SELECTION OF HARD RED WINTER WHEAT LINES WITH DIVERSE RESISTANCE TO LEAF SPOT DISEASES

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Title

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

MASTER OF SCIENCE

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ABSTRACT

Tan spot and Septoria nodorum blotch cause serious yield losses in winter wheat in North Dakota as the majority of commercially grown cultivars are susceptible. This study aimed to identify lines with improved resistance for use as breeding parents. First, advanced NDSU breeding lines and alternative sources of resistance were inoculated with fungal isolates and tested for necrotrophic effector sensitivity. Second, resistant lines were derived from a highly heterogeneous recurrent mass selection F_2 population using single seed descent inbreeding coupled with selection for resistance. Finally, the best performers from both experiments (total of 52 lines) were evaluated to confirm resistance. In addition the 52 lines were analyzed with markers that detect *Tsn1* and the 1RS rye translocation. Twenty lines were identified with simultaneous resistance to four or three fungal isolates and insensitivity to three, two, or one necrotrophic effectors (of which 11 can be used directly as new parents).

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LIST OF ABBREVIATIONS

A .....Absent (gel marker data)

AFLP .....Amplified fragment length polymorphism

AR.....AR CrossB10

ATG .....Translation start codon

bar .....Atmospheric pressure at sea level

BG .....BR34 x Grandin lines/population

CIMMYT .....International Maize and Wheat Improvement Center

cr .....cross

DarT .....Diversity Arrays Technology

DH .....Double haploid

DNA.....Deoxyribonucleic acid

dNTP .....Deoxynucleotide mix

EST .....Expressed sequence tag

$F_{2:5}$ .....Fifth generation derived from the second generation

F_5 .....Fifth selfed generation

FHB/Fhb .....Fusarium head blight

HRSW .....Hard red spring wheat

HRWW .....Hard red winter wheat

HWSW .....Hard white spring wheat

HWWW .....Hard white winter wheat

kDa .....Kilo dalton

L .....Ladder

LMW .....Low molecular weight

Lr .....Leaf rust

MAS .....Marker assisted selection

$MgCl_2$ .....Magnesium Chloride

MN .....Minnesota

Ms3 .....Male sterility gene 3

mya .....Million years ago

ND .....North Dakota

NDSU .....North Dakota State University

NE .....necrotrophic effector

NETS .....necrotrophic effector triggered susceptibility

ORF .....Open reading frame

P .....Present (gel marker data)

p= .....Probability

PCR .....Polymerase chain reaction

QTL .....Quantitative trait locus (loci)

R .....Rye chromatin

r= .....coefficient value

R1 .....Race 1

R3 .....Race 3

RAPD .....Randomly amplified polymorphic DNA

RFLP .....Restriction fragment length polymorphism

RICL .....Recombinant inbred chromosome line

RIL .....Recombinant inbred line

RMS .....Recurrent mass selection

RNA .....Ribonucleic acid

Rpm .....Revolutions per minute

s .....second

SA .....South African

SCoT .....Start codon targeted polymorphism

SNB .....Septoria nodorum blotch

SNP .....Single nucleotide polymorphism

SNPD .....Single nucleotide polymorphism detector

Sr31 .....Stem rust 31 (resistance gene)

Sr50 .....Stem rust 50 (resistance gene)

SRWW .....Soft red winter wheat

SSD .....Single seed descent

SSR .....Simple sequence repeat

SWSW .....Soft white spring wheat

SWWW .....Soft white winter wheat

Taq .....Taq polymerase

Taq Man .....Polymerase chain reaction

ToxA .....Ptr ToxA/SnToxA

TS .....Tan spot

USA .....United States of America

USDA .....United States Department of Agriculture

V8-PDA .....V8 potato dextrose agar

YPD .....Yeast potato dextrose

LIST OF SYMBOLS

≈ .....Approximately

µL .....Microliter

mL .....Milliliter

10^6 1,000,000

ha.....Hectare

^oC.....Degrees Celsius

“ .....Inch

% .....Percent

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1. INTRODUCTION

Numerous environmental conditions, pests, and diseases can adversely affect wheat yield in North Dakota. These include poor seedling establishment under hot and dry fall conditions; inadequate snow cover and low winter temperatures resulting in winter kill; spring flooding; lodging under windy, wet conditions; and heat and drought stress. Dominant diseases of the region include Fusarium head blight, the wheat rusts, tan spot, and Septoria nodorum blotch. Insect pests such as aphids and wheat curl mite occur more sporadically, while the wheat stem sawfly is becoming a more regular problem in the western parts of the state. Winter wheat breeding in the state is directed at the selection of lines that have broad adaptation to the prevailing environmental conditions and parasites, yield well, and have good processing quality.

Tan spot and Septoria nodorum blotch can each cause significant yield losses of up to 50% in severe cases (Chu et al., 2008), while both fungi can infect other species such as wild grasses, have a sexual reproduction phase, and have similar looking symptoms. Resistance to both diseases was found to be complex and primarily under polygenic control, but some single genes with major effects were also encountered (Friesen et al., 2008; Frecha, 1973). Reduced infection may not only result from resistance to fungal infection and spread per se, but also from insensitivity to necrotrophic effectors. These necrotrophic effectors aid fungal infection by killing leaf tissue for the invading fungus to feed on.

North Dakota produces 14% of the total US wheat production and is the second largest wheat producing state (after Kansas). Winter wheat, within North Dakota, was seeded on 750,000 acres in 2014 which was about 1.79% of the 41.9 million acres seeded in the US (USDA, 2014; Bangsund and Leistritz, 2005). Increased popularity of winter wheat in recent years due to its ability to out yield spring wheat while catching more moisture and spreads out

the field work (North Dakota Wheat Commission). This motivated the decision to introduce a new winter wheat breeding program at NDSU in 2011. The founder germplasm of the new program had limited disease resistance and winter hardiness; thus additional genes needed to be found and combined with broad adaptation, good yield, and end-use quality in order to establish a productive breeding population. It is furthermore imperative to continually acquire new and useful winter wheat germplasm through pre-breeding efforts. A first comprehensive attempt to quantify resistance to the two diseases among commercially grown winter wheat cultivars and advanced breeding lines was made by Liu et al. (2015) who found promising resistance in SY Wolf and Decade. However, the need for a broader and more diverse range of breeding parents with good resistance coupled with winter hardiness remained and prompted this study.

The purpose of this study was therefore to evaluate a group of advanced breeding lines from the breeding program in order to assess the scope of currently available resistance; evaluate potentially new sources of resistance; and to develop additional resistant lines from a highly diverse pre-breeding population through single seed descent inbreeding with concurrent selection for resistance.

2. LITERATURE REVIEW

2.1. Introduction

2.1.1. Wheat

World-wide, wheat is grown on 218.4 million ha producing 713 million tons per year (FAOSTAT, 2014). Wheat provides about 20% of the food calories consumed by people worldwide and is a staple in many countries with about one-third of the world's people relying on wheat for their nourishment (Oklahoma Cooperative Extension Service et al., 2008). Wheat is a versatile crop, well adapted to grow in harsh environments. Wheat production in China, India, Russia, France, Australia, and the United States was 121.7, 93.5, 52, 38.6, 22.8, and 57.9 million tons, respectively, as of 2013 (FAOSTAT 2014).

Bread consumption transcends race, culture, and religion. One acre of wheat is able to produce enough wheat to make about 2,500 loaves of wheat bread, able to feed a family of four for about 10 years, and only requires nine seconds for a combine to harvest enough wheat for 70 loaves (Oklahoma Cooperative Extension Service et al., 2008). Besides bread, wheat can also be used to make pasta, various baked goods, and feed for animals. Some non-food products made from wheat are wallboard, cosmetics, pet foods, various paper products, paste, oil, gluten, alcohol, concrete, trash bags, and soap (Oklahoma Cooperative Extension Service et al., 2008).

Common wheat (*Triticum aestivum*, 2n=6x=42) is an allohexaploid which arose as the result of natural hybridization events. A domesticated form of allotetraploid wild emmer (*Triticum turgidum* ssp. *dicoccoides*, 2n=4x=28, AABB) hybridized with diploid *Aegilops tauschii* (2n=2x=14, DD), and the resulting progeny underwent a natural chromosome doubling event to have two copies of each genome. Emmer wheat in turn was derived from a cross between diploid Einkorn wheat (*T. urata*, 2n=2x=14) and an unknown goatgrass (2n=2x=14)

(Petersen et al., 2006 and Cox, 1998). Progeny from this hybridization underwent natural chromosome doubling to result in wild emmer wheat. The types of wheat grown both world-wide and within the United States are durum, hard red spring wheat (HRSW), soft red winter wheat (SRWW), hard white wheat (both spring and winter wheat, HWSW and HWWW respectively), soft white wheat (spring and winter, SWSW and SWWW), and hard red winter wheat (HRWW) (Oklahoma Cooperative Extension Service et al., 2008). Wheat type is classified mainly by seed hardness and color. In the USA an example of some states which grow hard red winter wheat include: Oklahoma, Kansas, Colorado, Texas, North Carolina, North Dakota, and other states. In North Dakota alone, 750,000 acres were seeded with winter wheat in 2014 which was approximately 1.79% of the 41.9 million acres seeded in the United States (USDA, 2014).

Winter wheat is usually planted in the fall and harvested around May or early in the summer. During the growing season, plants require some cold period to vernalize; to trigger the plants to start the flowering process. If they do not get this cold period, they will stay vegetative longer than usual. The time between planting and winter allows the plants to grow and prepare for their dormant period during the winter. This is why it is very important in breeding winter wheat with other germplasm sources to make sure the progeny have the winter growth habit and are winter hardy. However, winter wheat still experiences winter kill under extreme temperature conditions, especially if plants do not get covered with snow during the winter.

Regardless of what type of wheat is grown or where it is grown, during the growing season it encounters yield limiting biotic and abiotic stresses during the growing season. These include foliar diseases such as leaf rust (caused by *Puccinia triticina* Eriks.), stem rust (caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. E. Henn.), Fusarium head blight [caused by

Fusarium graminearum (Schwabe), *Gibberella zeae* teleomorph (Schw.) Petch.; referred to as FHB or scab], tan spot (caused by *Pyrenophora tritici-repentis* (Died.) Drechsler; asexual stage: *Drechslera tritici-repentis* and referred to as TS), and Septoria nodorum blotch (caused by *Parastagonospora nodorum* (Berk.) Quaedvlieg, Verkley & Crous, and referred to as SNB). Each of the latter diseases is capable of causing yield losses up to 50% (Chu et al., 2008). Tan spot was the most commonly occurring disease in North Dakota during 2015 and was more prevalent during tillering and flag leaf emergence (NDSU Extention Service, 2015) while no incidences of glume blotch were reported. Tan spot disease incidence was highest (over 50%) in the southeastern and western parts of the state and ranged from 0 to over 50% in the central portion (from north to south). Thus, the occurrence of tan spot within North Dakota in 2015 was high, but the severity percentage was between 6-15%, with rare incidences of 26-50%. In 2014 the tan spot incidence exceeded 50% in roughly half of the state, being concentrated in the mid and western parts. However, tan spot severity rarely amounted to 26-50% or more than 50%, and the majority of the state experienced 1-5% severity. In 2014 no glume blotch was reported for the majority of the state (NDSU Extension Service). Both tan spot and SNB diseases are major concerns when growing winter wheat in North Dakota and will be focused on in this study.

Tan spot disease was first described in 1823, but has been identified in the USA, Europe, and Japan during the early 1900s (Diedicke, 1902; Dreshler, 1923; and Hosford, 1982). *Pyrenophora tritici-repentis* was first believed to be a saprophytic fungus that occasionally caused minor to severe spotting on wheat in the field. Since the 1940s more severe outbreaks were observed (Hosford, 1982) and since the 1970s, epidemics began to be recorded in the USA, Canada, the southern cone of Africa, and Australia (Rees and Platz, 1992; Tekauz, 1976; Hosford, 1971). The more severe outbreaks in the 1940s were due to the fungus acquiring the

necrotrophic effector (NE) Ptr ToxA from *Parastagonospora nodorum* (causal agent of Septoria nodorum blotch) through a horizontal gene transfer event (Friesen et al., 2006).

