCAGCTATGACGCCGAATCCTTCCATCCC
SC2.322KAMAS2		GACGCAAGTGAGCAGTATGACGCCGAATCCTTCCACTCA
SC2.322KRev		AACAGGCAAGAGCAAGTGGT
SC2.658KAMAS1	M	GCAACAGGAACCAGCTATGACACTGGGTCGAGACATCCG
SC2.658KAMAS2		GACGCAAGTGAGCAGTATGACACTGGGTCGAGACATCCA
SC2.658KRev		GCCAGTCGAAGAAACTCTGG
SC2.1442KAMAS1	P	GCAACAGGAACCAGCTATGACCGGTGCTTCAGAGACAGTTG
SC2.1442KAMAS2		GACGCAAGTGAGCAGTATGACCGGTGCTTCAGAGACAACTA
SC2.1442KRev		AGTGCTCATTTCCGTGCAGT
SC2.2003KAMAS1	P	GCAACAGGAACCAGCTATGACCAGAGATAGCAGGTCTCGCC
SC2.2003KAMAS2		GACGCAAGTGAGCAGTATGACCAGAGATAGCAGGTCTTACT
SC2.2003KRev		TTGGAGGTAACCGATTGCAG
SC2.2358KAMAS1	P	GCAACAGGAACCAGCTATGACGAAGCCAACACACACGAAAG

Primer name	Polymorphism[a]	Primer sequence
SC2.2358KAMAS2		GACGCAAGTGAGCAGTATGACGAAGCCAACACACACGCGAA
SC2.2358KRev		CCAACTCCGAGAAACAAAGG
SC2.2524KAMAS1	P	GCAACAGGAACCAGCTATGACAAAAATCCACCGAAGCCCTG
SC2.2524KAMAS2		GACGCAAGTGAGCAGTATGACAAAAATCCACCGAAGCTTTT
SC2.2524KRev		GGCGATACGTTGCGACTG
SC2.2720KAMAS1	P	GCAACAGGAACCAGCTATGACGCGTTTGGACAGCCACCG
SC2.2720KAMAS2		GACGCAAGTGAGCAGTATGACGCGTTTGGACAGCCCTCA
SC2.2720KRev		GCTTGTTGAGCAAATGGAGA
SC3.60kAMAS1	P	GCAACAGGAACCAGCTATGACAGAGGATGGTGAAGGACT
SC3.60kAMAS2		GACGCAAGTGAGCAGTATGACAGAGGATGGTGAAGAGCA
SC3.60kRev		CCCATGAACCAACCACAGAT
SC30.37KAMAS1	P	GCAACAGGAACCAGCTATGACCGCAACATAAGCAAACAATC
SC30.37KAMAS2		GACGCAAGTGAGCAGTATGACCGCAACATAAGCAAACGCTT
SC30.37KRev		ATCCCAGGCCATTCCTACAT
SC3.163KAMAS1	P	GCAACAGGAACCAGCTATGACCGAAAAAGAAGAAGAGGTCG
SC3.163KAMAS2		GACGCAAGTGAGCAGTATGACCGAAAAAGAAGAAGAGACCA
SC3.163KRev		CCACCACTCTACAACGCTACC
SC3.404KAMAS1	P	GCAACAGGAACCAGCTATGACACGGTCTACAAGTTTAGGGCCG
SC3.404KAMAS2		GACGCAAGTGAGCAGTATGACACGGTCTACAAGTTTAGGATCA
SC3.404KRev		GTCTCCTCGCTCTGGTGAAC
SC3.804KAMAS1	P	GCAACAGGAACCAGCTATGACTCGACCCTACGACATTTGCAGC
SC3.804KAMAS2		GACGCAAGTGAGCAGTATGACTCGACCCTACGACATTTGACGT
SC3.804KRev		GTGGTAGCGAGGGCATCAC
SC3.1402KAMAS1	P	GCAACAGGAACCAGCTATGACGTGTTTCTGATTCGTGGC
SC3.1402KAMAS2		GACGCAAGTGAGCAGTATGACGTGTTTCTGATTCGCAGT
SC3.1402KRev		GTGCCTAACGCAACGTGTC
SC3.2029KAMAS1	P	GCAACAGGAACCAGCTATGACAGAGCATCCCCATTCAATTC
SC3.2029KAMAS2		GACGCAAGTGAGCAGTATGACAGAGCATCCCCATTCAGCTT
SC3.2029KRev		CTAAAACGCTTGTCGTGTGC
SC3.2135KAMAS1	P	GCAACAGGAACCAGCTATGACCTAGATAGTTCCTAGCTGTTCCATC
SC3.2135KAMAS2		GACGCAAGTGAGCAGTATGACCTAGATAGTTCCTAGCTGTTCACTT
SC3.2135KRev		AGGTGGGTGAAAGTCCACAG
SC3.2326KAMAS1	P	GCAACAGGAACCAGCTATGACAGTGTGCTTACAGACTCGTAG
SC3.2326KAMAS2		GACGCAAGTGAGCAGTATGACAGTGTGCTTACAGACTCACAA
SC3.2326KRev		TAGGGCTAGAGGGGTTGAGC
SC3.2710KAMAS1	P	GCAACAGGAACCAGCTATGACCTTCGATACGGGTAGAGTCTGC
SC3.2710KAMAS2		GACGCAAGTGAGCAGTATGACCTTCGATACGGGTAGAGTTCGT
SC3.2710KRev		GGTCTTCCCAACGAAATGAA
SC3.2919KAMAS1	P	GCAACAGGAACCAGCTATGACTTGGAATTTGCCCAATATG
SC3.2919KAMAS2		GACGCAAGTGAGCAGTATGACTTGGAATTTGCCCAACGTA
SC3.2919KRev		GTACGTCGGCACGCTTAGAT
SC5.743KAMAS1	P	GCAACAGGAACCAGCTATGACCCAACCTACCCAGTATCCC
SC5.743KAMAS2		GACGCAAGTGAGCAGTATGACCCAACCTACCCAGTACTCT

Primer name	Polymorphism[a]	Primer sequence
SC5.743KRev		GAGCTGGAAACGCAGGTTAC
SC5.878KAMAS1	P	GCAACAGGAACCAGCTATGACGCGGATTGAGGTGTTAGG
SC5.878KAMAS2		GACGCAAGTGAGCAGTATGACGCGGATTGAGGTGTCGGA
SC5.878KRev		TCACTATCCCTACCCCCACA
SC5.1045KAMAS1	P	GCAACAGGAACCAGCTATGACGCTGGTCTATGCAAATTCG
SC5.1045KAMAS2		GACGCAAGTGAGCAGTATGACGCTGGTCTATGCAAACCCA
SC5.1045KRev		ACAGGCGAAGCAGAAATGTT
SC5.1294KAMAS1	P	GCAACAGGAACCAGCTATGACCCAAAGGAAGTTTTGCCCTG
SC5.1294KAMAS2		GACGCAAGTGAGCAGTATGACCCAAAGGAAGTTTTGCTTTA
SC5.1294KRev		CCTCCAGTAACCACGACGAT
SC5.1457KAMAS1	P	GCAACAGGAACCAGCTATGACAGAAGAAGACTGATTCGGTATC
SC5.1457KAMAS2		GACGCAAGTGAGCAGTATGACAGAAGAAGACTGATTCGGCCTT
SC5.1457KRev		ATGACTTCGCCCATGATCTC
SC5.2380KAMAS1	P	GCAACAGGAACCAGCTATGACTTGTTCCACATGGGCCCTG
SC5.2380KAMAS2		GACGCAAGTGAGCAGTATGACTTGTTCCACATGGGCTTTA
SC5.2380KRev		CGCAACAAATACCACGTCAA
SC4.124KAMAS1	P	GCAACAGGAACCAGCTATGACATCCCCGAACCCGACACC
SC4.124KAMAS2		GACGCAAGTGAGCAGTATGACATCCCCGAACCCGATCCT
SC4.124KRev		GTAGTCTCGGACGGAAATCG
SC4.259KAMAS1	P	GCAACAGGAACCAGCTATGACGAAAGAGGTTTGGGAGATGG
SC4.259KAMAS2		GACGCAAGTGAGCAGTATGACGAAAGAGGTTTGGGAGCCGA
SC4.259KRev		TCCAACCCCAGATACACCAT
SC4.408KAMAS1	P	GCAACAGGAACCAGCTATGACCGACCGACGACGACAGCAG
SC4.408KAMAS2		GACGCAAGTGAGCAGTATGACCGACCGACGACGACAATAA
SC4.408KRev		GTTGCGTTATCAGCAGCGTA
SC4.804KAMAS1	P	GCAACAGGAACCAGCTATGACGCAACCTCCTTCAATTCC
SC4.804KAMAS2		GACGCAAGTGAGCAGTATGACGCAACCTCCTTCAACCCT
SC4.804KRev		AAGCATTGGAGAGTCGGAAA
SC4.1122KAMAS1	P	GCAACAGGAACCAGCTATGACCTACCTTTGCCTACGCACTC
SC4.1122KAMAS2		GACGCAAGTGAGCAGTATGACCTACCTTTGCCTACGCCTTA
SC4.1122KRev		TCGACACTCCTGGACAGACT
SC4.1383KAMAS1	P	GCAACAGGAACCAGCTATGACGGTCATTCATGTCGTTAGCCGG
SC4.1383KAMAS2		GACGCAAGTGAGCAGTATGACGGTCATTCATGTCGTTAGTAGA
SC4.1383KRev		CCTTCTCCCTGATTGTGCTC
SC4.1845KAMAS1	P	GCAACAGGAACCAGCTATGACCGGTTGAGGAAGCTCCTG
SC4.1845KAMAS2		GACGCAAGTGAGCAGTATGACCGGTTGAGGAAGCTTATA
SC4.1845KRev		TGGTCCCCTACATTGTCGTT
SC4.1955KAMAS1	M	GCAACAGGAACCAGCTATGACAGATGGAGTCTGTGCTAGC
SC4.1955KAMAS2		GACGCAAGTGAGCAGTATGACAGATGGAGTCTGTGCCCGT
SC4.1955KRev		ACGCCGACAAGCTAGAGAAA
SC4.2190KAMAS1	P	GCAACAGGAACCAGCTATGACACGGTGCATCAGTCCCTG
SC4.2190KAMAS2		GACGCAAGTGAGCAGTATGACACGGTGCATCAGTCTATA
SC4.2190KRev		TCGTCTCGTGGAAGTGTACG

Primer name	Polymorphism[a]	Primer sequence
SC4.2364KAMAS1	P	GCAACAGGAACCAGCTATGACACCGACTGGGCCATAAAG
SC4.2364KAMAS2		GACGCAAGTGAGCAGTATGACACCGACTGGGCCATGCAT
SC4.2364KRev		GAACGTGGAAGAAACCGAGA
SC4.2541KAMAS1	P	GCAACAGGAACCAGCTATGACCAAATCCAGTCCAGAAATGT
SC4.2541KAMAS2		GACGCAAGTGAGCAGTATGACCAAATCCAGTCCAGAACCGA
SC4.2541KRev		TCGCAAGTCCATGTCAGAAG
SC4.2703KAMAS1	P	GCAACAGGAACCAGCTATGACCTCCTGGTTCTACGTCGC
SC4.2703KAMAS2		GACGCAAGTGAGCAGTATGACCTCCTGGTTCTACGCTGT
SC4.2703KRev		CGGTAATAGCGTCCATTGGT
SC6.669KAMAS1	P	GCAACAGGAACCAGCTATGACTGCCACGTCAACAAAACAAC
SC6.669KAMAS2		GACGCAAGTGAGCAGTATGACTGCCACGTCAACAAAAACAT
SC6.669KRev		GCCAGTGCTTCTGAAAGACA
SC6.1444KAMAS1	P	GCAACAGGAACCAGCTATGACCGCAAGTTAAATAAGTAGGTACGGG
SC6.1444KAMAS2		GACGCAAGTGAGCAGTATGACCGCAAGTTAAATAAGTAGGTAAAGT
SC6.1444KRev		AGTCTCGTAAGCGGAGCGTA
SC6.1955KAMAS1	P	GCAACAGGAACCAGCTATGACGGCAATATCCTCATAGGTAGGC
SC6.1955KAMAS2		GACGCAAGTGAGCAGTATGACGGCAATATCCTCATAGGTGAGT
SC6.1955KRev		GGACTAGCGGCATCGTCAAG
SC7.101KAMAS1	P	GCAACAGGAACCAGCTATGACGACGTAGTCGTCGTGTAGAC
SC7.101KAMAS2		GACGCAAGTGAGCAGTATGACGACGTAGTCGTCGTGTGAAT
SC7.101KRev		GCTCATTCTCACCACCGATT
SC7.333KAMAS1	P	GCAACAGGAACCAGCTATGACCTCGACGAAGCAGACTACCG
SC7.333KAMAS2		GACGCAAGTGAGCAGTATGACCTCGACGAAGCAGACTCTCA
SC7.333KRev		CAGTCGTTGCTGTGCCTTCT
SC7.727KAMAS1	P	GCAACAGGAACCAGCTATGACTCTCACAGTCTTGGAGCC
SC7.727KAMAS2		GACGCAAGTGAGCAGTATGACTCTCACAGTCTTGGGACT
SC7.727KRev		GTGGAGCCAGAGTCGTTCTC
SC7.851KAMAS1	P	GCAACAGGAACCAGCTATGACCGAGACAAATCACTATCGACCCT
SC7.851KAMAS2		GACGCAAGTGAGCAGTATGACCGAGACAAATCACTATCGATACA
SC7.851KRev		GTTTGGGTGATGGTGCTGAT
SC7.1564KAMAS1	P	GCAACAGGAACCAGCTATGACGAAGGCGAGACCGAAACC
SC7.1564KAMAS2		GACGCAAGTGAGCAGTATGACGAAGGCGAGACCGAGCCT
SC7.1564KRev		CCAACATCCTAGCTCTGAAAGAA
SC7.1643KAMAS1	P	GCAACAGGAACCAGCTATGACAGAAAGCTGGTCTGCACG
SC7.1643KAMAS2		GACGCAAGTGAGCAGTATGACAGAAAGCTGGTCTGTGCA
SC7.1643KRev		CGGTGGGTCTCTCTGATAGC
SC7.1853KAMAS1	P	GCAACAGGAACCAGCTATGACGCCCTCCATATCTTCGATATAGC
SC7.1853KAMAS2		GACGCAAGTGAGCAGTATGACGCCCTCCATATCTTCGATACCGT
SC7.1853KRev		TTACCTCAACACTGGCGAGC
SC7.1870KAMAS1	P	GCAACAGGAACCAGCTATGACCAGATTGGTGGTGCTGCTG
SC7.1870KAMAS2		GACGCAAGTGAGCAGTATGACCAGATTGGTGGTGCTATTA
SC7.1870KRev		GAGCATCGTCGAGTTCCTTC
SC8.16KAMAS1	P	GCAACAGGAACCAGCTATGACCATTCAAACCCTACATTGTGGCAG