2.1.2. Tan spot

Since the earliest studies of this fungus, it has been identified as being a necrotrophic fungus which can cause a lot of damage to wheat during the growing season while in its parasitic phase but can also survive on dead/dying plant tissue (Singh et al., 2010). This foliar disease is able to infect durum, bread wheat, other types of wheat, smooth bromegrass, and others. According to previous studies, *Pyrenophora tritici-repentis* has the widest host range on grasses and is able to infect at least 26 species, but is non-pathogenic on oat and barley while only weakly pathogenic on rye (Krupinsky, 1992; Morrall and Howard, 1975; Maraite et al., 1992; and Weise, 1987). *P. tritici-repentis* has occurred worldwide and is a significant disease everywhere wheat is grown, provided the environmental conditions are conducive for disease development. Hosford and Busch (1974) did a study comparing the field performance of three wheat cultivars which were artificially infected, then subjected to different wet period durations and found that the cultivar with the highest yield loss was affected after shorter wet period exposures than those with more resistance. Since the 1970's the adoption of practices to retain residue as well as conservation tillage or no-till farming has led to an increase in incidence of this disease (and caused the epidemics previously seen). *Pyrenophora tritici-repentis* over winters in both wheat stubble as well as on other gramineous hosts and undergoes sexual reproduction to form pseudothecia which mature during the fall and winter seasons. Once spring comes, the ascospores are actively ejected from the pseudothecia and serve as the primary source of inoculum (Hosford, 1982; Morrall and Howard, 1975; Solomon et al., 2006a; and Rees et al., 1982). Once on the leaves, the ascospores germinate and penetrate the epidermal cell layer

directly. Upon entering the leaf tissue, hyphae grow intercellularly in the leaf's mesophyll layer. The big lesions formed on susceptible hosts give rise to the growth of asexual conidia producing conidiophores which are the secondary source of inoculum during the growing season. Following maturation, the conidia are dispersed over longer distances via the wind. These conidia then infect leaves of other plants and produce more conidia in a number of repeated cycles during the growing season (Schilder and Bergstrom, 1992; and Rees and Platz, 1980). Symptoms which can show up on susceptible wheat lines include large, tan-colored lesions which are often surrounded by chlorotic haloes. If the lines are highly susceptible these lesions which start off small get bigger in size as they coalesce and cause the leaf to die. However if the lines are resistant, the symptoms are either very tiny pin point black dots without a chlorotic halo or a black spot which appears as though the plant killed off the fungus. The dead leaf tissue, from highly susceptible lines, can serve as an additional food supply for the fungus and decreases the photosynthetic area. This resulting decreased photosynthetic area causes plant stress and decrease yield potential of the infected plants.

Fungal isolates of *P. tritici-repentis* are genetically diverse and were classified into eight races utilizing a differential set of lines to distinguish each race (Lamari et al. 2003) which began by evaluating the isolate's ability to cause necrosis, chlorosis, or both on these individual lines. Four pathotypes were characterized this way until isolates were found which were able to cause the same symptoms, but on different wheat lines which previously showed no sensitive reaction (Lamari et al., 1995). Race 1 isolates produce both necrosis and chlorosis on sensitive wheat genotypes, Glenlea and 6B365 (sensitive to tan necrosis and chlorosis). Race 2 isolates only induce necrosis on Glenlea while race 3 isolates only induce chlorosis on 6B365. Isolates representing the last race, race 4, are unable to induce either symptom on Glenlea and 6B365 so

are considered avirulent. Race 5 was the first additional race identified with a new virulence pattern varying from the previously identified four. Isolates of race 5 are only able to induce chlorosis, but on 6B662 not 6B365. Races 3, 5, and combinations induce chlorosis in hexaploid wheat but necrosis in tetraploid wheat; like cultivar Coulter (Lamari et al., 2003; Singh et al., 2008). Race 6 isolates have the virulence patterns of races 5 and 3 with only inducing chlorosis, but on 6B365 and 6B662. In a study done by Lamari et al. (2003), races 7 and 8 were identified using lines and cultivars, including 6B365, 6B662, Glenlea, Salamouni, Coulter, and 4B1149. To identify them, Lamari et al. (2003) checked three isolates' virulence patterns to see if they infected the lines in a similar manner as previously identified isolates of known races. They grew culture filtrates of the three isolates with unknown virulence patterns (Az35-5, TS93-71B, and TS93-71F) along with seven isolates of known patterns [ASC1, 86-124, Hy 331-9, 90-2, Alg3-24, AlgH2 (races 1-6), and Az35-1]. All ten isolates were subjected to Southern and Western blotting analyses to look for similarities among the isolates for common necrotrophic effector (NE) producing genes. In conclusion, Lamari et al. (2003) determined that the virulence pattern of isolate Az35-5 was a combination of the race 2 and 5 virulences because this isolate was capable of inducing necrosis and chlorosis on Glenlea and 6B662. Both TS93-71B and TS93-71F, like Az35-5, expressed a different virulence pattern combination than what was previously seen. This new pattern combined the virulence patterns of races 2, 3, and 5. However, unlike Az35-5, TS93-71B and TS93-71F caused chlorosis on 6B365 but not on 6B662. Isolate Az35-5 was then classified as race 7 while TS93-71B and TS93-71F were grouped together as isolates of race 8. A study done by Singh et al. (2006b) looking for new sources of resistance to tan spot, septoria tritici blotch [caused by *Mycosphaerella graminicola* (Fückl) J. Schröt in Cohn], and Septoria nodorum blotch mentions the existence of eleven races which *Pyrenophora tritici-*

repentis isolates are classified into. Published findings by Ali and Francl (2003) reported races 1 to 5 as well as race 9 to occur in North America while North Africa and the Middle East see races 6, 7 and 8 (as reported in Lamari et al. (2003)). Finally, in South America, isolates representing races 10 and 11 occur (Ali et al., 2002; and Ali and Francl, 2002). The additional three races may have very little known information about them considering how rare it is to find any mention of them. Ali and Francl (2002), as reported in their abstract, observed a set of *P. tritici-repentis* isolates which induced necrosis reactions on Glenlea, Salamouni, Katepwa, ND495, and 6B365 (previously classified races either induced chlorosis or nothing on 6B365). The line M-3 was noted as expressing a resistant reaction and did not suffer from either of the two common symptoms produced by *P. tritici-repentis* isolates. Ali and Francl (2003) did not mention race 9 of *P. tritici-repentis* in their study while looking at the race structure prevalent on wheat as well as non-cereal grasses. Their findings identified isolates representing races 1, 2, 4, and 5 on wheat and durum while only seeing races 1 and 4 on non-cereal grasses. Even though they focused on *P. tritici-repentis* isolates, many *P. bromi* isolates were found from smooth bromegrass. Within the brief abstract by Ali et al. (2003), an isolate named ARD-1 is said to have the ability to produce Ptr ToxA, Ptr ToxC , and Ptr ToxD. They thus recommended ARD1 to be classified as race 7, based on its capabilities and the new NE to be called Ptr ToxD.

Previous studies on tan spot have shown that yield losses due to this disease are highest when older plants are infected during the boot and flowering stages compared to when juvenile plants are infected (Rees and Platz 1983; Shabeer and Bockus 1988).Yield loss mainly results from reduction in kernel size and number of kernels per head, but other yield components such as the number of tillers, amount of dry matter, leaf area index, and grain size may also be reduced (Shabeer and Bockus, 1988; Rees and Platz 1983). *Pyrenophora tritici-repentis* also

affects the wheat grain quality as infection may result in lower test weight and smaller amounts of flour being produced from the fewer and smaller kernels. The fungus furthermore physically contaminates the grain by causing pink smudge on the kernels (Schilder and Bergstrom, 1994). Under severe infection, yield losses due to tan spot disease have been reported to be either approaching or at 50%.

Tan spot disease can be and has been partially controlled using different cultural practices, fungicides, or biological controls. Adopting crop rotations to avoid constantly growing wheat on wheat makes it harder for *P. tritici-repentis* to overwinter in previous wheat crop stubble, thus decreasing the total amount of primary inoculum to infect the next round of wheat. One other practice that can be used is reducing the amount of wheat crop stubble left above or on the soil surface by tilling the land (Sutton and Vyn, 1990; Bockus and Claasen, 1992). Fungicides have been used to provide cost effective control of the fungus. However, fungicides are harmful to the environment and have to be applied more than once. This can also be said for using biological controls with the exception that biological controls tend not to be harmful to the environment. Using resistant wheat cultivars requires less time than multiple fungicide applications.

2.1.3. Septoria nodorum blotch

In addition to the tan spot disease which negatively impacts wheat during the growing season, another foliar disease called Septoria nodorum blotch (SNB) occurs at the same time. The teleomorph of the fungus responsible for causing Septoria nodorum blotch was first called *Leptosphaeria nodorum* (Cunfer, 1997), initially described in the field, and cultured by Weber (1922). Weber (1922) reported that this disease caused severe grain loss in some areas of the United States. After this point, other reports came in from all over the world and Machacek

(1945) found the fungus to be abundant on seeds in Canada and described “seedling attack” (Hewett, 1965). Seedling attack was later named “seedling blight” of wheat which was observed in several European countries during 1954. Ponchet (1956) noticed that most of the seed lots in France were either infected with *L. nodorum*, *G. nivalis*, or both fungi with several lots containing over 60% of *L. nodorum* infected seeds. Following the initial naming of the fungus by Weber (1922) the teleomorph was transferred to the genus *Phaeosphaeria* and became *Phaeosphaeria nodorum* (Cunfer, 1997). The anamorph of this fungus was named *Stagonospora nodorum* (Cunfer, 1997). Then in more recent years, the names were updated to be *Parastagonospora nodorum* for both the sexual and asexual stages. *P. nodorum* is a major pathogen of wheat which, as stated in the review by Oliver et al. (2012), has emerged as a model for the necrotrophic fungus class Pleosporales. This model is being used by breeders as a way to advance more resistant cultivars and facilitates the understanding of other necrotrophic fungi which make up the Pleosporales group.

Weber (1922) collected *P. nodorum* isolates on wheat, rye, and *Poa pratensis.* Derevyankin (1969) found a slight infection of *P. nodorum* on rye. In 1969, Baker noticed a slight infection on Cocksfoot which lacked pycnidia growth. In a study by Williams and Jones (1973), they infected eight grass species (*Poa trivialis*, *P. pratensis*, *Agropyron repens*, *Bromus sterilis*, *Holcus lanatus*, *Avena ludoviciana*, *A. fatua*, and *Agrostis tenuis*) with *P. nodorum* after the plants were 11 weeks old with a spore concentration of 10^6 spores per mL. *P. nodorum* pycnidia were found on all species following the incubation of lower older leaves. In another experiment involving *Lolium perenne* and *L. multiflorum*, *P. nodorum* pycnidia were also found on the older leaves after being incubated. These studies gave evidence that *P. nodorum* can infect and grow on at least some grass species. *P. nodorum* was found by Osbourn et al. (1986) to be

capable of infecting barley (*Hordeum vulgare*) (Solomon et al., 2006b). *P. nodorum* is also heterothallic, having a sexual stage of reproduction involving two mating types. Halama (2002) studied mating-type ratios among 56 *P. nodorum* isolates (originating from Europe, USA, Israel, Morocco, and Australia) to discover that 92% of them had the MAT1-1 allele while the remaining 8% had the MAT1-2 allele. Mating-type ratios were discovered to be different by other researchers in different locations (Bennett et al., 2003 and Sommerhalder et al., 2006). *P. nodorum* is one of the important phytopathogenic species within the group Dothideomycetes. Just like *P. tritici-repentis*, *P. nodorum* is also able to overwinter in previous wheat crop stubble and has increased in incidence due to the adoption of conservation tillage methods, increased wheat production, increased use of nitrogen fertilizers, and growing wheat cultivars which are susceptible to *P. nodorum* (Crook et al., 2012). After *P. nodorum* conidia come into contact with the leaf surface, multiple germ tubes germinate from both the ends and middle of an individual conidia. These multiple germ tubes are capable of getting into the leaves via entering the stomata or directly through the leaf's cuticle (Solomon et al., 2006b). Yellowing of the leaves at the penetration points followed by protruding pycnidia is indicative of *P. nodorum* infection. Fungus hyphae grow inside the leaf tissue parallel to the leaf's vasculature. On susceptible plants, asexual pycnidia can form in the lesions roughly after a week. Whether growing the fungus in culture or it growing in nature, the pycnidia structures begin as a light color, but darkens and swells as it grows and matures (Douaiher et al., 2004). When growing on the leaf surface, the swelling pycnidia structure ruptures the cuticle layer. The new conidia contained within the pycnidia can spread via splash-dispersal. According to Shah and Bergstrom (2002), in order for a significant amount of disease and glume infection to occur, it is likely that about 2-4 cycles of infection with asexual conidia are needed. When the host dies, the fungus lives as a saprotroph in

infested wheat crop stubble via the conidia and undergo sexual reproduction. The sexual pseudothecia structures overwinter in the crop stubble, then during the next growing season, ascospores are actively ejected from them (Solomon et al., 2006a). Once the ascospores land on the leaf tissue and infect the plants, they produce pycnidia in the infected lesions (Bathgate and Loughman, 2001). Thus, the cycle continues each year if previous wheat crop stubble is left and if conducive weather is present for fungal growth. *P. nodorum* is closely related to *P. tritici-repentis* and causes similar lesions on susceptible wheat lines. Lesions of this fungus tend to be lens-shaped necrotic and chlorotic, but on resistant lines the lesions tend to be small and restricted with some resistant lines even lacking lesions (Liu et al., 2004a). Sources of inoculum for *P. nodorum* infections include: infected wheat seeds, conidia or ascospores from previous infected wheat debris, and conidia from infected alternative gramineous hosts (Harrower, 1974; Cunfer, 1978; Holmes and Colhoun, 1974; and Mehta, 1975).