Primer name	Polymorphism[a]	Primer sequence
SC8.16KAMAS2		GACGCAAGTGAGCAGTATGACCATTCAAACCCTACATTGTGAAAA
SC8.16KRev		CCGTAGAAGATGGCGAGACG
SC8.155KAMAS1	P	GCAACAGGAACCAGCTATGACACATAATCCAATCAGTTCCCGC
SC8.155KAMAS2		GACGCAAGTGAGCAGTATGACACATAATCCAATCAGTTCTTGT
SC8.155KRev		ATTATTGGGGAGAGGGAGGA
SC8.278KAMAS1	P	GCAACAGGAACCAGCTATGACGAACAGTGAGTTCAGACATAAACG
SC8.278KAMAS2		GACGCAAGTGAGCAGTATGACGAACAGTGAGTTCAGACATACGCA
SC8.278KRev		GTGAAGGACTGTGGCAGTGA
SC8.437KAMAS1	P	GCAACAGGAACCAGCTATGACCCGACAAACCACACTACAAAGC
SC8.437KAMAS2		GACGCAAGTGAGCAGTATGACCCGACAAACCACACTACAGCGT
SC8.437KRev		GCTTGAGGGACAGTTCTGCT
SC8.741KAMAS1	P	GCAACAGGAACCAGCTATGACTGGATAGGTGTGTCTACCTAGG
SC8.741KAMAS2		GACGCAAGTGAGCAGTATGACTGGATAGGTGTGTCTACCCGGA
SC8.741KRev		ACGAGGATCTGCCTGAGAAG
SC8.1100KAMAS1	P	GCAACAGGAACCAGCTATGACCTCCTCTGCATGTGAAAGAGTTG
SC8.1100KAMAS2		GACGCAAGTGAGCAGTATGACCTCCTCTGCATGTGAAAGAACTA
SC8.1100KRev		ACTGAACTGTTGGGGAGTGG
SC8.1356KAMAS1	P	GCAACAGGAACCAGCTATGACCGTTTTGCGACGGTTGTCGT
SC8.1356KAMAS2		GACGCAAGTGAGCAGTATGACCGTTTTGCGACGGTTGCTGA
SC8.1356KRev		CGACGGTCCCTACTCGAATG
SC8.1612KAMAS1	M	GCAACAGGAACCAGCTATGACACATTCACTCTGACCTCACATTG
SC8.1612KAMAS2		GACGCAAGTGAGCAGTATGACACATTCACTCTGACCTCACCCTA
SC8.1612KRev		GCATACCGAAATGACTGCCC
SC8.1750KAMAS1	P	GCAACAGGAACCAGCTATGACTCAAAGACTCCTCTCAAGCAACAG
SC8.1750KAMAS2		GACGCAAGTGAGCAGTATGACTCAAAGACTCCTCTCAAGCACAAA
SC8.1750KRev		CATCGGGAAGTGACACCGTT
SC9.144KAMAS1	P	GCAACAGGAACCAGCTATGACTCGTTGTCTTGAGGAATGAGT
SC9.144KAMAS2		GACGCAAGTGAGCAGTATGACTCGTTGTCTTGAGGAATAGGA
SC9.144KRev		GTCGGTCCTTACGTTCTGCT
SC9.405KAMAS1	P	GCAACAGGAACCAGCTATGACGACTCTTCTGAAATCGACG
SC9.405KAMAS2		GACGCAAGTGAGCAGTATGACGACTCTTCTGAAATCAGCA
SC9.405KRev		TGCCTGTCATTCCAAACAAA
SC9.628KAMAS1	P	GCAACAGGAACCAGCTATGACGAAGGAGGCTTGTCAGGCGG
SC9.628KAMAS2		GACGCAAGTGAGCAGTATGACGAAGGAGGCTTGTCAGAAGA
SC9.628KRev		GCCTTTCCATCTGCTTTGTT
SC9.1040KAMAS1	P	GCAACAGGAACCAGCTATGACAGGCGTAACAAGGACCCCC
SC9.1040KAMAS2		GACGCAAGTGAGCAGTATGACAGGCGTAACAAGGACATCG
SC9.1040KRev		GGATCGTGGTATTGGTTGCT
SC9.1609KAMAS1	P	GCAACAGGAACCAGCTATGACGTGCCGAGAAGTTCGATCATC
SC9.1609KAMAS2		GACGCAAGTGAGCAGTATGACGTGCCGAGAAGTTCGATACTT
SC10.707KAMAS1	P	GCAACAGGAACCAGCTATGACTTCGTCGTTCTGCTCACCC
SC9.1609KRev		GGCAGCAATGGTATGGAAGT
SC10.707KAMAS2		GACGCAAGTGAGCAGTATGACTTCGTCGTTCTGCTCCACA

Primer name	Polymorphism[a]	Primer sequence
SC10.707KRev		AACTTCCGGTCCTCAAATCC
SC10.1568KAMAS1	P	GCAACAGGAACCAGCTATGACGGGGTGGTGTTAGTTTGCGTC
SC10.1568KAMAS2		GACGCAAGTGAGCAGTATGACGGGGTGGTGTTAGTTTGTATT
SC10.1568KRev		AGAATGGTCCGACTGTCCAC
SC11.409KAMAS1	P	GCAACAGGAACCAGCTATGACTAGATCATGGGTTGGAGG
SC11.409KAMAS2		GACGCAAGTGAGCAGTATGACTAGATCATGGGTTGAGGA
SC11.409KRev		GGGAAAGTTGAACCAGGACA
SC11.429KAMAS1	P	GCAACAGGAACCAGCTATGACGCCGCAGTCCTTTACCGC
SC11.429KAMAS2		GACGCAAGTGAGCAGTATGACGCCGCAGTCCTTTAATGT
SC11.429KRev		TCAATATGGCGTGGGGTATT
SC17.519kAMAS1	P	GCAACAGGAACCAGCTATGACAGCAGGAGAGTAGGTATACG
SC17.519kAMAS2		GACGCAAGTGAGCAGTATGACAGCAGGAGAGTAGGTACGCA
SC17.519KRev		TGCTTTGGGTTCGTCAAGGA
SC17.611KAMAS1	P	GCAACAGGAACCAGCTATGACAAGAAGAGGACGAGTCCGGC
SC17.611KAMAS2		GACGCAAGTGAGCAGTATGACAAGAAGAGGACGAGTCAAGT
SC17.611KRev		CCAATGGCACACACAATCAT
SC18.91KAMAS1	P	GCAACAGGAACCAGCTATGACGTCCTCGATAATGCTTCTCTGC
SC18.91KAMAS2		GACGCAAGTGAGCAGTATGACGTCCTCGATAATGCTTCTTCGT
SC18.91KRev		GCTGTTTGCCGTCTTCGTA
SC18.211KAMAS1	P	GCAACAGGAACCAGCTATGACTGCGTGGCTTTCCCTATG
SC18.211KAMAS2		GACGCAAGTGAGCAGTATGACTGCGTGGCTTTCCCCGTA
SC18.211KRev		GGCGACCTTTTTCCTTCTCT
SC16.630KAMAS1	P	GCAACAGGAACCAGCTATGACCACAGATTACACTCTCCCACCTC
SC16.630KAMAS2		GACGCAAGTGAGCAGTATGACCACAGATTACACTCTCCCAATTT
SC16.630KRev		TTTCGACAAAATACTCCAGGCG
SC25.84KAMAS1	P	GCAACAGGAACCAGCTATGACAGGAAAATCCACAAACCTTTCC
SC25.84KAMAS2		GACGCAAGTGAGCAGTATGACAGGAAAATCCACAAACCTCCCG
SC25.84KRev		AGAGTTCTTTGAGGCGGTCG
SC12.505KAMAS1	P	GCAACAGGAACCAGCTATGACCGAAGAGCAGACGCCTGC
SC12.505KAMAS2		GACGCAAGTGAGCAGTATGACCGAAGAGCAGACGCTCGT
SC12.505KRev		CTATCCTTTGGGCGATTGAA
SC12.1062KAMAS1	P	GCAACAGGAACCAGCTATGACCCGAAGCCAACACAATAG
SC12.1062KAMAS2		GACGCAAGTGAGCAGTATGACCCGAAGCCAACACACCAA
SC12.1062KRev		AAGGAAGGAGCATTCAGACG
SC13.433KAMAS1	P	GCAACAGGAACCAGCTATGACGGGATACGATACAGCCTCCTG
SC13.433KAMAS2		GACGCAAGTGAGCAGTATGACGGGATACGATACAGCCTTTTA
SC13.433KRev		ATGTGTCGGAGATGGTGAGC
SC13.753KAMAS1	P	GCAACAGGAACCAGCTATGACCGATGTGGTACGCAGGCTTC
SC13.753KAMAS2		GACGCAAGTGAGCAGTATGACCGATGTGGTACGCAGGTCTT
SC13.753KRev		AGTTCATCCACGTCCTCCAC
SC13.887KAMAS1	P	GCAACAGGAACCAGCTATGACGAGTCGAGTAGTAAGAGGTTATCCG
SC13.887KAMAS2		GACGCAAGTGAGCAGTATGACGAGTCGAGTAGTAAGAGGTTACACA
SC13.887KRev		GTGTACGTGTCGGTGTACGG

Primer name	Polymorphism[a]	Primer sequence
SC14.840KAMAS1	P	GCAACAGGAACCAGCTATGACGCCCTTCTTGGTTGTTATC
SC14.840KAMAS2		GACGCAAGTGAGCAGTATGACGCCCTTCTTGGTTGTCCTT
SC14.840KRev		CCAGTCCTCTTGATGGCAGT
SC14.950KAMAS1	P	GCAACAGGAACCAGCTATGACGACCTCAGCGAAACAACTCC
SC14.950KAMAS2		GACGCAAGTGAGCAGTATGACGACCTCAGCGAAACAAACCT
SC14.950KRev		AGGGGAAGGATTGTGAGATG
SC15.243KAMAS1	P	GCAACAGGAACCAGCTATGACACACTCCAACACCACTGAC
SC15.243KAMAS2		GACGCAAGTGAGCAGTATGACACACTCCAACACCACCAAT
SC15.243KRev		AGCCGCTGCCTATGAAGTCT
SC15.323KAMAS1	P	GCAACAGGAACCAGCTATGACACGGATAGTAGCAGCGAATG
SC15.323KAMAS2		GACGCAAGTGAGCAGTATGACACGGATAGTAGCAGCGCGTA
SC15.323KRev		TATGGCTACGGAATGGGCTA
SC15.476KAMAS1	P	GCAACAGGAACCAGCTATGACACATTCTGTCCAAAGTAAGATCAG
SC15.476KAMAS2		GACGCAAGTGAGCAGTATGACACATTCTGTCCAAAGTAAGACTAT
SC15.476KRev		GTTGCTGTCAACTTCGATGC
SC24.41KAMAS1	P	GCAACAGGAACCAGCTATGACGGGCAGGCTTTGTCGCTG
SC24.41KAMAS2		GACGCAAGTGAGCAGTATGACGGGCAGGCTTTGTCAATA
SC24.41KRev		GGGTTGTGTCGTGGGATACT
SC24.101KAMAS1	P	GCAACAGGAACCAGCTATGACTCCGAGCCTGGTTTTGTC
SC24.101KAMAS2		GACGCAAGTGAGCAGTATGACTCCGAGCCTGGTTTCATT
SC24.101KRev		CTCCTGCACTTACCGAATCC
SC26.55KAMAS1	P	GCAACAGGAACCAGCTATGACGACAAGCGTTCAACACTACC
SC26.55KAMAS2		GACGCAAGTGAGCAGTATGACGACAAGCGTTCAACACCCCT
SC26.55KRev		TTGTAGAAGTCGTCCCATCG
SC11.1338KAMAS1	P	GCAACAGGAACCAGCTATGACTTAACGCCCACACTATTAGAACCTC
SC11.1338KAMAS2		GACGCAAGTGAGCAGTATGACTTAACGCCCACACTATTAGAATTTA
SC11.1338KRev		AAGGGCATCACCGCATACTA
SC22.88KAMAS1	P	GCAACAGGAACCAGCTATGACTGGAGTTTTTGAGACAGACGGC
SC22.88KAMAS2		GACGCAAGTGAGCAGTATGACTGGAGTTTTTGAGACAGATAGT
22.88KRev		GCGTAAGAGCACATCCCGTA
SC19.272KAMAS1	P	GCAACAGGAACCAGCTATGACTCTGCAATAGACGCTACTC
SC19.272KAMAS2		GACGCAAGTGAGCAGTATGACTCTGCAATAGACGCTGTTT
SC19.272KRev		AGGAATGCTTTTGCCCAAC
SC21.54KAMAS1	N	GCAACAGGAACCAGCTATGACGTACTACAAAGCAGTATCGCTTT
SC21.54KAMAS2		GACGCAAGTGAGCAGTATGACGTACTACAAAGCAGTATCGTCTA
SC21.54KRev		TTTTCCTCCATTCGATGTAATATCT
SC28.7KAMAS1	P	GCAACAGGAACCAGCTATGACGGCAGGGTGAAGGAGCAC
SC28.7KAMAS2		GACGCAAGTGAGCAGTATGACGGCAGGGTGAAGGAAAAA
SC28.7KRev		GCTCCTCTTGTTGTGGGTTG
SC25.91KAMAS1	P	GCAACAGGAACCAGCTATGACACCGGCACCATTTGTAGCATC
SC25.91KAMAS2		GACGCAAGTGAGCAGTATGACACCGGCACCATTTGTAGTCTT
SC25.91KRev		AGCTGCAAAGGAAGAGCTTG
SC25.151KAMAS1	N	GCAACAGGAACCAGCTATGACCATTTAGAATCATTGCGTCACCAAC