The severity of *P. nodorum* infection was shown to increase with longer durations of a wet period post inoculation. Even in resistant lines, more lesions showed up when exposed to a longer time under mist (Eyal et al., 1977). Regardless of the wet period duration, the amount of time required by the fungus for successful host infection is different among the various cultivars involved (Brönniman et al., 1972). In addition to the wet period duration, temperature also affects conidia germination, fungal growth, and disease development (Rapilly and Skajenikoff, 1974; Wiese, 1977; Da Luz and Bergstrom, 1986). Temperature and wet period duration are not the only factors, leaf tissue age also affects disease susceptibility. In a study done by Baker and Smith (1979), they observed that secondary leaves were significantly more affected than the flag leaf and had more *P. nodorum* lesions while the size of the lesions were not significantly different. Djurle et al. (1996) studied the relationship between the duration of leaf wetness and

SNB disease progress of glume blotch in winter wheat utilizing currently available standard weather data. They found that they could predict an increase in the rate of disease by how long leaves stayed wet either in the top, middle, or bottom canopy areas. How long leaves remained wet was dependent upon the temperature, rainfall, the number of hours that humidity was above 90%, and the amount of wind present. In discussing the results, they mention that younger leaves are less susceptible as well as that it is likely for them to dry faster. These younger leaves would be at the top of the canopy which would be exposed to more light and wind. The lower leaves would be closer to pycnidia containing leaves from well-developed infection. Conidia, if splash-dispersed, could have a limited travel distance thus being unable to reach the upper canopy with the younger leaves. This would then cause the rate of SNB disease progress to be slower than leaves which are at the bottom and older (Aust and Hau, 1983; Jöensson, 1985). Many years later in 2012, Bnejdi et al. conducted a study looking at the relationship between leaf stages and resistance to *P. nodorum* controlled by epistatic genes in durum wheat. The 2-3 leaf stages showed a higher disease incidence than the 6-7 leaf stages.

P. nodorum isolates are genetically diverse, but unlike *P. tritici-repentis* isolates, they are not classified into separate races based on the symptoms they produce on a set of differential wheat lines. Observed genetic variation among isolates was due to differences in conidia sizes, cultural characteristics, host vegetative compatibility, and aggressiveness (Scharen and Krupinsky, 1970; Caten and Newton, 2000; Shipton et al., 1971; Krupinsky, 1997; and Mebrate and Cooke, 2001). Ali and Adhikari (2008) compared the aggressiveness and virulence of 40 *P. nodorum* isolates collected within North Dakota. The isolates differed significantly when they were used to infect four wheat lines (ND495, Alsen, Erik, and Salamouni; first two are susceptible while the remaining two are resistant). Four of the 40 isolates had low aggressiveness

(causing between 18% and 26% necrotic leaf area), whereas the majority (83%) had medium aggressiveness, and three lines were highly aggressive (causing between 57% and 59% of the leaf area to be necrotic). It was concluded that the wide range of variability among the different *P. nodorum* isolates was due to sexual recombination. Scharen et al. (1985) found that *P. nodorum* isolates from Canada, Europe, and South America, were not as virulent as the isolates from the United States. Isolates used in research studies include NOD-00, NOD-94, NOD-99, SN15, SN2000, SN2000KO6-1, SN1501, SN4, and many others (Kim and Bockus, 2003; Tan et al., 2014; Friesen et al., 2012; and current study).

By itself, *P. nodorum* is capable of causing significant yield loss, ranging from 31%-53% (Babadoost and Herbert, 1984 and Eyal, 1981). Yield losses due to *P. nodorum*, in highly susceptible plants, result from the complete destruction of leaf tissue and resultant loss of photosynthetic area. In addition to wreaking havoc on leaves, *P. nodorum* is also able to infect wheat glumes, thus causing Septoria nodorum glume blotch. *P. nodorum* is also able to reduce grain quality and test weight (Bhathal et al., 2003; and Solomon et al., 2006b).

Ways to help control *P. nodorum* include using seed which is free from previous *P. nodorum* infection, burning wheat crop residue, deep plowing, fungicides, or crop rotations. Fungicides can partially control this fungus, but are not environmentally friendly like host resistance which, in addition, provides long term protection.

In some studies focusing on virulence factors, transgenes were used to knock out specific genes of an isolate, thus creating new strains such as SN2000K06-1. This was done by Faris and Friesen (2009) in order to evaluate the effect ToxA in conferring susceptibility to SNB in the durum wheat cultivar Langdon (LDN). Along with eliminating the *SnToxA* gene in strains SN2000K028 and SN2000K06-1, they also used five ToxA-insensitive LDN recombinant inbred

chromosome lines (RICLs), a RICL called LDN-DIC 5B, LDN, and a RICL population derived from the cross LDN x LDN-DIC 5B. The LDN RICLs possess a disrupted *Tsn1* gene. Post inoculation and infiltration, they found LDN to be susceptible to the wild SNn2000 strain while the RICL parent, LDN-DIC 5B was resistant. The resulting progeny segregated. Overall, they found that all lines with ToxA sensitivity were also susceptible and that ToxA was the only virulence factor, also referred to as necrotrophic effector (NE), needed for conferring susceptibility in the population they made.

2.2. Necrotrophic effectors (NEs)

Both *P. tritici-repentis* and *P. nodorum* isolates produce necrotrophic effectors (NEs), formerly called host-specific toxins. Each NE can elicit a sensitivity reaction in the host provided the host possesses a dominant allele of the gene needed in order to cause a sensitive reaction. If the host does not possess the dominant sensitivity allele, then there will be an incompatible reaction and the individual plant will be insensitive to the NE. Faris et al. (2011) describes this interaction better by stating that the recognition of the NE by the host's single dominant allele causes a compatible sensitive reaction that leads to disease susceptibility. This is referred to as necrotrophic effector-triggered susceptibility (NETS), and results in leaf tissue death which is then used as a source of nutrients for the invading fungal mycelium. In essence, the NEs trigger the "normal" resistance response present in some genotypes which would normally interact with the trigger to kill the infected area thus restricting the fungus from growing further. The "normal" resistance response interacts in a gene-for-gene manner and is usually seen for leaf rust resistance. However with the NE interactions, which aid disease development, these are the inverse of the classical resistance mechanism. Wolpert et al. (2002) states that the NEs are important disease determinants. This can clearly be seen in the findings of Faris et al. (2011),

where they studied the interactions of the NEs SnToxA and SnTox2 from the *P. nodorum* isolates SN4 and SN5. Their study found that these NEs (SnToxA and SnTox2) act independently to explain about 25% or 26% of the disease variation in the plants being studied, but together they explain 50% of the variation. Thus, even though the two NEs can act independently, they act in an additive manner if present together. Host genotypes with the dominant sensitivity allele for both NEs expressed a significantly higher amount of disease than host genotypes which only had the dominant allele for either one of the two NEs.

2.2.1. The *P. nodorum* NE by host gene interaction

SnToxA and SnTox2 are two NEs which have been identified and characterized from *P. nodorum*. However, they are not the only ones which have been characterized. SnTox1, SnTox3, SnTox4, SnTox5, and the two newest, SnTox6 and SnTox7, have also been identified from different *P. nodorum* isolates (Liu et al., 2004a; Faris et al., 2010; Friesen et al., 2006; Friesen et al., 2007; Liu et al., 2009; Abeysekara et al., 2009; Zhang et al., 2011; Friesen and Faris, 2009; Friesen et al., 2012; Gao et al., 2014; and Shi et al., 2015). SnTox1, a 10.3kDa protein, was the first NE to be identified and characterized with sensitivity being controlled by a single dominant gene called *Snn1* (Liu et al., 2004a and Liu et al., 2012). The SnTox1-*Snn1* interaction was shown by Liu et al. (2004a) to account for 58% of disease variation. *Snn1* mapped to the distal end of chromosome arm 1BS, and SnTox1 was identified in about 85% of *P. nodorum* isolates collected worldwide (Liu et al., 2004a and Liu et al., 2012). SnTox2 was the third NE, 7-10kDa in size, to be identified and confers sensitivity in genotypes which possess the dominant *Snn2* gene located on chromosome 2D (Friesen et al., 2007). SnTox3 is the fourth NE; a mature protein ~18kDa in size that interacts with the *Snn3* gene which was found on chromosome arm 5BS of wheat (Oliver et al., 2012). A study looking at the interactions between SnTox3,

SnToxA, and SnTox2 found that both SnToxA and SnTox2 were epistatic to SnTox3, but SnToxA and SnTox2 were highly additive (Friesen et al., 2008). In a study done by Zhang et al. (2011), a second SnTox3 sensitivity gene was discovered on chromosome arm 5DS of *Aegilops tauschii*. The two host homoeo-loci are being distinguished with the gene symbols *Snn3-B1* and *Snn3-D1* to represent the two mapped locations. Zhang et al. (2011) concluded that the *Snn3-D1* gene was only present in *Ae. tauschii* accessions and not in polyploid wheat genotypes while the *Snn3-B1* gene is found in tetraploid and hexaploid wheat genotypes. Thus, from an evolutionary perspective *Snn3-B1* derives from the B genome donor, *Ae. speltoides*. In addition to this, it was observed that *Snn3-B1* results in weaker sensitivity effects compared to *Snn3-D1*. Chromosome 1A was found to be the location of the dominant *Snn4* gene which confers sensitivity to the next identified NE, SnTox4, which is light dependent (like SnToxA, SnTox1, and SnTox2) and estimated to be between 10 and 30kDa (Abeysekara et al., 2009). Friesen et al. (2012) identified and partially characterized a sixth NE, SnTox5, and compared its interaction in genotypes with SnToxA and SnTox3. Again, the SnTox5 NE was estimated to be between 10 and 30kDa in size and confers sensitivity in genotypes which have the dominant *Snn5* gene. This study determined that SnTox5 is also light dependent. When comparing interactions, it appeared that both SnTox5 and SnToxA can act independently (explained 37% and 31% of the variation in disease symptoms, respectively) or act additively to explain 60% of the symptom variation. Thus, wheat genotypes that have the dominant gene for sensitivity to either or both SnTox5 and SnToxA will produce a significantly higher disease reaction than homozygous recessive genotypes. Genotypes with either dominant gene produced about the same amount of disease. Friesen et al. (2012) also observed that the SnTox5-*Snn5* interaction masked the SnTox3-*Snn3* interaction in the genotype used in their study. This was similar to how both SnToxA and SnTox2 interacted with SnTox3.

Since SnTox5, two additional NEs, SnTox6 and SnTox7, were identified (Gao et al., 2014 and Shi et al., 2015). In the latter studies and in Oliver et al. (2012) evidence was presented for the occurrence of many more additional NEs. The isolate SN4 produces all known NEs, including both SnToxA and SnTox2 (Liu et al., 2015).

SnToxA, was the second NE identified and characterized from *P. nodorum* isolates. This same NE was first identified and characterized in *P. tritici-repentis* (Lamari and Bernier, 1989 and Lamari and Bernier, 1991). The gene *Tsn1*, found on the long arm of chromosome 5B, is responsible for conferring sensitivity to ToxA in *P. nodorum* and *P. tritici-repentis* if the dominant form of this gene is present in wheat genotypes (Oliver et al., 2012). Friesen et al. (2006) speculated that ToxA in *P. tritici-repentis* was obtained through a horizontal gene transfer from *P. nodorum.* This conclusion was based on the observation that the *SnToxA* gene from the SN15 *P. nodorum* isolate shares more than 99% identity with the *ToxA* gene from *P. tritici-repentis* (Stukenbrock and McDonald, 2007).

2.2.2. The *P. tritici-repentis* NE by host gene interaction

In addition to NEs produced by *P. nodorum, P. tritici-repentis* isolates also produce NEs, such as the previously mentioned Ptr ToxA common between the two fungi. Ptr ToxA was the first identified and characterized NE in the fungus causing tan spot. This NE produces a necrotic symptom in sensitive hosts. The *Tsn1* gene confers sensitivity in sensitive genotypes if present in the dominant form and the gene is located on the long arm of chromosome 5B (Anderson et al., 1999 and Ballance et al., 1989). Ptr ToxB is the second *P. tritici-repentis* NE to be identified and characterized. Similar to Ptr ToxA, this NE is a protein as well, but smaller in size and produces extensive leaf chlorosis in hosts having the dominant *Tsc2* gene located on the short arm of chromosome 2B (Friesen and Faris, 2004 and Strelkov et al., 1999). The third identified and

characterized NE is Ptr ToxC which is a nonionic, polar, low molecular weight molecule which produces chlorosis. *Tsc1* is the corresponding dominant gene which confers sensitivity to Ptr ToxC and is located on the short arm of chromosome 1A. One other NE, Ptr ToxD, was briefly mentioned by two studies and was observed to induce either chlorosis or necrosis according to each (Ciuffetti et al., 2003; and Meinhardt et al., 2003). However since then there has been no new information about this NE. Similar to the situation with *P. nodorum*, *P. tritici-repentis* fungi have been observed to produce more NEs than only the ones characterized so far. Within the *P. tritici-repentis* races, race 1 isolates (Pti-2) tend to produce both Ptr ToxA and Ptr ToxC while race 2 isolates only produce Ptr ToxA. Then race 3 isolates (331-9) only produce Ptr ToxC, but have been noted to cause necrosis symptoms instead of chlorosis in tetraploid wheat (Lamari et al., 2003; and Singh et al., 2008). Race 4 isolates do not produce any of the three currently characterized NEs and are considered to be avirulent. Race 5 isolates, like Dw5, tend to only produce Ptr ToxB (Effertz et al., 2002 and Singh et al., 2006b). Race 6 isolates produce the NEs Ptr ToxB and Ptr ToxC while isolates within race 7 produce Ptr ToxA and Ptr ToxB. Finally, isolates belonging to race 8 are able to produce all three known *P. tritici-repentis* NEs.

Aboukhaddour et al. (2012) conducted a study to investigate the effect Ptr ToxB had in causing disease by silencing the *ToxB* gene in the wild race 5 isolate Alg3-24. In a study done in 2009, Aboukhaddour et al. found that the *ToxB* gene is represented as multiple copies within different race 5 *P. tritici-repentis* isolates and even in race 3 and 4 isolates which do not produce this NE (Martinez et al., 2004; Strelkov et al., 2006). In order to silence the multiple copies of *ToxB*, they utilized both sense and anti-sense mediated RNA gene silencing focused on the open reading frame (ORF) of the gene's sequence. From the five transformed strains of Alg3-24 they obtained, two of them produced the least amount of Ptr ToxB while one produced the most

despite its slower growth rate on V8-PDA media. Conidia germination and growth rates were not affected by the reduced number of *ToxB* copies. *ToxB* copy number reduction only negatively impacted the amount of Ptr ToxB each transformed strain produced which translated into reduced amounts of chlorosis seen in the Ptr ToxB sensitive line 6B662 (Aboukhaddiur et al., 2012).

2.3. Resistance

2.3.1. Sources of resistance

Resistance to both *P. tritici-repentis* and *P. nodorum* has been found in tetraploid and hexaploid wheat, wheat relatives, and synthetic hexaploid wheat (Singh et al., 2011). Kim et al. (2004) referenced previous studies done to find resistance in species related to wheat. These species included *Triticum timopheevii, Ae. speltoides, Ae. longissim*a, *Ae. tauschii*, and *T. monococcum* (Ecker et al., 1990ab; and Ma and Hughes, 1993 and 1995). Previous studies have shown durum wheat to be highly susceptible to both fungi (Xu et al., 2004). Even though resistance occurs in winter wheat, there is a need for germplasm that combines resistance with good winter-hardiness characteristics and the ability to survive the harsh winters in North Dakota. Available winter-hardy germplasm lines at NDSU are generally susceptible to *P. tritici-repentis* and *P. nodorum*. A recent study by Liu et al. (2015), evaluated winter wheat lines and cultivars from North Dakota and other winter wheat growing states for resistance and insensitivity to *P. nodorum* and *P. tritici-repentis* isolates and NEs. Cultivars such as Jerry, CDC Falcon, Danby, and Fuller were found to be susceptible to both diseases. Both Wesley and SY Wolf were highly resistant to all the isolates while cultivars like Hawken and Decade lacked resistance to *P. tritici-repentis* isolate 331-9 (race 3) only. Even though race 3 has not yet been detected in North Dakota (Liu et al., 2015; Ali et al., 2003), it has been detected in Canada when

collected from durum wheat in the 1980s (Friesen et al., 2005 and Lamari et al., 1995). One isolate, OH99, representing race 3 was used in a study by Faris and Friesen (2005) when looking for race-nonspecific resistance QTL. OH99 was recently identified and collected from Ohio by Pat Lipps and Jessica Engle who are at Ohio State University in Columbus Ohio (as mentioned by Faris and Friesen 2005). Isolates representing races 1, 4, and 5 have been found within North Dakota from 1969-2002, but conidia of other races can be blown or infected seeds can be moved from other locations to here (Friesen et al., 2005). Generally, few HRWW cultivars and lines showed a wide range of resistance/NE insensitivity. It is difficult to foretell how increased resistance in new wheat cultivars is going to impact the evolution of new virulence in the two pathogens. The evolution of new virulence can also be expected to happen more readily in both *P. nodorum* and *P. tritici-repentis* whose life cycles include a sexual phase (Singh et al., 2010). *P. tritici-repentis*, being a homothallic fungus, readily goes through a sexual reproduction cycle on field stubble (Friesen et al., 2005). In order to ensure longevity of resistance in newly released cultivars, involving diverse sources of resistance is necessary in breeding. Resistance may be incorporated from these through crosses and back-crosses, or other means of pre-breeding such as recurrent selection.

Fortunately, good resistance is obtainable from germplasm sources, although un-adapted to North Dakota, such as spring wheat, less winter-hardy winter wheat, synthetic derivatives, and wheat relatives. Spring wheat germplasm tends to have more resistance genes that are easy to transfer into winter wheat. However, the transfer of resistance QTL from spring wheat needs to be accompanied by pre-breeding to simultaneously raise the winter-hardiness and adaptation of new germplasm before it can be involved in elite crosses. Transfer of QTL from less winter-hardy winter wheat would obviously require less pre-breeding. Synthetic wheat has been

produced to facilitate the transfer of useful genes from the immediate progenitors of hexaploid wheat, i.e. tetraploid *T. turgidum* and diploid *Ae. tauschii*. Synthetics are created through artificial synthesis of hexaploid wheat in a way which is similar to how common wheat originally evolved from these two ancestors. The synthetic's donor parent commonly has been either wild emmer or common durum wheat which contributes the AABB genome. This donor parent is then crossed with an *Ae. tauschii* accession which contributes the DD genome. The synthetics act as a bridge, which can then be used by breeders to improve their target germplasm. Crosses of synthetics with common wheat followed by backcrosses of hybrid progeny carrying the desired trait to common wheat are then used to transfer targeted genes. Such transfers will require much more extensive pre-breeding to remove undesirable donor germplasm associated with the target genes through linkage drag. Although gene transfer through synthetics requires a lot of work to get the newly created population to resemble something which can be worked with, their use is often justified due to the variety of useful primary gene pool traits that can be accessed. In this manner, numerous genes for disease and pest resistance, environmental stress tolerance, and morphological diversity have been transferred and they are still considered as potential building blocks for the germplasm base found in hexaploid wheat (Xu et al., 2004; Trethowan and Mujeeb-Kazi, 2008; and Van Ginkel and Ogbonnaya, 2007).

Before a program for the genetic improvement of resistance to tan spot and Septoria nodorum blotch can be implemented, it is necessary to first assess the genetic diversity for resistance in the current breeding population. Detailed resistance and NE insensitivity information makes it possible to do a targeted search for additional, useful resistance genes. If useful QTL such as *tsn1* already occur within the breeding population, albeit at low frequency, a first objective may be to raise its frequency through focused selection. Molecular markers have

been developed for some of the most notable resistance QTL and can be used when aiming to improve the general resistance of a population. The best available lines with a base level of resistance will also be the most logical recurrent parents when introgressing additional resistance genes from un-adapted sources.

2.3.2. Genetics of resistance to *P. tritici-repentis*

The production of NEs makes breeding for resistance to these two fungi difficult. As of 2004, only a handful of resistance genes had been identified and mapped. During 1996 and 1997, Faris et al. confirmed that tan spot necrosis insensitivity was controlled by a single recessive nuclear gene mapped on chromosome 5BL in the synthetic line W-7976. Additionally it was observed, through progeny segregation ratios as well as identical culture filtrate and pure NE results, that isolate 86-124 (race 2) produced only a single NE (Ptr ToxA). The identified recessive insensitivity gene was named *tsn1*, and later called *tsr1* because it was found to confer resistance against *P. tritici-repentis* isolates with Ptr ToxA producing ability. In 1997 a gene on chromosome 1AS was identified that contributed a major resistance effect (later named *tsc1* which if dominant confers Ptr ToxC sensitivity) as well as a minor effect gene on the long arm of chromosome 4A. There was also an interaction between the markers *XGli1* on 1AS and *Xbcd120* on 2DL. In conclusion, their findings suggested that the chlorosis symptom was quantitatively controlled in some populations, but primarily by the QTL on 1AS. This QTL was named *QTs.ndsu-1A* and gives insensitivity to chlorosis caused by both race 1 and 3 isolates [(Ptr ToxA and Ptr ToxC) Faris et al., 2013]. In 2004, Friesen and Faris used a population of recombinant inbred lines derived from the W-7976 and Opata85 cross to map resistance QTL for Ptr ToxB and isolate Dw5 (race 5). They found four genomic areas which showed significant association with resistance. The *tsc2* gene at the distal end of chromosome 2BS was the most significant

factor they observed. Then, two minor QTL were also mapped to 2B with one on the short arm and the other on the long arm. *Tsc2*, in the dominant form, confers Ptr ToxB sensitivity. The minor QTL close to *tsc2* on the short arm of 2B was determined to not be independent from *tsc2*. Another minor QTL was found on the short arm of 2A, contributed by Opata85. They also detected the previously seen resistance QTL on the long arm of 4A. In the end, only *tsc2* and the 4AL QTL were determined to be the true sources of resistance causing the observed variation. The results they got from inoculating with race 5 conidia is one of many pieces of evidence showing that even though a line is insensitive to a NE, it can still be susceptible to *P. tritici-repentis*. Opata85 was shown to be insensitive to Ptr ToxB, the only NE produced by race 5, but averaged a 3.11 disease reaction score. Previous work by Faris et al. (1997) also found a resistance QTL for race 1 of *P. tritici-repentis* while Effertz et al. (2001) identified and mapped a race 3 resistance QTL. The 4A QTL was identified for race 1, race 3, and race 5 resistance. Effertz et al. (2002) mapped resistance to race 1 chlorosis on 3A, which was not associated with the Ptr ToxC chlorosis, and named it *QTs.ksu-3AS*. Cheong et al. (2004) identified and mapped a resistance QTL on chromosome 5B, close to the *tsn1* locus utilizing inoculum of mixed *P. tritici-repentis* races and inoculum representing a single race in a set of Australian wheat cultivars.