Primer name	Polymorphism[a]	Primer sequence
SC25.151KAMAS2		GACGCAAGTGAGCAGTATGACCATTTAGAATCATTGCGTCACTCAT
SC25.151KRev		TGCTATTCCAGCACCTCAAA
SC34.9KAMAS1	M	GCAACAGGAACCAGCTATGACATGACCTGGTTGCAGATGCTG
SC34.9KAMAS2		GACGCAAGTGAGCAGTATGACATGACCTGGTTGCAGATATTA
SC34.9KRev		TTATCCCTTGGCTCCTCCTT
SC23.168KAMAS1	M	GCAACAGGAACCAGCTATGACCGTGCCACTTATACTGACAAC
SC23.168KAMAS2		GACGCAAGTGAGCAGTATGACCGTGCCACTTATACTGAACAT
SC23.168KRev		TAAGCAGCCTTGCCTTCTGT
SC25.84KAMAS1	P	GCAACAGGAACCAGCTATGACAGGAAAATCCACAAACCTTTCC
SC25.84KAMAS2		GACGCAAGTGAGCAGTATGACAGGAAAATCCACAAACCTCCCG
SC25.84KRev		AGAGTTCTTTGAGGCGGTCG
SC23.10KAMASA1	P	GCAACAGGAACCAGCTATGACCGTGCAACACAATCCAGCAGC
SC23.10KAMAS2		GACGCAAGTGAGCAGTATGACCGTGCAACACAATCCAGTCGT
SC23.10KRev		GGAGAAGGAGACCGCGATAG
SC23.38KAMAS1	P	GCAACAGGAACCAGCTATGACAGAAATGCTGTGTCTTTGGCAT
SC23.38KAMAS2		GACGCAAGTGAGCAGTATGACAGAAATGCTGTGTCTTTGAAAA
SC23.38KRev		TTCGTCGCTCTCCTCCTAAA
SC23.84KAMAS1	P	GCAACAGGAACCAGCTATGACGCTAGTCCAGTTTCTAACAACC
SC23.84KAMAS2		GACGCAAGTGAGCAGTATGACGCTAGTCCAGTTTCTAACGCCG
SC23.84KRev		TGAACTCATGTGCGGTAGGA
SC25.36KAMAS1	M	GCAACAGGAACCAGCTATGACATTGGCAGCCTCAAACAC
SC25.36KAMAS2		GACGCAAGTGAGCAGTATGACATTGGCAGCCTCAAGTAT
SC25.36KRev		GGCAAGCAACCAGAACTAGC
SC1.23KAMAS1	P	GCAACAGGAACCAGCTATGACAGCAACAAAGCTGGTATC
SC1.23KAMAS2		GACGCAAGTGAGCAGTATGACAGCAACAAAGCTGGCCTG
SC1.23KREV		CAACAACCCAAGTCTCAACG
SC1.319KAMAS1	P	GCAACAGGAACCAGCTATGACTTACTGCGGCTTGTATAAATATCTG
SC1.319KAMAS2		GACGCAAGTGAGCAGTATGACTTACTGCGGCTTGTATAAATACATA
SC1.319KREV		GTACGCCCGTCGGTGTAT
SC1.4563KAMAS1	P	GCAACAGGAACCAGCTATGACTCGGGTTGTGGTATCCCG
SC1.4563KAMAS2		GACGCAAGTGAGCAGTATGACTCGGGTTGTGGTATTTCA
SC1.4563KREV		GTACTTCCAACGCTCGTGTC
SC1.4718KAMAS1	P	GCAACAGGAACCAGCTATGACGCAGAGACCCGACGTCTAC
SC1.4718KAMAS2		GACGCAAGTGAGCAGTATGACGCAGAGACCCGACGTACAT
SC1.4718KREV		GAACCTTGAAAGCTGCCATA
SC1.3535KAMAS1	P	GCAACAGGAACCAGCTATGACGCAACGGTATGTATGGAATCAG
SC1.3535KAMAS2		GACGCAAGTGAGCAGTATGACGCAACGGTATGTATGGAACAAA
SC1.3535KREV		ATCCAGTTAATCGGTGTGGA
SC1.2617KAMAS1	P	GCAACAGGAACCAGCTATGACGGTGTTGAGAACAGGTGACAG
SC1.2617KAMAS2		GACGCAAGTGAGCAGTATGACGGTGTTGAGAACAGGTGCTAA
SC1.2617REV		ACACCATAGGTCCGCTTGTA
SC1.2238KAMAS1	P	GCAACAGGAACCAGCTATGACCGTGACAGCAGCAACAAC
SC1.2238KAMAS2		GACGCAAGTGAGCAGTATGACCGTGACAGCAGCAAACAT

Primer name	Polymorphism[a]	Primer sequence
SC1.2238KREV		CGTCCTACAAACACCTCCTG
SC1.1616KAMAS1	P	GCAACAGGAACCAGCTATGACTATACTAGGGGAGGAGCGGC
SC1.1616KAMAS2		GACGCAAGTGAGCAGTATGACTATACTAGGGGAGGAGTAGT
SC1.1616KREV		GACGAGCAGGTCATTCAAGT
SC10.1488KAMAS1	P	GCAACAGGAACCAGCTATGACAGAATACTAGGAAGTACGTAGCCAC
SC10.1488KAMAS2		GACGCAAGTGAGCAGTATGACAGAATACTAGGAAGTACGTAGATAT
SC10.1488KREV		GAGCTATGGTGCCAGTTACG
SC10.106KAMAS1	P	GCAACAGGAACCAGCTATGACGTACGTACATTCCATCGCC
SC10.106KAMAS2		GACGCAAGTGAGCAGTATGACGTACGTACATTCCATAACT
SC10.106KREV		TTTTCTGCGCTAGTTTCCTG
SC17.59KAMAS1	P	GCAACAGGAACCAGCTATGACAAGTAGTCATCGTCGTCCTCC
SC17.59KAMAS2		GACGCAAGTGAGCAGTATGACAAGTAGTCATCGTCGTCACCT
SC17.59KREV		CCACATACGATACCGGCTAC
SC16.106KAMAS1	P	GCAACAGGAACCAGCTATGACAAAAGGCAAGCCGACCAC
SC16.106KAMAS2		GACGCAAGTGAGCAGTATGACAAAAGGCAAGCCGATTAT
SC16.106KREV		GCACTTGATGCGAAGAAAAT
SC2.22KAMAS1	P	GCAACAGGAACCAGCTATGACCGTCTAACGTTACCTCAAAC
SC2.22KAMAS2		GACGCAAGTGAGCAGTATGACCGTCTAACGTTACCTCGCAT
SC2.22KREV		GATGACCTGATTCGTTACGG
SC2.408KAMAS1	P	GCAACAGGAACCAGCTATGACGAAAGAGCGAAAGTGTGCAAC
SC2.408KAMAS2		GACGCAAGTGAGCAGTATGACGAAAGAGCGAAAGTGTGTCAT
SC2.408KREV		TCAGCCTTTCTTTTGTTTCC
SC2.573KAMAS1	P	GCAACAGGAACCAGCTATGACAAATGTGCGCGATCCAAC
SC2.573KAMAS2		GACGCAAGTGAGCAGTATGACAAATGTGCGCGATCTCAT
SC2.573KREV		GTGCCGTCGTCTTTGTAGTC
SC2.1447KAMAS1	P	GCAACAGGAACCAGCTATGACCATCACAACAGTGAGCCCG
SC2.1447KAMAS2		GACGCAAGTGAGCAGTATGACCATCACAACAGTGAGTACA
SC2.1447KREV		TGATGGTAGGAGGATTGGTG
SC2.1512KAMAS1	P	GCAACAGGAACCAGCTATGACATGACCGAGATGCAACGG
SC2.1512KAMAS2		GACGCAAGTGAGCAGTATGACATGACCGAGATGCACAGA
SC2.1512KREV		GCATTCCGGCTATACAGACA
SC2.1193KAMAS1	P	GCAACAGGAACCAGCTATGACAGTTGGAGGGCTTGTTGCAG
SC2.1193KAMAS2		GACGCAAGTGAGCAGTATGACAGTTGGAGGGCTTGTTATAA
SC2.1193KREV		AGACAGCAATGAAGCGAAAC
SC13.570KAMAS1	P	GCAACAGGAACCAGCTATGACGGTTGTAGCCAGCGCATC
SC13.570KAMAS2		GACGCAAGTGAGCAGTATGACGGTTGTAGCCAGCGACTT
SC13.570KREV		TGTCCGGTGTACTGTCTTGA
SC15.524KAMAS1	P	GCAACAGGAACCAGCTATGACCCATAAACATATTTCAATGTCCTTC
SC15.524KAMAS2		GACGCAAGTGAGCAGTATGACCCATAAACATATTTCAATGTCTCTT
SC15.524KREV		AGGACCTCCACCAAAATCAT
SC15.744KAMAS1	P	GCAACAGGAACCAGCTATGACGTATCTATGGACGCGAAGC
SC15.744KAMAS2		GACGCAAGTGAGCAGTATGACGTATCTATGGACGCGGCGT
SC15.744KREV		GCCATCCCTTTTATTTTTGG

Primer name	Polymorphism[a]	Primer sequence
SC26.104KAMAS1	M	GCAACAGGAACCAGCTATGACTTCTCACGTCCTTTGAAATC
SC26.104KAMAS2		GACGCAAGTGAGCAGTATGACTTCTCACGTCCTTTGAGCTT
SC26.104KREV		TAACTAATGCGCGACTGACA
SC5.84KAMAS1	P	GCAACAGGAACCAGCTATGACCAGACGGGTCTTTATGAG
SC5.84KAMAS2		GACGCAAGTGAGCAGTATGACCAGACGGGTCTTTACAAT
SC5.84KREV		GTCTTCCTCCTTCCGCTTAC
SC7.550KAMAS1	P	GCAACAGGAACCAGCTATGACGGTTGAGTGGTGGAAGCGG
SC7.550KAMAS2		GACGCAAGTGAGCAGTATGACGGTTGAGTGGTGGAACTGA
SC7.550KREV		AGCCCATATCCACTGCACTA
SC6.1147KAMAS1	M	GCAACAGGAACCAGCTATGACCAAAACGGGAAAACAACTG
SC6.1147KAMAS2		GACGCAAGTGAGCAGTATGACCAAAACGGGAAAACACATG
SC6.1147KREV		CACCTTCCTTCTTCCCTCTC
SC4.1650KAMAS1	P	GCAACAGGAACCAGCTATGACGTTGGGGTGATGACCTCG
SC4.1650KAMAS2		GACGCAAGTGAGCAGTATGACGTTGGGGTGATGACACCT
SC4.1650KREV		TTGCATTGTATCCACCATGA
SC18.313KAMAS1	P	GCAACAGGAACCAGCTATGACACTAGCCACTCGGACTGC
SC18.313KAMAS2		GACGCAAGTGAGCAGTATGACACTAGCCACTCGGATCGT
SC18.313KREV		TGCTTCACTCCTTCGATCAT
SC7.1132KAMAS1	P	GCAACAGGAACCAGCTATGACAATCTCATAGACAAGATGTTACG
SC7.1132KAMAS2		GACGCAAGTGAGCAGTATGACAATCTCATAGACAAGATGTCCCA
SC7.1132KREV		CACACACGGCTTTTCGATTA
SC7.1467KAMAS1	P	GCAACAGGAACCAGCTATGACCTTCAGTCGTCCCAGCGTC
SC7.1467KAMAS2		GACGCAAGTGAGCAGTATGACCTTCAGTCGTCCCAGAATG
SC7.1467KREV		CTTGCAGTACGAGAGGCAGT
SC14.670KAMAS1	P	GCAACAGGAACCAGCTATGACACGTCATTCAGATGTGAC
SC14.670KAMAS2		GACGCAAGTGAGCAGTATGACACGTCATTCAGATGCAAT
SC14.670KREV		GGCACTGTTGAAGAAGGAGA
SC13.200KAMAS1	P	GCAACAGGAACCAGCTATGACCAAACCTGTCGGTATTCTTTAC
SC13.200KAMAS2		GACGCAAGTGAGCAGTATGACCAAACCTGTCGGTATTCTCCAT
SC13.200KREV		GGGATGGAGGCTTTGTATTT
SC6.1607KAMAS1	P	GCAACAGGAACCAGCTATGACCCTATCCCGTTACACAGCAG
SC6.1607KAMAS2		GACGCAAGTGAGCAGTATGACCCTATCCCGTTACACAAAAA
SC6.1607KREV		TCGTCTTCTTCTTCGTCGTC
SC14.769KAMAS1	P	GCAACAGGAACCAGCTATGACACAAGGTAGAGGAGCCACAGC
SC14.769KAMAS2		GACGCAAGTGAGCAGTATGACACAAGGTAGAGGAGCCATCGT
SC14.769KREV		GAAAAAGGCGTTCTGGTTCA
SC14.654KAMAS1	P	GCAACAGGAACCAGCTATGACCATGTTCACATACAATCACCATC
SC14.654KAMAS2		GACGCAAGTGAGCAGTATGACCATGTTCACATACAATCACACTG
SC14.654KREV		CGAAGTGCCTAGCGGTATTT
SC34.9KAMAS1	P	GCAACAGGAACCAGCTATGACTGACCTGGTTGCAGATGCTG
SC34.9KAMAS2		GACGCAAGTGAGCAGTATGACTGACCTGGTTGCAGATATTA
SC34.9KREV		TTTACGGCCTAGCTCCTTCA
SC42.8KAMAS1	M	GCAACAGGAACCAGCTATGACCTCTCTACTGCTTCGCATG