Faris and Friesen (2005) identified race-nonspecific resistance QTL on chromosome arms 1BS and 3BL in BR34 using races 1, 2, 3, and 5. Singh et al. (2006a) identified and mapped the *tsn2* gene on 3BL, later called *tsr2* (synonymous with resistance gene *tsr6*) in a population of RILs [(*T. turgidum* no. 283 (TT283) x Coulter)]. The *tsn2*, also called *tsr2*, gene confers susceptibility to necrosis caused by isolate 331-9 when dominant, which may be the same gene Friesen and Faris found in 2005 (Faris et al., 2013). Tadesse et al. (2006a) identified and mapped resistance QTL on chromosome 3D in three synthetic lines (XX41, XX110, and XX45). In a

second study using Salamouni, Tadesse et al. (2006b) also identified the *tsn4* gene on chromosome 3A (subsequently renamed *tsr4*). Both *tsr3* and *tsr4* were thought to possibly be homoeologous genes that confer susceptibility. This conclusion was based on the finding that Chinese Spring lines nullisomic for chromosome 3A were resistant to the isolate, ASC1a, they tested with. However, this needed to be confirmed (Faris et al., 2013). In 2007, Tadesse et al. retested the same three synthetics to study inheritance, allelism, and genetic linkage. When retesting the synthetics, they produced three populations representing progeny from each synthetic crossed to Chinese Spring. Both XX41 and XX110 were found to have resistance controlled by a single recessive gene (*tsn3a* and *tsn3c*) while the resistance gene in XX45 was found to be dominant (*Tsn3b*), later renamed *tsr3a*, *Tsr3b*, and *tsr3c* respectively (Faris et al., 2013). All three of the genes were found to be tightly linked or to be alleles of each other, but only *tsn3a* and *Tsn3b* provide strong resistance while *tsn3c* provides moderate resistance (Faris et al., 2013). Singh et al. (2008) did a study utilizing the race 5 isolate DW13 on a population resulting from a Coulter (durum cultivar) x *T. turgidum* cross. The gene *tsr5* which gives resistance to the necrosis symptom produced by isolate DW13 was mapped close to *tsr2* on 3BL. Faris et al. (2012) identified and mapped four resistance QTL on 5BL, 5DL, 7BS, and 7DS, which were later named *QTs.fcu-5B*, *QTs.fcu-5D*, *QTs.fcu-7B*, and *QTs.fcu-7D*. Both *QTs.fcu-5B* and *QTs.fcu-7B*, associated with isolate Pti-2 (race 1) resistance, and the effects of the 5B QTL were due to the Ptr ToxA-*Tsn1* interaction. The other race 1 isolate used, Asc1, only elicited a significant resistance response in the presence of the 5D QTL, while the 5B and 7B QTL were ineffective. However, both the 5B and 7B QTL were significantly associated with resistance to isolate 86-124 (race 2). Both of the 7B and 7D QTL were significantly associated with resistance to the new Arkansas isolate, AR LonB2. Thus it was concluded that the 5B QTL is a

susceptibility QTL because of the Ptr ToxA sensitivity contributed by Katepwa as well as it having the largest effects. The 7B and 5D QTL were concluded to be possible race-nonspecific resistance QTL while the 7D QTL was found to be specific to the Arkansas isolate. Prior to this study, in 2010, Chu et al. reported a QTL on the long arm of 7B in tetraploid wheat which had minor effects. The 7B QTL in Faris et al. (2012) is on the short arm. Even though the 2012 study showed the 7D QTL to be specific for the new Arkansas isolate, the same chromosome location was previously found when looking at resistance to race 3 with 7D only showing minor effects.

2.3.3. Genetics of resistance to *P. nodorum*

Similar to tan spot, Septoria norodum blotch (SNB) disease resistance was found to be quantitatively inherited and complex (Czembor et al., 2003; and Friesen et al., 2008), but some studies found single genes to be responsible for high resistance levels in certain genotypes (Murphy et al., 2000). Frecha (1973) also found a single dominant seedling resistance gene in Atlas 66, on chromosome 1B, whereas Ma and Hughes (1995) identified single genes controlling resistance to SNB. Ma and Hughes (1995) found a recessive gene in durum wheat which was temporarily named *SnbTM*, located on chromosome 3A. The gene found by Murphy et al. (2000) was found in an *Aegilops tauschii* accession, RL5271. Scharen and Eyal (1983) provided evidence showing that resistance in highly resistant cultivars might be governed by major resistance genes. Studies done by Nelson and Gates (1982), Fried and Meister (1987), and others showed evidence that adult plant resistance to *P. nodorum* was quantitatively or polygenically controlled. Some studies (Wicki et al., 1999; Nelson and Gates, 1982; Fried and Meister, 1987; and Botswick et al., 1993) found up to six genes, each of which had a minor effect but collectively gave a larger effect. Other studies (Aguilar et al., 2005; Shatalina et al., 2014; Nelson and Gates, 1982; Ecker et al., 1989; and Wicki et al., 1999) involving glume or leaf

blotch resistance and both similarly found resistance to be polygenically controlled. In 1989, Wong and Hughes conducted a study of the genetic control of seedling resistance to SNB. They looked at three winter wheat cultivars; 81IWWMN 2095, Coker 76-35, and Red Chief. The results they got pointed to a single gene being responsible for resistance, similar to what Frecha (1973) found in other germplasm. Messmer et al. (1997) found seven leaf as well as seven glume blotch resistance QTL in a wheat by spelt hybrid population. Nicholson et al. (1993) and Hu et al. (1996) found QTL or polygenes in the A, B, and D genomes which interacted for resistance.

Several studies, including Fried and Meister (1987) have reported that the resistance for leaf and glume blotch are controlled by separate sets of genes. This is also true when comparing resistance in seedlings to resistance during the adult leaf and glume blotch phases of SNB infection (Nelson and Gates, 1982; and Scharen and Eyal, 1980). The identification of resistance genes and QTL in wheat and wheat relatives began as early as 1973 (Frecha, 1973). In 2003, Schnurbusch et al. conducted a study looking for QTL while growing plants in different environments. The three leaf blotch resistance QTL identified in this study, were the only ones which showed significant effects when comparing the combined averages across all environments. These QTL were located on chromosomes 3BS, 4BL, and 5BL and accounted for 9-31.9% of the variation in disease symptoms. Toubia-Rahme et al. (2003) identified glume blotch resistance QTL on chromosomes 1B, 2A, 2B, 3A, and 5A in the spring wheat line CM82036. Czembor et al. (2003) reported leaf blotch resistance QTL on chromosomes 2B, 3B, 5B, and 5D. Aguilar et al. (2005) evaluated both leaf and glume blotch and found 10 glume and 11 leaf blotch resistance QTL. Hu et al. (1996) found leaf blotch QTL on chromosomes 3A, 4A, and 3B whereas glume blotch QTL were located on the same chromosomes as well as 7A. A study done by Kim et al. (2004) determined how many genes were responsible for controlling

resistance to *P. nodorum* in selected winter wheat lines from Kansas, while looking at the leaf blotch phase of the fungus, and comparing to see if any of the genotypes shared common genes. Among three sets of RIL populations they created, they determined that the resistant parents in each RIL population, contributed a single dominant gene which was later confirmed by the F_2 segregation ratio. They also found that the two resistant lines, Betty and Heyne, did not contain the same resistance gene. A single SNB disease resistance locus, *Sng.sfr-3BS*, known to be in European elite winter wheat (Tommasini et al., 2007) occurs in the same chromosome region as the *Fhb1* gene which confers Fusarium head blight resistance and has an Asian origin (Löffler et al., 2009).

Shankar et al. (2008) did a study looking for both seedling and adult plant resistance QTL to *P. nodorum* in wheat. In their study, one goal was to reduce the effects of morphological traits, such as height and heading date, on the disease severity level. A doubled haploid (DH) population, created from the parent lines 6HRWSN125 (resistant) and WAWHT2074 (susceptible), was used in field and greenhouse trials for adult and seedling resistance, respectively. Their results showed continuous variation for resistance and transgressive segregation for all the phenotypic traits observed, including relative grain weight. Correlations were calculated between the traits, and a low correlation was present between seedling and adult leaf blotch resistances as well as between seedling and glume blotch resistances. However, a high correlation was observed between glume and leaf blotch resistances (with the seedling and adult phases combined for leaf blotch). Five QTL were found for seedling resistance, explaining about 5-13% of the observed variation in phenotypes. These five QTL included *QSns.daw-2D*, *QSns.daw-4D*, *QSns.daw-5B.1*, *QSns.daw-5B2*, and *QSns.daw-6D*. A total of eight QTL were detected but only two of them (*QSng.daw-2B* and *QSng.daw-4B*) were found in both testing

years for SNB affecting the glumes. Finally, for adult leaf blotch resistance, five QTL were detected but only *QSnl.daw-2D* was detected during both years. In their results, they point out that no common QTL between seedling and adult glume blotch or between seedling and adult leaf blotch were found. However, even if the QTL are not the same, QTL were found on chromosomes 5B and 2D for all three resistance stages. Seedling resistance had two QTL detected on 5B. A QTL for both adult plant glume and leaf blotch SNB resistances occurred on chromosome 4B. However, the Shankar et al. (2008) study detected QTL for seedling resistance which did not align with known QTL for NE sensitivity. Thus they concluded that either different genes conferring NE sensitivity are in 6HRWSN125 or different NEs are produced by the *P. nodorum* isolates in Australia that are ineffective against the known insensitivity genes to date. In Liu et al. (2004 a, b), they reported that NE insensitivity was a factor aiding with seedling resistance.

Many of the studies done to identify resistance QTL used either recombinant inbred lines (RIL) or doubled haploid (DH) populations from crosses between susceptible and resistant parents. Other studies such as those of Adhikari et al. (2011) and Tommasini et al. (2007) used association mapping. Regardless of how the QTL were discovered, genotype x environment interaction complicated confirming the presence of previously detected QTL (Shankar et al., 2008).

2.4. Marker-assisted selection

One way to screen potential parents for resistance and predict NE reactions while avoiding laborious phenotypic data collection is by using genetic markers. Genetic markers can be of three types: morphological, biochemical, and DNA based. Morphological markers are phenotypic traits which are usually characterized visually (ie: plant height, seed color, and etc.).

Polymorphism in biochemical markers such as isozymes are detected through chemical assays. Variations in the makeup of enzymes result when the amino acid sequence of an enzyme is modified. The alterations can result in either or both a structural and electric charge change which can then be detected on an electrophoretic gel because these changes affect the molecule's mobility through the gel. DNA markers, unlike the other two types, focus on detecting variations in the genetic makeup of a specific organism. All of these markers have been used for genetic variability estimates between species or genera, population genetic studies, plant breeding, phylogenetic relationship studies, and other genome related studies. Heinz (1987) mentioned that isozymes were reliable genetic markers for use in breeding and genetic studies of plants because their expression was consistent regardless of environmental influences. However, Winter and Kahl (1995) and others have found both isozymes and morphological markers to be influenced by environmental conditions as well as being limited in number even though they were very useful for plant breeding (Eagles et al., 2001). The latter drawbacks compelled researchers to explore and develop DNA markers which were more abundant and required less investment. In addition, DNA markers progressed to being easier to use in automated or high throughput systems with methods which made it easier to interpret results and increase reproducibility.

DNA markers, commonly referred to or thought of as molecular markers, started to be used before 1990 (Williams et al., 1990). DNA markers are of three kinds, based on how they are detected. As reviewed by Collard et al. (2005) and Kalia et al. (2011), the three groups are: hybridization-based, polymerase chain reaction (PCR)-based, and DNA sequence-based. The very first type of DNA marker used, restriction fragment length polymorphisms (RFLPs), are detected using hybridization-based methods.