Primer name	Polymorphism[a]	Primer sequence
SC42.8KAMAS2		GACGCAAGTGAGCAGTATGACCTCTCTACTGCTTCGTGTA
SC42.8KREV		CTCAAGGGAATGACCAGGAA
SC13.316KAMAS1	P	GCAACAGGAACCAGCTATGACGGTTGATGACTCCACGATAC
SC13.316KAMAS2		GACGCAAGTGAGCAGTATGACGGTTGATGACTCCACGGCAT
SC13.316KREV		ATGCGGCTGATAACAAGGAC
SC1.6243KAMAS1	P	GCAACAGGAACCAGCTATGACCGCAAGAGGTGGAAGCTG
SC1.6243KAMAS2		GACGCAAGTGAGCAGTATGACCGCAAGAGGTGGAAAATA
SC1.6243KREV		TCTAGCGATGGGGTACTGCT
SC1.6663KAMAS1	P	GCAACAGGAACCAGCTATGACGATTTCCTCTACATACGATCAC
SC1.6663KAMAS2		GACGCAAGTGAGCAGTATGACGATTTCCTCTACATACGACTAT
SC1.6663KREV		ACATACAACAGCGGGGATTC
SC18.340KAMAS1	P	GCAACAGGAACCAGCTATGACGCGCTCTCAATAGAACTTCCAC
SC18.340KAMAS2		GACGCAAGTGAGCAGTATGACGCGCTCTCAATAGAACTTATAT
SC18.340KREV		GGAGAACAGTAGGGCCAAGA
SC5.562KAMAS1	P	GCAACAGGAACCAGCTATGACCATCGAAGACGAAGATAAGC
SC5.562KAMAS2		GACGCAAGTGAGCAGTATGACCATCGAAGACGAAGATGCGT
SC5.562KREV		TCAACAACCTTACGCAGCAG
SC5.1605KAMAS1	P	GCAACAGGAACCAGCTATGACCATCAAGAGAAGGACTATGTCCG
SC5.1605KAMAS2		GACGCAAGTGAGCAGTATGACCATCAAGAGAAGGACTATGCACA
SC5.1605KREV		CAGTACGAATGCCACTCTGG
SC1.6545KAMAS1	P	GCAACAGGAACCAGCTATGACCTCCATATACGAATACCC
SC1.6545KAMAS2		GACGCAAGTGAGCAGTATGACCTCCATATACGAATGTCT
SC1.6545KREV		CCTAGCCTGTAGCCTTGTCG
SC42.8KAMAS1	M	GCAACAGGAACCAGCTATGACCTCTCTACTGCTTCGCATG
SC42.8KAMAS2		GACGCAAGTGAGCAGTATGACCTCTCTACTGCTTCGTGTA
SC42.8KREV		CTCAAGGGAATGACCAGGAA
SC35.24KAMAS1	M	GCAACAGGAACCAGCTATGACGCGTCTAGTGGCTATGCC
SC35.24KAMAS2		GACGCAAGTGAGCAGTATGACGCGTCTAGTGGCTACACT
SC35.24KREV		GTTTCAAGACGCGCTAAAGG
SC34.9KAMAS1	N	GCAACAGGAACCAGCTATGACGACCTGGTTGCAGATGCTG
SC34.9KAMAS2		GACGCAAGTGAGCAGTATGACGACCTGGTTGCAGATATTA
SC34.9KREV		TTTACGGCCTAGCTCCTTCA
SC32.31KAMAS1	P	GCAACAGGAACCAGCTATGACGCGAAGCTCGGACGGC
SC32.31KAMAS2		GACGCAAGTGAGCAGTATGACGCGAAGCTCGGAAAGT
SC32.31KREV		AGAAGGGGACTTGGGTGTTC
SC31.43KAMAS1	M	GCAACAGGAACCAGCTATGACCAAATACCACGCAGTACC
SC31.43KAMAS2		GACGCAAGTGAGCAGTATGACCAAATACCACGCAGCCCT
SC31.43KREV		GAGCAGCTGCAGAGATCAGA
SC29.8KAMAS1	P	GCAACAGGAACCAGCTATGACGGAGTTTCGGAAGACGGC
SC29.8KAMAS2		GACGCAAGTGAGCAGTATGACGGAGTTTCGGAAGAAAGT
SC29.8KREV		GAGTGGATGCCCACCAAATA
SC29.61KAMAS1	P	GCAACAGGAACCAGCTATGACGACATTTGCTGAGGCTCCTC
SC29.61KAMAS2		GACGCAAGTGAGCAGTATGACGACATTTGCTGAGGCTATTT

Primer name	Polymorphism[a]	Primer sequence
SC29.61KREV		TACGGAGGTCCTTTTTAGGC
SC27.78KAMAS1	N	GCAACAGGAACCAGCTATGACTTCCAACCTCCGTCCAAG
SC27.78KAMAS2		GACGCAAGTGAGCAGTATGACTTCCAACCTCCGTCTCAT
SC27.78KREV		GAGGCCGGGAGACATAATTT
SC21.54KAMAS1	P	GCAACAGGAACCAGCTATGACAGCATAGCAGCGAGAGT
SC21.54KAMAS2		GACGCAAGTGAGCAGTATGACAGCATAGCAGCGAAGGA
SC21.54KREV		TGAGTCAGCAATTCCCACTG
SC21.64KAMAS1	P	GCAACAGGAACCAGCTATGACTCAGAAGGAGAGAGCCCATC
SC21.64KAMAS2		GACGCAAGTGAGCAGTATGACTCAGAAGGAGAGAGCCACTT
SC21.64KREV		CGACCGCAACTCTGGTTAAT
SC8.1549KAMAS1	P	GCAACAGGAACCAGCTATGACGAGCATCTCCACGCCTGC
SC8.1549KAMAS2		GACGCAAGTGAGCAGTATGACGAGCATCTCCACGCACGT
SC8.1549KREV		GGTACTCATTCGCCGTTGTT
SC10.109kAMAS1	P	GCAACAGGAACCAGCTATGACGTAAGGACAATCCAATTCTCTG
SC10.109kAMAS2		GACGCAAGTGAGCAGTATGACGTAAGGACAATCCAATTCCATA
SC10.109kRev		TCCCTTAATACACCCGTTATGG
SC10.507kAMAS1	P	GCAACAGGAACCAGCTATGACTGTGGAGTAGTCCGCTAC
SC10.507kAMAS2		GACGCAAGTGAGCAGTATGACTGTGGAGTAGTCCGTCAA
SC10.507kRev		CAATGGCCTCCACTGATACA
SC10.700kAMAS1	P	GCAACAGGAACCAGCTATGACACGACGACGACGACGCCG
SC10.700kAMAS2		GACGCAAGTGAGCAGTATGACACGACGACGACGACAACA
SC10.700kRev		CAGTCACTATTCGGCAGGTG
SC10.1307kAMAS1	P	GCAACAGGAACCAGCTATGACCGATGCTTGAGAGACTAG
SC10.1307kAMAS2		GACGCAAGTGAGCAGTATGACCGATGCTTGAGAGATCAA
SC10.1307kRev		GTTCTTCTGGTTGCCAATCTT
SC10.1445kAMAS1	P	GCAACAGGAACCAGCTATGACCTCTCCTGTACAACCTCC
SC10.1445kAMAS2		GACGCAAGTGAGCAGTATGACCTCTCCTGTACAACACCG
SC10.1445kRev		ATATCTTTGGCTGGGCTTGT
SC33.20kAMAS1	P	GCAACAGGAACCAGCTATGACGAACTCCCCACTGAGAAG
SC33.20kAMAS2		GACGCAAGTGAGCAGTATGACGAACTCCCCACTGAAGAA
SC33.20kRev		GAGTTCTCGTGGCTCTCGT
SC18.455kAMAS1	P	GCAACAGGAACCAGCTATGACGCAATGTTTGGAATGCGCTG
SC18.455kAMAS2		GACGCAAGTGAGCAGTATGACGCAATGTTTGGAATGCAATA
SC18.455kRev		TAGCCTAAACAGCACTAGTC
SC1.650kAMAS1	P	GCAACAGGAACCAGCTATGACAGCGGCGTGACGAAACGG
SC1.650kAMAS2		GACGCAAGTGAGCAGTATGACAGCGGCGTGACGAACTGA
SC1.650kRev		GTCGCGCCCAACTAAGGT
SC1.1218kAMAS1	P	GCAACAGGAACCAGCTATGACCGGCCGGAGAGCGGTGTC
SC1.1218kAMAS2		GACGCAAGTGAGCAGTATGACCGGCCGGAGAGCGGCATT
SC1.1218kRev		CACGGAAAGCGTACGTACTTG
SC1.2025kAMAS1	P	GCAACAGGAACCAGCTATGACTTACCTTTCGGACCTTTC
SC1.2025kAMAS2		GACGCAAGTGAGCAGTATGACTTACCTTTCGGACCCCTA
SC1.2025kRev		TATAATAGACTCGTGGTGGATGG

Primer name	Polymorphism[a]	Primer sequence
SC1.2727kAMAS1	P	GCAACAGGAACCAGCTATGACTAGCAACGGCGACAGTAG
SC1.2727kAMAS2		GACGCAAGTGAGCAGTATGACTAGCAACGGCGACAACAA
SC1.2727kRev		ACAAAGATGGCCAACGAAAC
SC1.2931kAMAS1	P	GCAACAGGAACCAGCTATGACGTAATTGTCGAAGGACCCG
SC1.2931kAMAS2		GACGCAAGTGAGCAGTATGACGTAATTGTCGAAGGATTCA
SC1.2931kRev		AAATCTCTACGGTCACGGCTA
SC1.3898kAMAS1	P	GCAACAGGAACCAGCTATGACTGCAAGCGATAAGATACTCG
SC1.3898kAMAS2		GACGCAAGTGAGCAGTATGACTGCAAGCGATAAGATATCCA
SC1.3898kRev		TATCGAAGAGCAAGCCAGGT
SC1.4785KAMAS1	M	GCAACAGGAACCAGCTATGACGTTGCTTCAACAGCACCG
SC1.4785KAMAS2		GACGCAAGTGAGCAGTATGACGTTGCTTCAACAGCCTCA
SC1.4785KRev		AGCCCAACTTTGCTTGCTAC
SC1.5253KAMAS1	P	GCAACAGGAACCAGCTATGACGACTAGATGCGCTAACGC
SC1.5253KAMAS2		GACGCAAGTGAGCAGTATGACGACTAGATGCGCTACAGA
SC1.5253KRev		TGTTCGCTTAAACACTTCCTG
SC1.5508KAMAS1	M	GCAACAGGAACCAGCTATGACCTTTCTTTCTTATTTGCCTAACATC
SC1.5508KAMAS2		GACGCAAGTGAGCAGTATGACCTTTCTTTCTTATTTGCCTAATCTT
SC1.5508KRev		GAGCAGATCGTTCTGTCGAA
SC1.5710KAMAS1	P	GCAACAGGAACCAGCTATGACCATGCTGTTGGCAGCTAG
SC1.5710KAMAS2		GACGCAAGTGAGCAGTATGACCATGCTGTTGGCAGTCAA
SC1.5710KRev		AGAGATCGGTCAGGTTGAGC
SC1.5935KAMAS1	P	GCAACAGGAACCAGCTATGACGAAGTGCGACGATGGCCG
SC1.5935KAMAS2		GACGCAAGTGAGCAGTATGACGAAGTGCGACGATGATCA
SC1.5935KRev		GTCAGCCTTTGCAGTTGTGA
SC1.6139KAMAS1	P	GCAACAGGAACCAGCTATGACCGAAAGTGGTGAAAAATATCCC
SC1.6139KAMAS2		GACGCAAGTGAGCAGTATGACCGAAAGTGGTGAAAAATACTCT
SC1.6139KRev		GGGCATAAGCTTCAAGAGCA
SC1.6351KAMAS1	N	GCAACAGGAACCAGCTATGACGTGGGGTTGATGAGGAAG
SC1.6351KAMAS2		GACGCAAGTGAGCAGTATGACGTGGGGTTGATGAGAGAA
SC1.6351KRev		CCAGCACAGAACCAACAAAA
SC1.6550KAMAS1	P	GCAACAGGAACCAGCTATGACGACTAGAGCACTAGGAGC
SC1.6550KAMAS2		GACGCAAGTGAGCAGTATGACGACTAGAGCACTAGAGGA
SC1.6550KRev		TTGAGCTGAGAAAACAGATGC
SC17.639KAMAS1	M	GCAACAGGAACCAGCTATGACGCGAGCTAGTCTTAAAAAC
SC17.639KAMAS2		GACGCAAGTGAGCAGTATGACGCGAGCTAGTCTTAAGCAT
SC17.639KRev		AGCACGCGAGGCTAAATACT
SC16.208KAMAS1	P	GCAACAGGAACCAGCTATGACGATTGGAGGAGGAGAGAC
SC16.208KAMAS2		GACGCAAGTGAGCAGTATGACGATTGGAGGAGGAGGAAT
SC16.208KRev		ACAGGGCCGATGATACTTCC
SC16.504KAMAS1	P	GCAACAGGAACCAGCTATGACGCTTAGGGAAAGGGTCTG
SC16.504KAMAS2		GACGCAAGTGAGCAGTATGACGCTTAGGGAAAGGGCATA
SC16.504KRev		TCAGCGATCTAGTTCGAGGTT