Restriction fragment length polymorphism (RFLP) markers are not true markers, like the other DNA markers. For example, when comparing two individual plants, the DNA from each is collected, extracted, and then cleaved using a restriction enzyme. One specific restriction enzyme is used to create DNA fragments which may differ in length between the two organisms being compared. The length differences tend to be due to evolutionary changes, and the banding patterns they create from gel electrophoresis can be used to distinguish the two plants. Following gel electrophoresis, the fragments of interest are transferred, via Southern blotting, to a membrane so they can be hybridized to a radioactively labeled probe. Once the fragments have been hybridized, the banding patterns can be visualized to compare/distinguish the two individual plants, different genotypes, or species (Karp et al., 1998). Unfortunately, RFLPs are time consuming, require laborious methodology, and a lot of DNA for finding the banding pattern differences and distinguishing the two individuals being compared.

PCR-based markers also require the use of genomic DNA, however some PCR-based markers require a lot less DNA than the RFLP method. One regularly used PCR-based marker type is called simple sequence repeats (SSRs).

Simple sequence repeat markers are created by amplifying a short stretch of DNA in a polymerase chain reaction. These markers tend to be co-dominantly inherited as well as amendable to high throughput sequencing procedures. SSR markers are also attractive to researchers because they have high levels of reproducibility and variability. Since their development, they have been used in very broad applications, for example: developing DNA fingerprints in soybeans (Rongwen et al., 1995); evaluating variation between cultivars in rice (Olufowote et al., 1997); studying genetic relationships (Provan et al., 1996; Liu et al., 1995); estimating genetic diversity in rice and wheat (Blouin et al., 1996; Plaschke et al., 1995);

anchoring markers on different genetic maps for a single crop; aide with identifying and mapping important genes/QTL to specific chromosomal locations (Beckmann and Soller, 1990; Liu et al., 1996; Panaud et al., 1996); marker-assisted selection or cloning (based on genetic maps) to transfer desired traits into preferred cultivars (Xiao et al., 1996; Tanksley and McCouch, 1997); and other applications. In addition to SSR markers, other PCR based markers exist such as randomly amplified polymorphic DNA (RAPD) and amplified fragment length polymorphisms (AFLPs) which also allow for the fine-scale genetic characterization of germplasm collections. Other PCR-based markers exist besides SSR, RAPD, and AFLP.

The last group of DNA markers are based on DNA sequences. The most commonly used marker type in this category is the single-nucleotide polymorphisms (SNPs) marker. SNPs are identified using computer software programs (for example, 'single nucleotide polymorphism detector' (SNPD)) to scan DNA sequences on a nucleotide by nucleotide basis for potential polymorphisms. A list of DNA primers is produced which are used to validate the polymorphisms in a mapping population to determine if they are true allelic variants. Validated SNPs are used to characterize progeny of the specific mapping population. Different detection platforms may be used and could range from conventional gel based to high throughput systems like microarrays (Chen-Cheng et al., 1996; and Chagne et al., 2008). In general, single-nucleotide polymorphisms have been found to be dispersed throughout an organism's genome and are very abundant which makes them a very attractive resource when performing marker-assisted selection or doing map-based gene cloning (Nasu et al., 2002; Garg et al., 1999; and Batley et al., 2003). Various SNP markers have been made utilizing pyrosequencing, fluorescence energy transfer, and Taq Man since about 1998 with other methods being discovered later on (Alderborn et al., 2000; Livak, 1999; and Chen et al., 1998). An allele-

specific PCR method is used as it allows for a more cost effective and efficient way to genotype SNPs (Newton et al., 1989). Following PCR the amplified products are scored on an absence/presence basis after running them through gel electrophoresis with stain. Allele-specific PCR markers were also applied by Drenkard et al. (2000) and Hayashi et al. (2004) to reliably assay SNP markers. Hayashi et al. (2004) determined that creating allele-specific PCR markers allowed SNPs to be a valuable tool in their gene mapping study as well as other studies through the ability to make fine-structured genetic maps. Again, SNPs are not the only sequence-based marker being used for genetic based studies such as marker-assisted selection, QTL locating, and etc.

Even though DNA markers are generally divided into three broad types, some marker types involve more than one detection strategy. For example amplified fragment length polymorphism (AFLP) markers combine both hybridization and PCR-based detection methods. Another example is start codon targeted polymorphism (SCoT) markers which are based on the translation start codon sequence ATG which codes for the amino acid methionine and initiates the translation process (Collard and Mackill., 2009). This sequence tends to be conserved. The PCR uses single primers which are designed to model the small region of DNA surrounding the start codon. Amplification products are separated by gel electrophoresis to be visualized.

Different types of markers were used in studies of SNB and tan spot disease aimed at identifying and locating individual QTL and/or genes responsible for disease resistance and NE sensitivity. When looking for SNB resistance in seedling and adult plants, Shankar et al. (2008) used simple sequence repeats (SSRs), diversity array technology (DArT), and expressed sequence tags (ESTs) among their group of 492 chosen molecular markers to make a genetic map. The marker filled map allowed them to pin point QTL like *QSnl.daw-2D, QSng.daw-4B,*

QSnl.daw-5A, and *QSnr.daw-5A* to the same marker intervals as for relative grain weight. Then, the *Rht-B1b* locus for plant height was observed to be linked to glume resistance QTL *QSng.daw-4B* and *QSnr.daw-4B.* Along with the markers used to locate *P. nodorum* resistance QTL by Shankar et al. (2008), markers were also used to locate and study NEs. The wheat gene, *Snn1*, that confers sensitivity to *P. nodorum* NE SnTox1, was mapped using expressed sequence tag markers and later Reddy et al. (2008) did high-resolution mapping of the locus. Two EST-based markers were found, during the work by Reddy et al. (2008), which was then followed by fine-mapping efforts. As a result of the fine-mapping, two microsatellite markers (*Xfcp618* and *Xpsp3000*) were found to be diagnostic for the presence of the *Snn1* gene and delineated the gene to an interval of 0.9cM (Friesen and Faris, 2010). Markers were also found for the detection of *Snn2*, *Snn3*, and *Snn4* (Abeysekara et al., 2009; Friesen et al., 2007, 2009; Zhang et al., 2009; and Liu et al., 2009). The *Tsn1* gene is very important for both *P. tritici-repentis* and *P. nodorum* because it confers sensitivity to Ptr ToxA/SnToxA. Markers including *Xfcp1*, *Xfcp2*, *Xfcp394*, and *Xfcp620* were found previously and both *Xfcp394* along with *Xfcp620* have been shown to be useful for determining if lines are homozygous or heterozygous for the gene. However the marker *Xfcp623*, more recently developed by Faris et al. (2010) is best used among a diverse group of lines. *Xfcp623* was developed using the *Tsn1* sequence and is considered to be a "perfect" marker to detect the presence of the dominant *Tsn1* gene.

Wheat lines homozygous for the 1BL.1RS translocation lack the *Snn1* locus on 1BS and will therefore be insensitive to *P. nodorum* NE SnTox1. The wheat line BR34 was used as a parent in the development of some of the breeding lines used in the present study and was confirmed by Simons et al. (2012) to have the 1BL.1RS translocation. Similarly, lines homozygous for the 1AL.1RS translocation will lack the *Snn4* wheat locus on 1AS that confers

sensitivity to SnTox4. Apart from the rye segment being beneficial in terms of resistance, its presence is associated with poor bread baking quality due to the co-introduced *Sec1* locus (Lee et al., 1995; McKendry et al., 1996; Johnson et al., 1999 and Graybosch, 2001). Along with *P. tritci-repentis* and *P. nodorum* related markers, those able to detect the 1RS rye segment will therefore provide useful information with respect to the presence/absence of the respective sensitivity loci.

The HRWW breeding program at NDSU was initiated in 2011 and it has been necessary to acquire breeding parents and develop hybrid populations for pure line selection. In this process a conventional pedigree breeding program is being established as well as parallel pre-breeding attempts. The purpose of this study was to assess the level of resistance/NE insensitivity with respect to the diseases tan spot and Septoria nodorum blotch in both the pedigree and pre-breeding populations. It was hoped that the data from the study would provide an indication of the occurrence of resistance in the better adapted current germplasm, suggest a number of lines that can be used as initial cross parents to improve the overall resistance within the breeding populations, and help to devise a strategy for purposeful introgression of additional resistance QTL that will complement the QTL already present.

3. MATERIALS AND METHODS

Two approaches were used in an attempt to identify and select winter-hardy wheat genotypes with good resistance to *P. tritici-repentis* and/or *P. nodorum*.

3.1. Initial line characterization (Part A)

In this part of the study a group of 161 genotypes consisting of current cultivars, advanced breeding lines from the NDSU HRWW breeding program, and diverse sources of possible additional resistance were evaluated for resistance/NE insensitivity with respect to both diseases.

3.1.1. Plant material

The 161 genotypes that were evaluated are listed in Table 2 (in the results section). Numbers 1-11 are HRWW cultivars currently grown in ND; entries 12-15 are exotic winter wheat cultivars which were reported to have good levels of *P. tritici-repentis* resistance but have not been tested in North Dakota. Entries 16-146 are advanced HRWW breeding lines from the NDSU breeding program that previously showed good winter survival. All of the lines had good resistance to one or more of the diseases FHB, leaf rust, stem rust and bacterial leaf streak but had not been evaluated for resistance to *P. trtici-repentis* and *P. nodorum*.

Some of the ND HRWW breeding lines within the group 104-140 carry the 1BL.1RS translocation. These lines derive from two crosses, i.e. cross 11M225 = RWG10/Jerry and cross 11M237 = RWG28/Norstar. Both RWG10 and RWG28 carry the 1BL.1RS translocation. Lines RWG10 and RWG28 are spring wheats with pyramided resistance to the diseases Fusarium head blight, tan spot, and SNB developed by Dr. Steven Xu (USDA, ARS, NCSL, 1307 18th Street North, Fargo, North Dakota, 58201). The genes present in RWG28 (pedigree = BG282/3*Alsen) include *tsn1*, *snn2*, *QTs.fcu-1BS*, and *Fhb1*. BG290 (pedigree = BG290/3*Alsen) has the same

genes as RWG10, plus an additional *P. tritici-repentis* race-nonspecific resistance/insensitivity QTL, i.e. *QTs.fcu-3BL*. The selections showed good winter survival in 2014 and each has *Fhb1*, yet they were not previously tested for the presence of *P. tritici-repentis* or *P. nodorum* resistance. The 1BL.1RS translocation also occurs in CM82036 which was used as a parent in cross 11M221 from which lines 54-100 derive. Lines homozygous for the 1RS.1BL translocation will lack the *Snn1* NE sensitivity locus on 1BS.

Lines 147-151 are HRSW from a South African breeding program based on recurrent selection and may have resistance to *P. nodorum*. Lines 152-158 are synthetic hexaploid spring wheat derivatives from crosses between hard red spring and synthetic wheat; kindly provided by Dr. Art Klatt (now retired, Oklahoma State University, Plant and Soil Sciences Department, 274 Agriculture Hall, Stillwater, Oklahoma, 74078). The seven synthetics have an *Aegilops tauschii* accession in their pedigrees. Dr. Klatt selected the lines based on their moderate to complete *P. tritici-repentis* resistance levels (field). However, none of these lines have been screened for resistance to *P. nodorum*. Finally, lines 159-161 are spring habit hexaploid tritipyrum lines (2n=42; AABBJJ) derived from crosses between *T. turgidum* ssp. durum (2n=28; AABB) and *Thinopyrum distichum* (2n=28; $J_1^dJ^dJ_2^dJ_2^d$, Marais et al., 2014).

3.1.2. Plant preparations

Prior to planting, 98 cone-tainers were placed into a tray and filled with Mix 1 Sunshine soil consisting of 70-80% Canadian Sphagnum peat moss, perlite, and dolomite limestone. All the lines were grown in a controlled greenhouse and disease was allowed to develop in controlled growth chambers. Three seeds per line were planted in a single cone-tainer and two cones, the experimental unit, of each line were placed among other lines in a 98 cone-tainer rack. Sixty of the lines were evaluated over two replications using a randomized complete block

design, whereas the remaining 101 lines were evaluated in a single replication. Immediately after planting, the cone-tainers were fertilized with osmocote plus 15-19-12 as well as an iron supplement and watered in. In addition to the 30 lines per 98 cone-tainer tray, Jerry was planted around the border to eliminate edge effects during *P. tritici-repentis* evaluations and the checks BR34, 6B365, 6B662, and Glenlea were included. For *P. nodorum* evaluations, Grandin replaced Jerry on the border, then both BR34 and Grandin were included as checks. BR34 is resistant to both tan spot and *P. nodorum*. The border and check cones were planted in the same manner as the lines being evaluated but utilizing only a single cone. Plants were grown in the greenhouse until they reached the two-to-three leaf stage at which point they were inoculated.