Primer name	Polymorphism[a]	Primer sequence
SC16.692KAMAS1	M	GCAACAGGAACCAGCTATGACAGCTAGATAGACCAGCCTG
SC16.692KAMAS2		GACGCAAGTGAGCAGTATGACAGCTAGATAGACCAGTTTA
SC16.692KRev		GGCACCTATAGTTCCCGC
SC2.224KAMAS1	P	GCAACAGGAACCAGCTATGACGAAATGCTGCAATGCATAAC
SC2.224KAMAS2		GACGCAAGTGAGCAGTATGACGAAATGCTGCAATGCACCAT
SC2.224KRev		CAGTTTTGACGGGAGAAAGC
SC2.870kAMAS1	P	GCAACAGGAACCAGCTATGACAGCCCCTGATATCGCACG
SC2.870kAMAS2		GACGCAAGTGAGCAGTATGACAGCCCCTGATATCGACCT
SC2.870kRev		ATCGAGGACAACAAGGCTGA
SC2.2861kAMAS1	P	GCAACAGGAACCAGCTATGACAGCACACTCGCGCTTAAC
SC2.2861kAMAS2		GACGCAAGTGAGCAGTATGACAGCACACTCGCGCTCCAT
SC2.2861kRev		TAGTCGCGTGAGCTGTTTTC
SC2.3124kAMAS1	P	GCAACAGGAACCAGCTATGACGCACGCAGCCTGACTACC
SC2.3124kAMAS2		GACGCAAGTGAGCAGTATGACGCACGCAGCCTGACCGCA
SC2.3124kRev		TCAGGATGAGAAACGGTCTG
SC25.15kAMAS1	P	GCAACAGGAACCAGCTATGACTGCTTCAGTAGCAGCATC
SC25.15kAMAS2		GACGCAAGTGAGCAGTATGACTGCTTCAGTAGCAGTCTT
SC25.15kRev		AAAATCCATCGAGGGTCTCC
SC23.47kAMAS1	P	GCAACAGGAACCAGCTATGACTACCTCTACATATGTGCCTG
SC23.47kAMAS2		GACGCAAGTGAGCAGTATGACTACCTCTACATATGTGTTTA
SC23.47kRev		TGAGTCTCGAGCGAGAAAGG
SC23.145kAMAS1	M	GCAACAGGAACCAGCTATGACGTTAGCGCCCCTGCATC
SC23.145kAMAS2		GACGCAAGTGAGCAGTATGACGTTAGCGCCCCTGACTT
SC23.145kRev		TGCCTCAAGATTACGTGGTG
SC13.95kAMAS1	P	GCAACAGGAACCAGCTATGACCACGTCCGGACAGCTCTC
SC13.95kAMAS2		GACGCAAGTGAGCAGTATGACCACGTCCGGACAGCCTTT
SC13.95kRev		CCAGCTTTTAGCAGCTCGTC
SC12.0.3kAMAS1	P	GCAACAGGAACCAGCTATGACCGCTTAAAAACTTTAGAGATATTG
SC12.0.3kAMAS2		GACGCAAGTGAGCAGTATGACCGCTTAAAAACTTTAGAGATCCTA
SC12.0.3kRev		ACGATTCAGATGCGCTGTTT
SC12.363kAMAS1	P	GCAACAGGAACCAGCTATGACGGAGCCCTGTGACCTTGG
SC12.363kAMAS2		GACGCAAGTGAGCAGTATGACGGAGCCCTGTGACCCCGA
SC12.363kRev		CGCCCGAAGTACATGAAGAT
SC12.798kAMAS1	P	GCAACAGGAACCAGCTATGACGCCTGACGTGCACTTACG
SC12.798kAMAS2		GACGCAAGTGAGCAGTATGACGCCTGACGTGCACTCGCA
SC12.798kRev		TGTCGTCTGCGACCTAAATG
SC12.943kAMAS1	P	GCAACAGGAACCAGCTATGACCTGGGGGTGGATAGGTTG
SC12.943kAMAS2		GACGCAAGTGAGCAGTATGACCTGGGGGTGGATAGACTA
SC12.943kRev		TGTGGCGACTACAACGTCTT
SC14.245kAMAS1	P	GCAACAGGAACCAGCTATGACACCTCGATATTGTATCCTTC
SC14.245kAMAS2		GACGCAAGTGAGCAGTATGACACCTCGATATTGTATCTCTT
SC14.245kRev		TCGCGATGATAGCCTTTTCT

Primer name	Polymorphism[a]	Primer sequence
SC3.101kAMAS1	P	GCAACAGGAACCAGCTATGACGCCCTGAAAGCCGTCGAG
SC3.101kAMAS2		GACGCAAGTGAGCAGTATGACGCCCTGAAAGCCGTAAAT
SC3.101kRev		AACGGGGCTAATCTCCAAGT
SC3.527kAMAS1	P	GCAACAGGAACCAGCTATGACTTATGCGGCTAGCAAATAAC
SC3.527kAMAS2		GACGCAAGTGAGCAGTATGACTTATGCGGCTAGCAAACCAT
SC3.527kRev		GCGTGCAACCGAGAATAGAT
SC3.968kAMAS1	P	GCAACAGGAACCAGCTATGACCGATAAAAGAGGAAGATACCG
SC3.968kAMAS2		GACGCAAGTGAGCAGTATGACCGATAAAAGAGGAAGATCACA
SC3.968kRev		GGCGAAGATAAAGCTGAACG
SC3.1170kAMAS1	P	GCAACAGGAACCAGCTATGACTTGGGAAGACGCGAGCGC
SC3.1170kAMAS2		GACGCAAGTGAGCAGTATGACTTGGGAAGACGCGAAAGA
SC3.1170kRev		TTCTCTCCCTCCCTCCCTTA
SC3.1528kAMAS1	P	GCAACAGGAACCAGCTATGACAAGGGCATATTCCAATTATCAC
SC3.1528kAMAS2		GACGCAAGTGAGCAGTATGACAAGGGCATATTCCAATTACTAT
SC3.1528kRev		GTCGGCGTAACAGTTCCTTG
SC3.1740kAMAS1	P	GCAACAGGAACCAGCTATGACGGCAGATGACCAATAACG
SC3.1740kAMAS2		GACGCAAGTGAGCAGTATGACGGCAGATGACCAATCGCA
SC3.1740kRev		ATCTCGAAGCTCGAATGGAA
SC3.1945kAMAS1	P	GCAACAGGAACCAGCTATGACGGCTGTGCCCGGTGTCAC
SC3.1945kAMAS2		GACGCAAGTGAGCAGTATGACGGCTGTGCCCGGTGCTAT
SC3.1945kRev		CGATGTTGCCCTTCTTCAA
SC3.2644kAMAS1	P	GCAACAGGAACCAGCTATGACGGATCAATTTTCTCATTTTCTACCAG
SC3.2644kAMAS2		GACGCAAGTGAGCAGTATGACGGATCAATTTTCTCATTTTCTAATAT
SC3.2644kRev		CGCCTATACCCGATCTTCCT
SC3.2983kAMAS1	P	GCAACAGGAACCAGCTATGACATGAGTTGGATGAGAGGG
SC3.2983kAMAS2		GACGCAAGTGAGCAGTATGACATGAGTTGGATGAGGAGT
SC3.2983kRev		CGTCTACTCGAGCTTAACACACA
SC5.205kAMAS1	P	GCAACAGGAACCAGCTATGACGCCAGTTTGTGTAGTTAGTC
SC5.205kAMAS2		GACGCAAGTGAGCAGTATGACGCCAGTTTGTGTAGTTGATT
SC5.205kRev		TCAAAAACACCAGCCTCTCC
SC5.1772kAMAS1	P	GCAACAGGAACCAGCTATGACCACGTGCGATTTGGATCG
SC5.1772kAMAS2		GACGCAAGTGAGCAGTATGACCACGTGCGATTTGGCCCA
SC5.1772kRev		AATGCAAACAGGAGAGCACA
SC5.2004kAMAS1	P	GCAACAGGAACCAGCTATGACTTCCTCTTTTCCAGCCAC
SC5.2004kAMAS2		GACGCAAGTGAGCAGTATGACTTCCTCTTTTCCAGTAAA
SC5.2004kRev		TCTGAAAACAGCCAGCTCAA
SC5.2218kAMAS1	P	GCAACAGGAACCAGCTATGACTTTTGCGTCCCGATTAAC
SC5.2218kAMAS2		GACGCAAGTGAGCAGTATGACTTTTGCGTCCCGATCCAT
SC5.2218kRev		AGTCACGGTACATGCGATCA
SC4.158kAMAS1	N	GCAACAGGAACCAGCTATGACCATGCTGTCAATATTCTATACG
SC4.158kAMAS2		GACGCAAGTGAGCAGTATGACCATGCTGTCAATATTCTACCCT
SC4.158kRev		AAGTCCACGTAGGCCATCC

Primer name	Polymorphism[a]	Primer sequence
SC4.530kAMAS1	P	GCAACAGGAACCAGCTATGACATCTTTGTGGACTTGAACG
SC4.530kAMAS2		GACGCAAGTGAGCAGTATGACATCTTTGTGGACTTGCGCA
SC4.530kRev		AAGCACCAAGCGTTTGAGAT
SC4.976kAMAS1	P	GCAACAGGAACCAGCTATGACCTCAGTCGTGGCCAATTG
SC4.976kAMAS2		GACGCAAGTGAGCAGTATGACCTCAGTCGTGGCCAGCTT
SC4.976kRev		GTTTGGGTATGACGGACACA
SC4.1245kAMAS1	N	GCAACAGGAACCAGCTATGACTCGAGTCAGCTCGTTTGG
SC4.1245kAMAS2		GACGCAAGTGAGCAGTATGACTCGAGTCAGCTCGTCCGA
SC4.1245kRev		AATCGCTCCTCCTCCTTCTC
SC4.1536kAMAS1	N	GCAACAGGAACCAGCTATGACATGTGTTAGTCTTTTATCTTTTTCTTC
SC4.1536kAMAS2		GACGCAAGTGAGCAGTATGACATGTGTTAGTCTTTTATCTTTTTTCTG
SC4.1536kRev		ATTTCGCACCCATGCTTCT
SC11.104kAMAS1	P	GCAACAGGAACCAGCTATGACTTCCTCTCTGCGAAGTAG
SC11.104kAMAS2		GACGCAAGTGAGCAGTATGACTTCCTCTCTGCGAAACAA
SC11.104kRev		AGCTTCCAACTCCACCCTTA
SC11.304kAMAS1	P	GCAACAGGAACCAGCTATGACGGCGAGTAGCATCCCGAC
SC11.304kAMAS2		GACGCAAGTGAGCAGTATGACGGCGAGTAGCATCCTAAT
SC11.304kRev		GGGGAACTAAGCCATCACTC
SC11.519kAMAS1	P	GCAACAGGAACCAGCTATGACCAGTTTCGCCATTGTACC
SC11.519kAMAS2		GACGCAAGTGAGCAGTATGACCAGTTTCGCCATTGCCCT
SC11.519kRev		GGCACAGCAACGTCCTACTA
SC11.732kAMAS1	P	GCAACAGGAACCAGCTATGACCTAAGCCCCCCCCAACGG
SC11.732kAMAS2		GACGCAAGTGAGCAGTATGACCTAAGCCCCCCCCACAGA
SC11.732kRev		TCAGAGGTCTGCCACTACCA
SC15.193kAMAS1	P	GCAACAGGAACCAGCTATGACGTACGACTCGAGTAACTG
SC15.193kAMAS2		GACGCAAGTGAGCAGTATGACGTACGACTCGAGTAGTTT
SC15.193kRev		CAGAACCATGGAGAGTCGAG
SC15.956kAMAS1	N	GCAACAGGAACCAGCTATGACGCCATTTTGAGAAGACGG
SC15.956kAMAS2		GACGCAAGTGAGCAGTATGACGCCATTTTGAGAAGCTGA
SC15.956kRev		CCATGTCTCTAGGCTGACCA
SC30.37kAMAS1	P	GCAACAGGAACCAGCTATGACGGAAACGACTAGCCAACG
SC30.37kAMAS2		GACGCAAGTGAGCAGTATGACGGAAACGACTAGCCCGCA
SC30.37kRev		TTGACGCGTTGTCTATGTTTG
SC8.6kAMAS1	N	GCAACAGGAACCAGCTATGACAAGCTCGAAATTATATAACCGCC
SC8.6kAMAS2		GACGCAAGTGAGCAGTATGACAAGCTCGAAATTATATAACTACT
SC8.6kRev		AGCGCCTATCCCTAAGCTC
SC8.115kAMAS1	M	GCAACAGGAACCAGCTATGACCACACGCACGAGCACAAC
SC8.115kAMAS2		GACGCAAGTGAGCAGTATGACCACACGCACGAGCAACAT
SC8.115kRev		GTTGTTTTGTGTAGGCGTCTG
SC8.696kAMAS1	M	GCAACAGGAACCAGCTATGACGACAGAGCAAAGAATATAATTACTG
SC8.696kAMAS2		GACGCAAGTGAGCAGTATGACGACAGAGCAAAGAATATAATTCATA

Primer name	Polymorphism[a]	Primer sequence
SC8.696kRev		ACGTTACGTATTGCTGAACCA
SC8.800kAMAS1	P	GCAACAGGAACCAGCTATGACGAGAAACGACCTGGTCCC
SC8.800kAMAS2		GACGCAAGTGAGCAGTATGACGAGAAACGACCTGGCACA
SC8.800kRev		TCGTTGCTCATTTGGAGTTG
SC8.1758kAMAS1	P	GCAACAGGAACCAGCTATGACCACCTCCACCACCACTTC
SC8.1758kAMAS2		GACGCAAGTGAGCAGTATGACCACCTCCACCACCATCTA
SC8.1758kRev		GCGATTTGAGTGATTTGGTG
SC26.45kAMAS1	N	GCAACAGGAACCAGCTATGACCAAAAGAATATTATAGGAAGAACTG
SC26.45kAMAS2		GACGCAAGTGAGCAGTATGACCAAAAGAATATTATAGGAAGACATA
SC26.45kRev		AAAAAGGGCGATGATCTAGG
SC27.41kAMAS1	M	GCAACAGGAACCAGCTATGACGCTAACGGGTTGCTCCTG
SC27.41kAMAS2		GACGCAAGTGAGCAGTATGACGCTAACGGGTTGCTTTTA
SC27.41kRev		AAGCTGGACATTGGCTTTGT
SC22.0.008kAMAS1	M	GCAACAGGAACCAGCTATGACACGCAAATTGATCCCCAC
SC22.0.008kAMAS2		GACGCAAGTGAGCAGTATGACACGCAAATTGATCCATAT
SC22.0.008kRev		GAACGGCTTTTGCTTTTGTC
SC22.168kAMAS1	P	GCAACAGGAACCAGCTATGACTTTCTATGTCGAGTCGGC
SC22.168kAMAS2		GACGCAAGTGAGCAGTATGACTTTCTATGTCGAGTTAGT
SC22.168kRev		TTTGTTGCGCACCTCTGTAG
SC9.5kAMAS1	N	GCAACAGGAACCAGCTATGACAAAGGAGGGGGGAGTCGG
SC9.5kAMAS2		GACGCAAGTGAGCAGTATGACAAAGGAGGGGGGAGCAGA
SC9.5kRev		TCTTTTTGACATCCGTGTCG
SC9.1043kAMAS1	M	GCAACAGGAACCAGCTATGACCCATTTGCGCCATCCTCC
SC9.1043kAMAS2		GACGCAAGTGAGCAGTATGACCCATTTGCGCCATCTCCT
SC9.1043kRev		TGCAGCATATCGACAAAAGA
SC9.1621kAMAS1	M	GCAACAGGAACCAGCTATGACTCCATCTCTATCACTACC
SC9.1621kAMAS2		GACGCAAGTGAGCAGTATGACTCCATCTCTATCACCCCG
SC9.1621kRev		GTTTGGTTTGGTTTGGTTGA
SC9.1750kAMAS1	M	GCAACAGGAACCAGCTATGACTGATAACTCGGTTGGCCG
SC9.1750kAMAS2		GACGCAAGTGAGCAGTATGACTGATAACTCGGTTGAACA
SC9.1750kRev		ATTGTTATCTCCGCCCTACC
SC6.7kAMAS1	M	GCAACAGGAACCAGCTATGACGTTGTCCAACGCTGTCCG
SC6.7kAMAS2		GACGCAAGTGAGCAGTATGACGTTGTCCAACGCTGCTCA
SC6.7kRev		TATTTTGGGTGGTGGACAAA
SC6.116kAMAS1	P	GCAACAGGAACCAGCTATGACGTTGCACTACGTAACCAG
SC6.116kAMAS2		GACGCAAGTGAGCAGTATGACGTTGCACTACGTAATTAA
SC6.116kRev		ACAATCGCCAAATCCTCTCT
SC6.220kAMAS1	P	GCAACAGGAACCAGCTATGACGTTTCTTCATCGTGTAGTTTC
SC6.220kAMAS2		GACGCAAGTGAGCAGTATGACGTTTCTTCATCGTGTAGCCTT
SC6.220kRev		GGCGTCTTCCAGGACTATGT
SC6.587kAMAS1	P	GCAACAGGAACCAGCTATGACTGTGGGCAAGTAGATTTATTG

Primer name	Polymorphism[a]	Primer sequence
SC6.587kAMAS2		GACGCAAGTGAGCAGTATGACTGTGGGCAAGTAGATTTCCTA
SC6.587kRev		GGTGCCTTGAATGAAATCTG
SC6.792kAMAS1	M	GCAACAGGAACCAGCTATGACGCGACATCGACAAAAGCC
SC6.792kAMAS2		GACGCAAGTGAGCAGTATGACGCGACATCGACAAAGACT
SC6.792kRev		GTTCACTTGCGAACTGGAAG
SC6.1006kAMAS1	P	GCAACAGGAACCAGCTATGACGCCAAAGATCATTTTGCGCTT
SC6.1006kAMAS2		GACGCAAGTGAGCAGTATGACGCCAAAGATCATTTTGCATTA
SC6.1006kRev		CGCAGAAGCCTTTCAACATA
SC6.1373kAMAS1	M	GCAACAGGAACCAGCTATGACTTCTTTCGGTACAGTTAGG
SC6.1373kAMAS2		GACGCAAGTGAGCAGTATGACTTCTTTCGGTACAGTCGGA
SC6.1373kRev		CCCATATCATCACCCCCTAT
SC6.1783kAMAS1	P	GCAACAGGAACCAGCTATGACGTGAGGGTGTTACTTATC
SC6.1783kAMAS2		GACGCAAGTGAGCAGTATGACGTGAGGGTGTTACTCCTT
SC6.1783kRev		GGTGCAGAAGTGAGGTTTTG
SC6.1956kAMAS1	M	GCAACAGGAACCAGCTATGACCCATGGGTTCCTGTGAGG
SC6.1956kAMAS2		GACGCAAGTGAGCAGTATGACCCATGGGTTCCTGTAGGA
SC6.1956kRev		TGCTACTCTCCCTCTTCTTCTGT
SC7.664kAMAS1	P	GCAACAGGAACCAGCTATGACAAAGGGGCGCGAAATTTC
SC7.664kAMAS2		GACGCAAGTGAGCAGTATGACAAAGGGGCGCGAAACCTT
SC7.664kRev		AGGTAGTTCGCGAAAGAAGTAAG
SC7.1097kAMAS1	P	GCAACAGGAACCAGCTATGACTTGCGATCGAAACGAAATG
SC7.1097kAMAS2		GACGCAAGTGAGCAGTATGACTTGCGATCGAAACGACGTA
SC7.1097kRev		CCATTCCACGTTCAAAAGAA
SC7.1291kAMAS1	P	GCAACAGGAACCAGCTATGACGTAAGCGCCGCCTTTCTC
SC7.1291kAMAS2		GACGCAAGTGAGCAGTATGACGTAAGCGCCGCCTTCTTT
SC7.1291kRev		AGACTGCGAAAAAGCATGAA
SC7.1537kAMAS1	N	GCAACAGGAACCAGCTATGACCCTTCATTCACACATCGG
SC7.1537kAMAS2		GACGCAAGTGAGCAGTATGACCCTTCATTCACACACTGA
SC7.1537kRev		CCGAATGCAGTGGAATAAAA
SC7.1723kAMAS1	N	GCAACAGGAACCAGCTATGACTGGCCTCTCCTAGCTCTG
SC7.1723kAMAS2		GACGCAAGTGAGCAGTATGACTGGCCTCTCCTAGCCTTT
SC7.1723kRev		GAATGGATGTGCAACTAGGC
SC19.101kAMAS1	P	GCAACAGGAACCAGCTATGACAATCGTTCGATCCCCCAC
SC19.101kAMAS2		GACGCAAGTGAGCAGTATGACAATCGTTCGATCCCATAT
SC19.101kRev		GATGAAAGACCGTGCAAAAC
SC19.240kAMAS1	P	GCAACAGGAACCAGCTATGACGCTTAAGTAATCGAGCCTG
SC19.240kAMAS2		GACGCAAGTGAGCAGTATGACGCTTAAGTAATCGAGTTTA
SC19.240kRev		ACGATGAGATGGTACGACGA
SC19.328kAMAS1	M	GCAACAGGAACCAGCTATGACTCTGGTGGGTGCTGCTGC
SC19.328kAMAS2		GACGCAAGTGAGCAGTATGACTCTGGTGGGTGCTGTCGT
SC19.328kRev		TCTGGGTTCAGTCCAGGGTA

Primer name	Polymorphism[a]	Primer sequence
SC20.113kAMAS1	P	GCAACAGGAACCAGCTATGACTTCAACAAACCAACAAAAGCG
SC20.113kAMAS2		GACGCAAGTGAGCAGTATGACTTCAACAAACCAACAAAGACT
SC20.113kRev		GCCCACTTCCTCTTCTTCTC
SC20.238kAMAS1	P	GCAACAGGAACCAGCTATGACAATAATCACCGAACAGCATC
SC20.238kAMAS2		GACGCAAGTGAGCAGTATGACAATAATCACCGAACAGACTG
SC20.238kRev		TCCTCGTCACCTGCTTACTC
SC21.236kAMAS1	M	GCAACAGGAACCAGCTATGACCGTAGAAGACTATAAACAACTC
SC21.236kAMAS2		GACGCAAGTGAGCAGTATGACCGTAGAAGACTATAAACAGTTT
SC21.236kRev		TGTTTGACACGTTGCATCTC
SC28.14kAMAS1	P	GCAACAGGAACCAGCTATGACACTTGAAGCACTGCTCCG
SC28.14kAMAS2		GACGCAAGTGAGCAGTATGACACTTGAAGCACTGCCTCA
SC28.14kRev		AGAGGACGCCGACAAGAT
SC28.48kAMAS1	N	GCAACAGGAACCAGCTATGACTCACGCGCACGGACCCGG
SC28.48kAMAS2		GACGCAAGTGAGCAGTATGACTCACGCGCACGGACATGT
SC28.48kRev		CATAGAACGGCTTAGCCAAA
SC29.53kAMAS1	M	GCAACAGGAACCAGCTATGACGATAATGTAAGCCTAAATTAGCGTC
SC29.53kAMAS2		GACGCAAGTGAGCAGTATGACGATAATGTAAGCCTAAATTAGAATT
SC29.53kRev		TTCTGGGATTTCTAAGCTCGT
SC34.6kAMAS1	P	GCAACAGGAACCAGCTATGACTAGGTAATCACTGCAAACATG
SC34.6kAMAS2		GACGCAAGTGAGCAGTATGACTAGGTAATCACTGCAAATGTA
SC34.6kRev		GCCTGTAGTGGAGGTTGATG
SC35.34kAMAS1	M	GCAACAGGAACCAGCTATGACGAGGTAAATAGCTTTGCCAG
SC35.34kAMAS2		GACGCAAGTGAGCAGTATGACGAGGTAAATAGCTTTGTTAT
SC35.34kRev		TAGCCTGGTTCGACGAAAT
SC36.15kAMAS1	P	GCAACAGGAACCAGCTATGACGCGAGACCTTACCTCTAC
SC36.15kAMAS2		GACGCAAGTGAGCAGTATGACGCGAGACCTTACCTACAT
SC36.15kRev		TATCGTGTTTCTTCCGCAAA
SC36.33kAMAS1	M	GCAACAGGAACCAGCTATGACACTTCTGTAGCTCGACCG
SC36.33kAMAS2		GACGCAAGTGAGCAGTATGACACTTCTGTAGCTCGCTCA
SC36.33kRev		CGAGCAGAAAGCAGCAAC
SC42.12kAMAS1	P	GCAACAGGAACCAGCTATGACGTGAGTGTGCCCTCCCTG
SC42.12kAMAS2		GACGCAAGTGAGCAGTATGACGTGAGTGTGCCCTCTATA
SC42.12kRev		AACCCCAACCCAAGACTG
SC1.500kAMAS1	M	GCAACAGGAACCAGCTATGACCAATCTTGATCTTACCATTCG
SC1.500kAMAS2		GACGCAAGTGAGCAGTATGACCAATCTTGATCTTACCACCCA
SC1.500kRev		GAAGGAGATGGGAGTGCAAA
SC1.583kAMAS1	P	GCAACAGGAACCAGCTATGACACTCGCATCCACCGCCGC
SC1.583kAMAS2		GACGCAAGTGAGCAGTATGACACTCGCATCCACCGTTGA
SC1.583kRev		CCTTTGAGACGATGCAGGA
SC1.3081kAMAS1	M	GCAACAGGAACCAGCTATGACTTGTAGAGGCGAGAAGGG
SC1.3081kAMAS2		GACGCAAGTGAGCAGTATGACTTGTAGAGGCGAGAGAGT

Primer name	Polymorphism[a]	Primer sequence
SC1.3081kRev		AATCCAACAGACACCGTCCT
SC1.3228kAMAS1	M	GCAACAGGAACCAGCTATGACAAGTGAGCTATGCTTCACC
SC1.3228kAMAS2		GACGCAAGTGAGCAGTATGACAAGTGAGCTATGCTTACCT
SC1.3228kRev		GCATGGGTCAAGCTCTTTGT
SC1.3338kAMAS1	P	GCAACAGGAACCAGCTATGACCCCCCCTCCCCCCCCTTC
SC1.3338kAMAS2		GACGCAAGTGAGCAGTATGACCCCCCCTCCCCCCCTCTT
SC1.3338kRev		AGGGTACTAGGCAACCTCCAA
SC1.4914kAMAS1	P	GCAACAGGAACCAGCTATGACTTTTGGTATCATTGGGAAGC
SC1.4914kAMAS2		GACGCAAGTGAGCAGTATGACTTTTGGTATCATTGGGGCGT
SC1.4914kRev		GCGACTACATTGCCACTTCA
SC1.5377kAMAS1	P	GCAACAGGAACCAGCTATGACGTGCAATGACCTTTGAATCGT
SC1.5377kAMAS2		GACGCAAGTGAGCAGTATGACGTGCAATGACCTTTGAACTGA
SC1.5377kRev		CGAAGCCATGTTCAGACCTC
SC1.5814kAMAS1	M	GCAACAGGAACCAGCTATGACCTGTGGTAGCCAGCCCCC
SC1.5814kAMAS2		GACGCAAGTGAGCAGTATGACCTGTGGTAGCCAGCATCT
SC1.5814kRev		TCGATCTCATGTCGCCTTTA
SC1.6551kAMAS1	P	GCAACAGGAACCAGCTATGACGACTAGAGCACTAGGAGC
SC1.6551kAMAS2		GACGCAAGTGAGCAGTATGACGACTAGAGCACTAGAGGA
SC1.6551kRev		GAAAACAGATGCCGTGGAAC
SC1.6752kAMAS1	P	GCAACAGGAACCAGCTATGACCTCCTCTATATTCTCTACCC
SC1.6752kAMAS2		GACGCAAGTGAGCAGTATGACCTCCTCTATATTCTCTCACA
SC1.6752kRev		AATAGCAGGAGCATCGCTAGA
SC12.499kAMAS1	M	GCAACAGGAACCAGCTATGACTAGGAGCAAGAGCACTCG
SC12.499kAMAS2		GACGCAAGTGAGCAGTATGACTAGGAGCAAGAGCATCCA
SC12.499kRev		CGTAGAGTGCCCTGCAAGTT
SC12.1155kAMAS1	M	GCAACAGGAACCAGCTATGACTTTCGGGTTCGCCTCAC
SC12.1155kAMAS2		GACGCAAGTGAGCAGTATGACTTTCGGGTTCGCCCTAT
SC12.1155kRev		TGCTAATGCCTTGTGAGACG
SC14.10kAMAS1	P	GCAACAGGAACCAGCTATGACGCTCGCGTCGGTAGTTC
SC14.10kAMAS2		GACGCAAGTGAGCAGTATGACGCTCGCGTCGGTAACTA
SC14.10kRev		GCTTAGTCAGATCGCTGATGC
SC14.114kAMAS1	P	GCAACAGGAACCAGCTATGACAGCAGGAAAGGGGCGCTG
SC14.114kAMAS2		GACGCAAGTGAGCAGTATGACAGCAGGAAAGGGGCAATA
SC14.114kRev		GGGGATGAGCGAGCTAAGA
SC14.850kAMAS1	M	GCAACAGGAACCAGCTATGACATGGTGATGCGATGTCATG
SC14.850kAMAS2		GACGCAAGTGAGCAGTATGACATGGTGATGCGATGTTGTA
SC14.850kRev		ACAGGGCAGGAAAGACAGAA
SC14.1022kAMAS1	M	GCAACAGGAACCAGCTATGACTGTGAGAGTGAAAGCGGC
SC14.1022kAMAS2		GACGCAAGTGAGCAGTATGACTGTGAGAGTGAAAGAAGT
SC14.1022kRev		CGAAGCCGCTAAAACAATTC
SC13.213kAMAS1	M	GCAACAGGAACCAGCTATGACCACTACACCTAAAAATCACCG