3.1.3. Tan spot (*P. tritici-repentis*) screening

Three *P. tritici-repentis* isolates were used for single isolate evaluations, which included Pti-2, 331-9, and AR CrossB10. These isolates represented *P. tritici-repentis* races 1, 3, and a new race which was previously studied by Ali et al. (2010). To culture each isolate, produce inoculum, and inoculate plants, the protocol used by Friesen et al. (2003) and Friesen and Faris (2012) was followed. Isolate growth began seven days prior to plants reaching the two-to-three leaf stage which was fourteen days after planting, but as early as nine or ten days after planting during June and July 2015. A single mycelium plug was placed in the middle of a petri dish containing V8-PDA media, using flame sterilized forceps. Following this, petri dishes were placed inside a drawer to grow in complete darkness at room temperature for roughly five days. On day five, if the fungus had grown enough, plates were flooded with sterile distilled water (filled till covering the mycelium). After flooding the plates, the mycelium was flattened by gently rubbing a flame sterilized test tube across the media surface where the mycelium was present. Water was then gently poured off and the plates were allowed to dry underneath a

laminar hood. Once plates were dry, the lids were placed back on and the plates were then placed on a shelf under a fluorescent light to grow for 24 hours with continuous light. Finally, plates were moved to a dark growth chamber at 16^{o}C approximately 12-14 hours prior to the desired time for plant inoculations the next day. Conidia produced during the 12-14 hour period were collected by flooding the plates with sterilized distilled water, then gently scraping the media surface with a flame sterilized metal wire loop. This scraping was repeated twice around the conidiophore area with the conidia and water being poured into a flask afterwards. Then, sterilized distilled water was again added to the plates, but less this time to rinse the plate in order to collect any extra conidia. The resulting conidia concentration was adjusted to 3,000 spores/mL and the surfactant agent Tween 20 was added at the rate of 2 drops per 100mL of inoculum prior to inoculation. Inoculum was also mixed using a magnetic stirrer in order to get the spores well suspended within the water. Post inoculum production and prior to plant inoculation, the suspension was stirred often by hand to keep the spores from collecting and clumping up. For each 98 cone-tainer rack inoculated, 100mL of inoculum was used. Plants were inoculated using a paint sprayer (Husky, Home Depot) with the air pressure being about 1.0 bar. Following inoculation, plants were moved into mist chambers for 24 hours with humidity at 100%. The chambers were set to have mist come on for 15 seconds, from humidifiers, once every four minutes. While plants were in the humidity chambers, they were under constant light, provided by fluorescent lights directly above the chamber area. Plants were then moved to a growth chamber at 21^{o}C and about 75% humidity after being in the misting chamber. Growth chambers contained metal trays filled with water to the top of the cone-tainer drainage holes to provide plants the ability for water uptake via capillary action. This arrangement allowed for the fungus to develop while avoiding possibly washing inoculum off the leaves the day after

inoculation. Plants were removed from the growth chambers seven days post inoculation. Observed disease symptoms were rated using the 1-5 scale developed and described by Lamari and Bernier (1989). The second leaf was used to determine the line's disease score, but the first leaf was at least looked at to aid with rating decisions. If a line was showing symptoms resembling a 2 on the 1-5 scale, but the first emerged leaves were displaying the lesion type qualifying a score of 5 it was then determined that the line was not resistant. Individual plants were scored in order to visualize any trends. The individual plant scores were later combined to get a single common score among the plants evaluated per line within each replicate. Scores for each line per replicate were then used in statistical analyses.

3.1.4. Septoria nodorum blotch (*P. nodorum*) screening

A single isolate, SN4, was used to screen the 161 lines. This isolate was found to be capable of producing all *P. nodorum* NEs known to date (Liu et al., 2015). Similar to the *P. tritici-repentis* isolates, SN4 growth was initiated seven days prior to inoculation, once the plants reached the two-to-three leaf stage. For culturing SN4 a method different from the previously described *P. tritici-repentis* culturing was used. A single mycelium plug was placed into a 2mL centrifuge tube following the addition of 1mL of sterile distilled water using a micropipetter. Once the plug was added, under a laminar flow hood utilizing flame sterilized forceps, the tube was closed and mixed for 30 seconds with a vortexer. Mixed tubes were returned to the laminar flow hood with V8-PDA media containing petri dishes waiting. The tube's contents were poured onto the media surface and the mycelium plug was removed using flame sterilized forceps. This mycelium containing liquid was spread across the entire media surface by tilting the petri dish to each side and turning it to make sure the liquid covered every part. Upon getting the surface covered, the closed petri dish was placed on a rack underneath a fluorescent light to grow at

room temperature in continuous light for seven days. Pycnidia were observed to form as early as four to five days and it was possible to collect conidia at this time as well, if needed. SN4 conidia were collected by adding 20mL of sterile distilled water to the plate, then allowing it to sit for five minutes in order to allow conidia to flow out of the pycnidia into the surrounding water. Following this, the media surface was gently scratched under a laminar flow hood using a flame sterilized wire loop. This broke the pycnidia open so the conidia could freely flow into the surrounding water. After letting the plate sit for a few minutes, the water containing conidia was poured into a flask and a single drop of Tween 20 was added to allow the conidia to separate. The solution was mixed using a magnetic stirrer. While stirring, a random 10μL sample of the solution was transferred to a hemacytometer and a spore count was done. The spore concentration was then diluted with water to 10^6conidia/mL. Then more Tween 20 was added at the rate of 2 drops per 100mL once the final volume was determined post spore counting. SN4 inoculations were done the same way as for the *P. tritici-repentis* inoculations including the plant growth stage, mist chamber, and growth chamber. However, SNB disease symptoms were rated seven days after inoculation using the 0-5 lesion scale as described by Liu et al. (2004b). A score of 0 indicated the absence of visible lesions; 1 meant there were few penetration points on the leaf which had either flecking or small dark spots; 2 was the presence of small dark spots with a small amount of either necrosis or chlorosis surrounding; 3 had 2 to 3mm dark lesions which were completely surrounded by necrosis or chlorosis; 4 consisted of 4mm or larger necrotic or chlorotic lesions which were coalescing a little, and 5 was indicated by leaves showing large coalescing lesions with very small regions of green leaf tissue. If the secondary leaf showed an equal amount of type 2 and 3 lesions, then the plant was assigned a score of 2.5. Every plant was scored and later these scores were adjusted to give a common score among plants per replicate

for each line being evaluated. Common scores were used in statistical analysis, unless lines were replicated. If lines were replicated then the common scores per replicate were averaged only if replicates were found to be homogeneous using the proc glm program in Statistical Analysis System version 9.30 (SAS, 2010).

3.1.5. Necrotrophic effector reaction phenotyping

Necrotrophic effector (NE) screening was conducted in a different greenhouse using plants that were at the two-to-three leaf stage. For infiltrations, only a single cone-tainer with three seeds per line sown into it was used. The NEs Ptr ToxA, SnTox3, and SnTox1 were evaluated separately on lines for their reactions. Ptr ToxA is a NE commonly produced by *P. tritici-repentis* isolates while SnToxA, SnTox3, and SnTox1 are produced by *P. nodorum* isolates. All four of these NEs were produced using the yeast (*Pichia pastoris*) strain X33 which expresses the fungal NE genes heterologously after the genes were cloned from *P. nodorum* and *P. tritici-repentis* (Liu et al., 2012 and Abeysekara et al., 2010). Liu et al. (2015) used these same strains to produce the NEs they used for infiltration which are the same ones used in this study.

Preparation of each individual NE involved first pouring 2mL of yeast potato dextrose broth (Liu et al., 2012), also called YPD for short, into a plastic tube then gently using a flame sterilized wire loop to gather a single colony of the X33 yeast strain. This single colony was then introduced into the tube containing YPD media and the lid was put on leaving a little space to allow air flow to the soon to be growing yeast. The tube was then placed in an agitator to provide constant shaking as the yeast grew at 28ºC for 24 hours. Following this 24 hour period, either 100 or 200µL of the yeast containing solution was added to pre-autoclaved glass flasks containing 50mL of YPD media. Upon adding the yeast solution, flasks were closed with foil. Flasks were then placed in the agitator for 24 hours at 28ºC. The tubes were then completely

closed and placed in the refrigerator for future use, if needed. Following the 24 hours of agitation in the flask, the contents were separated equally (by weight) into two 50mL plastic centrifuge tubes. Tube contents were spun down at 3,000rpm for 5 minutes and the supernatant decanted into new 50mL plastic centrifuge tubes which were immediately placed into a container containing ice. The NEs were kept on ice before and during the infiltration process. Infiltrations were done by utilizing a plastic needleless syringe and injecting about 20µL of the NE into newly growing leaves of plants at the two-to-three leaf growth stage, focusing on the second emerged leaf. Water soaked leaf areas, filled with the NE were marked on each end using a black permanent marker. Once infiltrations were complete, plants were moved into a growth chamber set at 21^{o}C. Plants were brought out three to five days after infiltration for reaction scoring using the 0 to 3 scale (Waters et al., 2011; and Faris et al., 2013). These scores were used in calculating correlations.

3.2. Resistant inbred line development (Part B)

3.2.1. Plant material

A highly diverse recurrent mass selection (RMS) population based on the male-sterility gene, *Ms3,* and developed by Bisek and Marais (2014) was used to derive SSD inbred lines. The origin and composition of the complex cross from which the RMS population derives is explained in Figure 1. In the first leg of the cross, 60 diverse hard red spring wheat lines (originating from a South African recurrent selection breeding program) carrying *Ms3* were pollinated with 26 hard red winter wheat breeding lines and eight cultivars (USA and Canada) to produce the F_{1A}. In the second leg of the complex cross, Norstar was hand-pollinated with 18 hard red spring wheat cultivars and lines (ex: Canada and North Dakota) to produce F_{1B}. The latter 18 parents included a wide range of known resistance genes to various diseases and parasites *(Lr16, Lr21, Lr34,*

Sr23, *tsn1*, *snn2*, *Fhb1*, and Hessian fly resistance to name some). The two F_1 groups were then crossed with a set of winter-hardy cultivars from North Dakota and Canada to respectively produce F_{1AC} and F_{1BC}. In the final cross, male-sterile F_{1AC} plants were pollinated with F_{1BC} plants to produce the final base population. Following this cross, the progeny were intercrossed for one cycle to promote further recombination of the pooled variation. As concluded in the diagram (Figure 1), the population's final parentage composition is approximately 62.5% winter-hardy winter wheat, 12.5% less hardy winter wheat, and 25% spring wheat. Inbreeding was initiated from random F_2 plants from this base population and was accompanied by seedling selection (Figure 2).