Primer name	Polymorphism[a]	Primer sequence
SC13.213kAMAS2		GACGCAAGTGAGCAGTATGACCACTACACCTAAAAATCCTCA
SC13.213kRev		AGCTTGCTTAGCTTGGTTGG
SC13.295kAMAS1	P	GCAACAGGAACCAGCTATGACAAAAAAACTCCGGTGACATAGAC
SC13.295kAMAS2		GACGCAAGTGAGCAGTATGACAAAAAAACTCCGGTGACATGAAT
SC13.295kRev		AACCACCACCCTCACAGAAC
SC3.255kAMAS1	M	GCAACAGGAACCAGCTATGACAGAGTATGAAGTGGTGATTG
SC3.255kAMAS2		GACGCAAGTGAGCAGTATGACAGAGTATGAAGTGGTGCCTA
SC3.255kRev		CGATCCACGTACAGCCTTCT
SC3.405kAMAS1	P	GCAACAGGAACCAGCTATGACTGGGATGCTTTCGCCCCG
SC3.405kAMAS2		GACGCAAGTGAGCAGTATGACTGGGATGCTTTCGCTACA
SC3.405kRev		GTTTTCCCCCGAGAAGATTT
SC3.716kAMAS1	P	GCAACAGGAACCAGCTATGACCATCATCACTCCAACGAC
SC3.716kAMAS2		GACGCAAGTGAGCAGTATGACCATCATCACTCCAATAAT
SC3.716kRev		CTTCATGGCCGAGTTTTCTC
SC3.2199kAMAS1	M	GCAACAGGAACCAGCTATGACAGATGAGGCTCTGTGCC
SC3.2199kAMAS2		GACGCAAGTGAGCAGTATGACAGATGAGGCTCTGCACG
SC3.2199kRev		GCAACCGGCTATTCCATCTA
SC3.2305kAMAS1	P	GCAACAGGAACCAGCTATGACGTTTTGCATGGGCTGCGCC
SC3.2305kAMAS2		GACGCAAGTGAGCAGTATGACGTTTTGCATGGGCTGTACT
SC3.2305kRev		CTGGACGTTCGGATAGAAGC
SC3.2836kAMAS1	P	GCAACAGGAACCAGCTATGACCTCCTGACATATCTACACC
SC3.2836kAMAS2		GACGCAAGTGAGCAGTATGACCTCCTGACATATCTAACCT
SC3.2836kRev		ATCCATGTTCAAAGCCAAGC
SC3.3070kAMAS1	P	GCAACAGGAACCAGCTATGACCAAACGTTACTCCAAAAGG
SC3.3070kAMAS2		GACGCAAGTGAGCAGTATGACCAAACGTTACTCCAAGCGT
SC3.3070kRev		TGCCTGTCAGTCGAAATGAA
SC5.936kAMAS1	M	GCAACAGGAACCAGCTATGACCATATTCTCGACCCATCTG
SC5.936kAMAS2		GACGCAAGTGAGCAGTATGACCATATTCTCGACCCAATTA
SC5.936kRev		ACTCATAATGCGGGGGATCT
SC5.1199kAMAS1	P	GCAACAGGAACCAGCTATGACTTTTGAGTTTACAGCAGCTTC
SC5.1199kAMAS2		GACGCAAGTGAGCAGTATGACTTTTGAGTTTACAGCAGACTG
SC5.1199kRev		AAGTCGCAAGCTGATTGACA
SC5.1349kAMAS1	P	GCAACAGGAACCAGCTATGACCCCACATCCCCAAAATAC
SC5.1349kAMAS2		GACGCAAGTGAGCAGTATGACCCCACATCCCCAAACCAA
SC5.1349kRev		GATGGTTGGGGGATAGGTGT
SC5.1460kAMAS1	M	GCAACAGGAACCAGCTATGACGTTTTGGATTCATGACCTTTT
SC5.1460kAMAS2		GACGCAAGTGAGCAGTATGACGTTTTGGATTCATGACCCCTA
SC5.1460kRev		GTTTGGATAGTGGGCGAGTT
SC5.2437kAMAS1	P	GCAACAGGAACCAGCTATGACTGTCTGCACCCTTCTTGG
SC5.2437kAMAS2		GACGCAAGTGAGCAGTATGACTGTCTGCACCCTTCCCGA
SC5.2437kRev		AGTAATGAGGGCGATGGTTG

Primer name	Polymorphism[a]	Primer sequence
SC5.2532kAMAS1	P	GCAACAGGAACCAGCTATGACGAATAATTAGGCGCGTTTTCAC
SC5.2532kAMAS2		GACGCAAGTGAGCAGTATGACGAATAATTAGGCGCGTTTCTAT
SC5.2532kRev		CGTATCAACCCATGGAGAGAA
SC4.272kAMAS1	P	GCAACAGGAACCAGCTATGACTTTCCATGCTCTTGCATAAAC
SC4.272kAMAS2		GACGCAAGTGAGCAGTATGACTTTCCATGCTCTTGCATGCAT
SC4.272kRev		ATCCGCAAAGAGCTGGAGT
SC4.634kAMAS1	M	GCAACAGGAACCAGCTATGACCTATAGGGCGTAATAACGG
SC4.634kAMAS2		GACGCAAGTGAGCAGTATGACCTATAGGGCGTAATACAGA
SC4.634kRev		AAGGTAGATGTGATCGCCGTA
SC4.1883kAMAS1	P	GCAACAGGAACCAGCTATGACTGCTCTCTGCCCCTCACC
SC4.1883kAMAS2		GACGCAAGTGAGCAGTATGACTGCTCTCTGCCCCTACCG
SC4.1883kRev		GGCAAGCTTGACTTGATGG
SC11.474kAMAS1	P	GCAACAGGAACCAGCTATGACATGCAGCAGCAACAGTAG
SC11.474kAMAS2		GACGCAAGTGAGCAGTATGACATGCAGCAGCAACAACAA
SC11.474kRev		GTGTGGCTGAGGAGTGTTCA
SC15.644kAMAS1	P	GCAACAGGAACCAGCTATGACGCCTGCATCAGACATTTG
SC15.644kAMAS2		GACGCAAGTGAGCAGTATGACGCCTGCATCAGACACCTT
SC15.644kRev		CGTTCCCTTTTCTGTTTGGA
SC8.131kAMAS1	M	GCAACAGGAACCAGCTATGACTGGAGCATGAGTTTTGAGG
SC8.131kAMAS2		GACGCAAGTGAGCAGTATGACTGGAGCATGAGTTTTAGGA
SC8.131kRev		CCTCCCATACTCCATCTCCA
SC8.262kAMAS1	P	GCAACAGGAACCAGCTATGACGATGCTTGACAGTGTAGG
SC8.262kAMAS2		GACGCAAGTGAGCAGTATGACGATGCTTGACAGTGCGGA
SC8.262kRev		GAGCACACCTGGGTCAAAGT
SC8.367kAMAS1	N	GCAACAGGAACCAGCTATGACATGGCTTACTACAGCCAG
SC8.367kAMAS2		GACGCAAGTGAGCAGTATGACATGGCTTACTACAGTAAA
SC8.367kRev		AGGCTGGCAGCTGTACTCAT
SC8.1036kAMAS1	M	GCAACAGGAACCAGCTATGACAACACGTATGGCGATCC
SC8.1036kAMAS2		GACGCAAGTGAGCAGTATGACAACACGTATGGCGGCCT
SC8.1036kRev		CTCATCCTGCTCAACGTCCT
SC24.171kAMAS1	M	GCAACAGGAACCAGCTATGACTCTATGTACTCAGCTCAGG
SC24.171kAMAS2		GACGCAAGTGAGCAGTATGACTCTATGTACTCAGCTTGGA
SC24.171kRev		CGCTCAGACCAGACCTCCTA
SC6.676kAMAS1	N	GCAACAGGAACCAGCTATGACAAGGCAGTCGCAACCCCG
SC6.676kAMAS2		GACGCAAGTGAGCAGTATGACAAGGCAGTCGCAACTACA
SC6.676kRev		TGCTGTGTTACCCGTACCTG
SC7.1422kAMAS1	M	GCAACAGGAACCAGCTATGACATGAAGCTGCTGGTACG
SC7.1422kAMAS2		GACGCAAGTGAGCAGTATGACATGAAGCTGCTGGCGCA
SC7.1422kRev		GACCTTATCACCCCTGCATT
SC9.700kAMAS1	P	GCAACAGGAACCAGCTATGACGGTAGGTGCCAGATGTC
SC9.700kAMAS2		GACGCAAGTGAGCAGTATGACGGTAGGTGCCAGACATT

Primer name	Polymorphism[a]	Primer sequence
SC9.700kRev		TGCTAACCACATCCAGAACG
SC9.885kAMAS1	N	GCAACAGGAACCAGCTATGACCTAGCCCCATCAATTCCCC
SC9.885kAMAS2		GACGCAAGTGAGCAGTATGACCTAGCCCCATCAATTTTCT
SC9.885kRev		GTGGATGGGCCAAGCTAATA
SC9.969kAMAS1	P	GCAACAGGAACCAGCTATGACCCGAAGCAAGAATCACCG
SC9.969kAMAS2		GACGCAAGTGAGCAGTATGACCCGAAGCAAGAATCCACA
SC9.969kRev		ACGCTGAGTCATGGGGATAA
SC9.1319kAMAS1	P	GCAACAGGAACCAGCTATGACAAGCAAAACATTCCTCCGG
SC9.1319kAMAS2		GACGCAAGTGAGCAGTATGACAAGCAAAACATTCCTTAGA
SC9.1319kRev		TCAACTCAGGGGCAAAAACT
SC9.211kAMAS1	P	GCAACAGGAACCAGCTATGACCGCTCTTCCACCTTGCCG
SC9.211kAMAS2		GACGCAAGTGAGCAGTATGACCGCTCTTCCACCTTAACA
SC9.211kRev		CTTCATCAGCGCCATTGATT
SC24.48k.2AMAS1	N	GCAACAGGAACCAGCTATGACTTGCGCCCCGGGGCACG
SC24.48k.2AMAS2		GACGCAAGTGAGCAGTATGACTTGCGCCCCGGGGTGCA
SC24.48k.2Rev		CTTTGGTCACTCGTCCCTGT
SC.1.40.7k.2AMAS1	M	GCAACAGGAACCAGCTATGACCCTGTAGCTTGTTTCCCGC
SC.1.40.7k.2AMAS2		GACGCAAGTGAGCAGTATGACCCTGTAGCTTGTTTCTAGA
SC.1.40.7k.2Rev		GACATAATCACAGCCGCGTA
SC13.1044kAMAS1	P	GCAACAGGAACCAGCTATGACCAGAAGTAGCGCCCATTG
SC13.1044kAMAS2		GACGCAAGTGAGCAGTATGACCAGAAGTAGCGCCCCCTA
SC13.1044kRev		ACGATACCTGCTGGAAGAGG
SC15.25kAMAS1	P	GCAACAGGAACCAGCTATGACGAGGAAGATGAAGTAGATATC
SC15.25kAMAS2		GACGCAAGTGAGCAGTATGACGAGGAAGATGAAGTAGACCTT
SC15.25kRev		GCTAAAGCACTCCCCTGTCT
SC9.553kAMAS1	P	GCAACAGGAACCAGCTATGACCACGTGGGTCTTTCATAG
SC9.553kAMAS2		GACGCAAGTGAGCAGTATGACCACGTGGGTCTTTCCCAA
SC9.553kRev		AAGAATAGGAGCCTGGCACA
SC7.891kAMAS1	P	GCAACAGGAACCAGCTATGACCATTCGAACAGGTGTATAC
SC7.891kAMAS2		GACGCAAGTGAGCAGTATGACCATTCGAACAGGTGTGCAT
SC7.891kRev		GAGTTTAAAACGCGGAGAATC
SC32.20kAMAS1	P	GCAACAGGAACCAGCTATGACAAGGAAAGGGGGTGATGG
SC32.20kAMAS2		GACGCAAGTGAGCAGTATGACAAGGAAAGGGGGTGCCGA
SC32.20kRev		CTTCCTGTTCGCCCTACAAC
SC1.6475kAMAS1	P	GCAACAGGAACCAGCTATGACGCTGCGCAGTCGGGCAG
SC1.6475kAMAS2		GACGCAAGTGAGCAGTATGACGCTGCGCAGTCGGATAA
SC1.6475kRev		GCCGTAAGGAACAGGTTCTG
SC3.2090kAMAS1	P	GCAACAGGAACCAGCTATGACGAAGCGTGCCGTATCGAC
SC3.2090kAMAS2		GACGCAAGTGAGCAGTATGACGAAGCGTGCCGTATTAAT
SC3.2090kRev		CGCAGAAACCCCACAAATAA
SC4.2508kAMAS1	P	GCAACAGGAACCAGCTATGACGAGGAGTCGCAACCCCAG

Primer name	Polymorphism[a]	Primer sequence
SC4.2508kAMAS2		GACGCAAGTGAGCAGTATGACGAGGAGTCGCAACCTAAA
SC4.2508kRev		GATGCCGTTTGTGACATTTG
SC9.1515kAMAS1	P	GCAACAGGAACCAGCTATGACAAAGAACCGACAGACACG
SC9.1515kAMAS2		GACGCAAGTGAGCAGTATGACAAAGAACCGACAGAACCT
SC9.1515kRev		TACGGCATCTGACCACTGAG

[a] "P" means primer set generated polymorphic amplicons between 86-124 and DW5; "M" means produce mono-morphic amplicon; "N" means no amplification.

APPENDIX C. TESTING THE PRESENCE OF *TOXA*, MATING TYPE GENES AND DIFFERENT LOCI OF *TOXB* IN THE POPULATION DERIVED FROM 86-124Δ*MAT1-2-1* × DW5Δ*MAT1-1-1*

Progeny[a]	*ToxB*	*ToxA*	*MAT1-1-1*	*MAT1-2-1*	*ToxB1*	*ToxB2*	*ToxB3*	*ToxB4*	*ToxB5*	*ToxB6*
C-1	Y	N	Y	N	N	N	N	N	N	Y
C-2	Y	Y	Y	N	N	N	N	N	N	Y
C-3	Y	Y	Y	N	N	N	N	N	N	Y
C-4	Y	Y	Y	N	Y	Y	Y	Y	Y	Y
C-5	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-6	Y	Y	Y	N	Y	Y	Y	Y	Y	N
C-7	N	N	Y	N	N	N	N	N	N	N
C-8	Y	N	Y	N	Y	Y	Y	Y	Y	N
C-9	N	N	N	Y	N	N	N	N	N	N
C-10	Y	Y	Y	N	N	N	N	N	N	Y
C-11	N	Y	Y	N	N	N	N	N	N	N
C-12	Y	Y	Y	N	Y	Y	Y	Y	Y	N
C-13	Y	N	Y	N	Y	Y	Y	Y	Y	N
C-14	Y	N	Y	N	N	N	N	N	N	Y
C-15	Y	Y	Y	N	Y	Y	Y	Y	Y	N
C-16	N	N	Y	N	N	N	N	N	N	N
C-17	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-18	Y	Y	Y	N	N	N	N	N	N	Y
C-19	Y	Y	N	Y	N	N	N	N	N	Y
C-20	N	Y	Y	N	N	N	N	N	N	N
C-21	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
C-22	Y	N	Y	N	N	N	N	N	N	Y
C-23	Y	N	Y	N	N	N	N	N	N	Y
C-24	Y	N	N	Y	N	N	N	N	N	Y
C-25	Y	N	Y	N	N	N	N	N	N	Y
C-26	Y	N	Y	N	Y	Y	Y	Y	Y	Y
C-27	Y	N	N	Y	Y	Y	Y	Y	Y	Y
C-28	Y	N	N	Y	Y	Y	Y	Y	Y	Y
C-29	Y	Y	Y	N	Y	Y	Y	Y	Y	N
C-30	N	Y	N	Y	N	N	N	N	N	N
C-31	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-32	N	N	Y	N	N	N	N	N	N	N
C-33	Y	N	N	Y	Y	Y	Y	Y	Y	Y
C-34	Y	N	Y	N	Y	Y	Y	Y	Y	N
C-35	N	Y	Y	N	N	N	N	N	N	N
C-36	N	Y	Y	N	N	N	N	N	N	N

Progenya	ToxB	ToxA	MAT1-1-1	MAT1-2-1	ToxB1	ToxB2	ToxB3	ToxB4	ToxB5	ToxB6
C-37	Y	Y	N	Y	N	N	N	N	N	Y
C-38	Y	Y	N	Y	N	N	N	N	N	Y
C-39	Y	N	N	Y	Y	Y	Y	Y	Y	Y
C-40	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-41	Y	N	Y	N	Y	Y	Y	Y	Y	N
C-42	Y	N	N	Y	N	N	N	N	N	Y
C-43	Y	N	N	Y	Y	Y	Y	Y	Y	Y
C-44	N	N	Y	N	N	N	N	N	N	N
C-45	Y	N	N	Y	N	N	N	N	N	Y
C-46	Y	Y	N	Y	Y	Y	Y	Y	Y	N
C-47	Y	N	Y	N	N	N	N	N	N	Y
C-48	Y	Y	N	Y	N	N	N	N	N	Y
C-49	Y	Y	Y	N	N	N	N	N	N	Y
C-50	Y	N	Y	N	Y	Y	Y	Y	Y	N
C-51	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-52	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
C-53	Y	Y	N	Y	Y	Y	Y	Y	Y	N
C-54	Y	N	Y	N	Y	Y	Y	Y	Y	Y
C-55	N	N	Y	N	N	N	N	N	N	N
C-56	Y	N	N	Y	Y	Y	Y	Y	Y	Y
C-57	N	N	N	Y	N	N	N	N	N	N
C-58	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
C-59	Y	N	Y	N	N	N	N	N	N	Y
C-60	Y	Y	N	Y	N	N	N	N	N	Y
C-61	Y	N	N	Y	Y	Y	Y	Y	Y	Y
C-62	Y	N	Y	N	N	N	N	N	N	Y
C-63	Y	N	Y	N	N	N	N	N	N	Y
C-64	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-65	Y	Y	N	Y	Y	Y	Y	Y	Y	N
C-66	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-67	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-68	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-69	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-70	Y	Y	N	Y	N	N	N	N	N	Y
C-71	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-72	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-73	Y	N	Y	N	N	N	N	N	N	Y
C-74	Y	N	Y	N	N	N	N	N	N	Y
C-75	Y	Y	N	Y	N	N	N	N	N	Y
C-76	Y	N	N	Y	Y	Y	Y	Y	Y	Y
C-77	Y	N	Y	N	N	N	N	N	N	Y

Progeny[a]	*ToxB*	*ToxA*	*MAT1-1-1*	*MAT1-2-1*	*ToxB1*	*ToxB2*	*ToxB3*	*ToxB4*	*ToxB5*	*ToxB6*
C-78	N	N	N	Y	N	N	N	N	N	N
C-79	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-81	N	N	Y	N	N	N	N	N	N	N
C-82	N	N	Y	N	N	N	N	N	N	N
C-84	Y	Y	Y	N	Y	Y	Y	Y	Y	Y
C-85	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-86	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-88	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-89	Y	Y	Y	N	Y	Y	Y	Y	Y	N
C-90	Y	Y	Y	N	Y	Y	Y	Y	Y	Y
C-92	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
C-93	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
C-94	Y	Y	Y	N	Y	Y	Y	Y	Y	N
C-95	Y	Y	N	Y	N	N	N	N	N	Y
C-96	Y	N	N	Y	Y	Y	Y	Y	Y	Y
C-97	N	N	Y	N	N	N	N	N	N	N
C-98	Y	N	Y	N	Y	Y	Y	Y	Y	Y
C-99	N	N	N	Y	N	N	N	N	N	N
C-100	Y	Y	Y	Y	Y	Y	Y	Y	Y	N
C-101	Y	Y	Y	N	Y	Y	Y	Y	Y	N
C-102	Y	N	Y	N	Y	Y	Y	Y	Y	Y
C-103	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
C-104	N	Y	N	Y	N	N	N	N	N	N
C-105	Y	N	Y	N	Y	Y	Y	Y	Y	Y
C-106	N	N	Y	N	N	N	N	N	N	N
C-107	N	N	Y	N	N	N	N	N	N	N
C-108	N	N	Y	N	N	N	N	N	N	N
C-109	Y	Y	Y	N	N	N	N	N	N	Y
C-110	Y	Y	N	Y	Y	Y	Y	Y	Y	Y
C-111	Y	Y	Y	N	N	N	N	N	N	Y
C-112	Y	Y	N	Y	N	N	N	N	N	Y
C-113	Y	N	N	Y	Y	Y	Y	Y	Y	N
C-114	N	Y	N	Y	N	N	N	N	N	N
C-115	Y	N	N	Y	N	N	N	N	N	Y
C-116	Y	N	Y	N	Y	Y	Y	Y	Y	N
C-117	Y	N	Y	N	Y	Y	Y	Y	Y	N
C-118	Y	N	Y	N	Y	Y	Y	Y	Y	N
C-119	Y	Y	Y	N	Y	Y	Y	Y	Y	N
C-120	Y	Y	N	Y	N	N	N	N	N	Y
C-121	N	N	Y	N	N	N	N	N	N	N

[a]Y means the gene is present, N means the gene is absent.

APPENDIX D. PHENOTYPIC DATA FOR REACTION OF CDC_OSLER TO POPULATION DERIVED FROM 86-124Δ*MAT1-2-1* × DW5Δ*MAT1-1-1*

Isolate	Rep1	Rep2	Rep3	Rep4	AVE
C-1	2	2.5	1.5	3.5	2.4
C-2	3	4	2.5	3	3.1
C-3	2	3	2	3.5	2.6
C-4	2	2.5	2	1.5	2.0
C-5	3.5	4.5	4	5	4.3
C-6	4	4.5	3.5	5	4.3
C-7	1	3.5	2.5	2.5	2.4
C-8	2	2.5	2.5	4	2.8
C-9	2	2	2	3.5	2.4
C-10	2.5	3	2.5	3.5	2.9
C-11	3.5	4.5	3.5	3.5	3.8
C-12	2	2	2	2	2.0
C-13	2	3.5	1.5	3	2.5
C-14	1	2	-	1.5	1.5
C-15	2	4	3.5	3.5	3.3
C-16	3.5	3.5	3.5	4.5	3.8
C-17	3	4.5	4	3	3.6
C-18	2	2	1	1.5	1.6
C-19	1.5	3	1.5	3	2.3
C-20	1	3.5	2.5	4	2.8
C-21	-	4.5	2.5	4.5	3.8
C-22	2	3.5	3.5	3.5	3.1
C-23	2	3.5	2	4	2.9
C-24	2.5	-	1	2	1.8
C-25	-	-	-	-	-
C-26	4	2	1	3.5	2.6
C-27	3.5	-	2	3	2.8
C-28	3.5	-	2	3.5	3.0
C-29	-	3.5	2	2	2.5
C-30	-	3.5	3.5	4	3.7
C-31	3.5	4.5	4	5	4.3
C-32	-	4.5	4	2.5	3.7
C-33	-	2	2	3.5	2.5
C-34	-	4.5	4	5	4.5
C-35	-	4	3.5	3.5	3.7
C-36	3.5	3.5	4.5	4.5	4.0
C-37	2.5	3	4	4.5	3.5

Isolate	Rep1	Rep2	Rep3	Rep4	AVE
C-38	2.5	4	3.5	4.5	3.6
C-39	2	2.5	2	3	2.4
C-40	3	4.5	4	4.5	4.0
C-41	2.5	3.5	4.5	5	3.9
C-42	2	3.5	2.5	2.5	2.6
C-43	1.5	3	2.5	3	2.5
C-44	1.5	3	2	2	2.1
C-45	1	2	1	2	1.5
C-46	4	4.5	4.5	5	4.5
C-47	4	3.5	-	2.5	3.3
C-48	2	1.5	2	2	1.9
C-49	2.5	4	-	3	3.2
C-50	4	4.5	4.5	4	4.3
C-51	4.5	3.5	-	5	4.3
C-52	3.5	3	-	5	3.8
C-53	4	3.5	-	3.5	3.7
C-54	4	4	4.5	4.5	4.3
C-55	2	3.5	2	2.5	2.5
C-56	2	4.5	2	3	2.9
C-57	-	3.5	1	2	2.2
C-58	-	-	-	-	-
C-59	2	3	2	-	2.3
C-60	1	2	2	-	1.7
C-61	2.5	2.5	2	-	2.3
C-62	1.5	-	-	1	1.3
C-63	1.5	2	-	2	1.8
C-64	2	3.5	-	2	2.5
C-65	4	4.5	2.5	4	3.8
C-66	4	4.5	-	4.5	4.3
C-67	-	-	-	-	-
C-68	3.5	4.5	-	3.5	3.8
C-69	4	4.5	-	3.5	4.0
C-70	-	4.5	-	4	4.3
C-71	-	3	-	4.5	3.8
C-72	2	2.5	-	1.5	2.0
C-73	4.5	5	-	5	4.8
C-74	4.5	4.5	-	4	4.3
C-75	3.5	4	-	4.5	4.0
C-76	3	2.5	-	2.5	2.7
C-77	4.5	5	-	5	4.8
C-78	2	2.5	-	1.5	2.0

Isolate	Rep1	Rep2	Rep3	Rep4	AVE
C-79	2	2.5	-	2.5	2.3
C-81	4.5	5	-	3.5	4.3
C-82	-	-	-	-	-
C-84	4.5	4.5	-	4	4.3
C-85	5	5	-	4	4.7
C-86	4	4	-	5	4.3
C-88	4.5	5	-	4.5	4.7
C-89	4	5	-	3	4.0
C-90	4	4.5	-	3.5	4.0
C-92	2	2.5	-	1	1.8
C-93	2.5	1	-	1.5	1.7
C-94	4.5	3.5	-	2.5	3.5
C-95	2.5	2	-	1	1.8
C-96	4.5	4	-	4	4.2
C-97	2	2.5	-	2	2.2
C-98	2.5	3.5	-	4	3.3
C-99	1.5	2.5	-	1	1.7
C-100	4.5	4	-	4.5	4.3
C-101	4	3	-	3	3.3
C-102	4.5	4.5	-	3.5	4.2
C-103	4	5	-	3.5	4.2
C-104	4.5	-	-	3.5	4.0
C-105	5	4.5	-	3	4.2
C-106	2	1	1	-	1.3
C-107	2	1.5	3	-	2.2
C-108	4.5	3.5	3	-	3.7
C-109	3.5	4.5	4	-	4.0
C-110	4.5	3	1.5	-	3.0
C-111	2	2.5	2	-	2.2
C-112	1.5	2.5	3.5	-	2.5
C-113	2	1	2	-	1.7
C-114	2	2	2	-	2.0
C-115	2.5	3.5	2.5	-	2.8
C-116	-	-	-	-	-
C-117	4.5	4	2	-	3.5
C-118	4	4.5	3	-	3.8
C-119	3	2.5	1	-	2.2
C-120	3	3.5	2.5	-	3.0
C-121	2.5	3.5	2	-	2.7