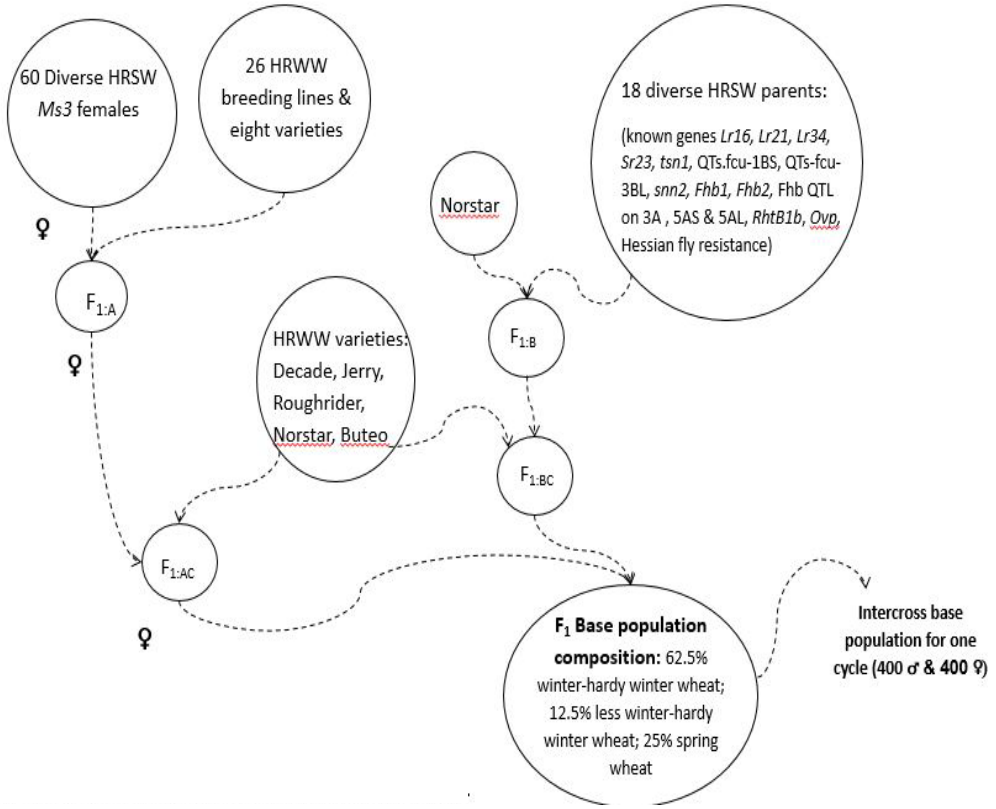

Figure 1: Outline of a complex cross made by Bisek and Marais (2014) in order to derive a highly heterogeneous base population for recurrent mass selection.

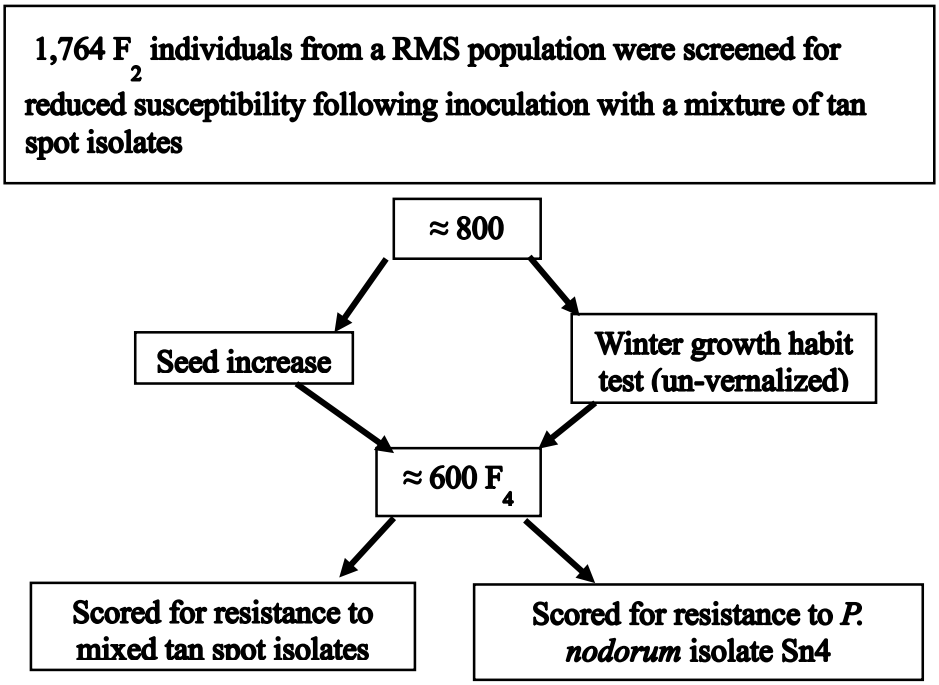

Figure 2: Outline of selection and inbreeding done utilizing the F_2 of a recurrent mass selection base population in order to derive inbred lines with resistance to either or both of *P. tritici-repentis* and *P. nodorum*.

3.2.2. Plant evaluation

For all generations/tests except for the F_3 seed increase and winter growth habit evaluations, plants were grown in cone-tainers with three seeds sown in each per family. The F_3 seed increase stage utilized 8" pots with three seeds per family and five or six families grown per pot. Finally for the winter growth habit screening, plants were grown in 4" square pots using four seeds per family with one seed sown at each of the four corners. All the plants were given osmocote plus 15-19-12 and an iron supplement following planting, unless they needed to be vernalized first. If plants needed to be vernalized, the fertilizer was given after the 56 day vernalization period ended and prior to placing them in the greenhouse. Only the F_2 and F_3 families were vernalized prior to screening and for seed increase respectively. However, the F_4 families were not vernalized until after being screened and the F_3 individuals used in the growth habit test were not vernalized. During the *P. tritici-repentis* and *P. nodorum* screenings at the F_2

and F_4 generations, the checks used in Part A were included, but the borders with Jerry or Grandin were not planted.

3.2.3. F_2 P. tritici-repentis screening

All the *P. tritici-repentis* isolates in Part A were used in this screening, but the isolate Dw5 representing race 5 was also included. A total of 1,764 F_2 individuals were screened using inoculum prepared as mentioned in Part A, except spores representing each isolate were included in the inoculum. Four un-replicated cone-tainer racks were inoculated at one time once the plants reached the two-to-three leaf stage. *P. tritici-repentis* isolates were grown in V8-PDA media, but with only a single plate per isolate which after combined was adjusted to a final volume of 400mL with 3,000 spores/mL. Plants were not given a disease symptom score, but instead were either kept if lacking symptoms or discarded if showing susceptibility and/or expressing chlorosis sensitivity (from Ptr ToxC). No phenotypic data was collected and the resistant and intermediate lines (≈ 800) were advanced to the next stage via a modified single seed descent (SSD) method involving harvesting and threshing a single spike per family after selection.

3.2.4. F_3 seed increase and winter growth habit screening

F_3 seed were kept for at least 20 days post-harvest to break seed dormancy before re-planting and vernalizing the ≈ 800 lines in 8” pots. While plants were growing, another test began for growth habit type. For determining whether the plants had spring or winter growth habit, a total of four seeds per family were sown into a 4” square pot in a greenhouse without prior vernalization. Plants with a winter growth habit remained in the vegetative stage with prostrate growth whereas the spring types directly entered the reproductive stage. If individuals were spring types, then those particular individuals were also removed from the seed increase

pots. Remaining plants from the seed increase pots were harvested with only a single spike per family being kept to move on to the F_4 and the rest of the spikes being harvested in bulk.

3.2.5. F_4 *P. tritici-repentis* and *P. nodorum* screening

The ≈ 600 lines harvested from the F_3 seed increase were grown in two identical trials and in unreplicated cone-tainers. In each cone-tainer, three seeds were sown per family. *P. tritici-repentis* screening of the F_4 involved using inoculum containing a mix of the previously used *P. tritici-repentis* isolates. *P. nodorum* screening was done using isolate SN4 of the same F_4 families which were planted separately from the *P. tritici-repentis* evaluation group. Fungal growth and inoculum preparation, inoculation, and disease symptom assessment was done the same as outlined in Part A. Families to be kept were first based off the range of scores gotten by scoring each individual plant and if none of the scores were higher than 2.5. These plants were then trimmed of their diseased leaves, watered well, and placed into the vernalization chamber for 56 days for seed increase as well as to obtain F_5 seeds. The selected individuals were therefore $F_{4:5}$ families with each family originating from a different male fertile F_2 plant from the RMS base population.

3.3. Final evaluation of the best selections from Part A and Part B for resistance/NE sensitivity to *P. tritici-repentis* and *P. nodorum*

The best individuals from Part A and Part B with good resistance capabilities were evaluated in final evaluation trials with checks included. Six trials were conducted with the entries and checks. Four trials evaluated reactions to inoculation with *P. tritici-repentis* isolates Pti-2, 331-9, and AR CrossB10; and *P. nodorum* isolate SN4, respectively. Two trials evaluated sensitivity of the entries when infiltrated with *P. tritici-repentis* NE Ptr ToxA (one trial) and *P. nodorum* NEs SnTox1 and SnTox3 (one trial), respectively. Each trial took the form of a

randomized complete block design with three replicates for each of the four inoculated trials and two replicates for each of the two NE infiltrated trials. Individual replicates were mostly planted and evaluated at different times. For the inoculated experiments an experimental unit consisted of two cone-tainers in each of which three seeds of an individual was planted for a total of six seeds per individual. These were placed into 98 cone-tainer racks with Jerry planted along the border to avoid edge effects. The checks Grandin, BR34, Glenlea, 6B626, and 6B365 were used and purposely planted in the middle of each rack prior to individuals being randomized. For the two NE reaction evaluation trials, an experimental unit consisted of three seeds per individual planted in a single cone-tainer. Every cone-tainer was fertilized using osmocote plus 15-19-12 and an iron supplement right after being planted and then were watered in. Both NE sensitivity and disease scores were averaged among the replicates after found to be homogeneous. Statistical analysis was then performed using the averages.

Inoculum preparation, inoculation, disease development, and evaluation of the symptoms of the two diseases were done as is described in Part A. Similarly, NE preparation, infiltration, and sensitivity assessment also followed the methodology described in Part A.

3.3.1. Validating the Ptr ToxA phenotypic data with markers

Leaves were collected from 1-2 seedlings of each of the individuals in the final evaluation trial. The leaves were freeze-dried for three days. Three dried leaf pieces of equal size were cut into a 2mL centrifuge tube. These tubes were previously filled with three metal beads along with enough plexiglass to cover the beads. The two components aided in grinding the dry leaf tissue into a fine powder which was then used to extract the DNA following the protocol by Diversity Array Technologies (2013). Once extraction was completed, the DNA was run on a 1.2% electrophoresis gel for DNA quality testing as well as determining how much to dilute the

samples. Next, 2µL of DNA from each sample was amplified using PCR with the reverse and forward primer sequences of *Xfcp623* (in Table 1). A total of 18µL of a master mix (4µL buffer, 1.6 µL $MgCl_2$, 2µL dNTP, 1µL primer, 0.2µL taq and 8.9µL distilled water per sample) was added to each 2µL DNA sample. Then samples were amplified. An amplification cycle involved denaturation at 95 ºC for four minutes, then re-annealing at 60 ºC for one minute, and extension at 72 ºC for one minute. Thirty five cycles were completed followed by a final extension at 72 ºC for five minutes which was followed by an infinite hold at 10ºC. Following amplification, the 20 µL of resulting PCR product was loaded into wells of a 3% gel and ran with a voltage of 77 for 2 hours and 30 minutes. A picture of the gel was taken afterwards to visualize the bands. The marker, *Xfcp623* (Faris et al., 2010) was used in order to determine if lines possessed the dominant *Tsn1* gene or not. Results were scored on a present or absent basis for the gene. This was repeated to confirm results.

3.3.2. Confirming the presence of the 1RS.1BL rye translocation

DNA of RWG28 and Norstar was used as checks along with Grandin, Jerry, BR34, and Glenlea. The marker *IB-267* (Mago et al., 2002) was used to detect the presence of the rye 1RS translocation. The PCR parameters were set following protocols used by Mago et al. (2002) and Sharma Poudel et al. (2015) with a modification to the denaturing temperature which was increased one degree to 95ºC. Conditions used were an initial step of denaturing the DNA at 95ºC for 3 minutes followed by 30 cycles of 95ºC for 30s, 55ºC for 1 min., and 72ºC for 1 minute. After the 30 cycles, a single cycle of 25ºC for 1 minute was used then once the PCR finished the samples were placed into an infinite hold at 10ºC till stored in a freezer if not loaded into wells of a 3% agarose gel. When samples were loaded into the 3% agarose gel, the gel was

run for 2 hours and 30 minutes at 77 volts. Following this, gel pictures were taken. The process was repeated in order to confirm results which were scored on a presence/absence basis.

Table 1: The forward and reverse DNA primer sequences for the Xfcp623 (Tsn1) and IB-267 (1RS) markers.

Marker		Sequence	Reference
Xfcp623	Forward	CTATTCGTAATCGTGCCTTCCG	Faris et al. (2010)
	Reverse	CCTTCTCTCTCACCGCTATCTCATC	
IB-267	Forward	GCAAGTAAGCAGCTTGATTTAGC	Mago et al. (2002)
	Reverse	AATGGATGTCCCGGTGAGTGG	

3.4. Statistical Analysis

Common scores gathered for each replicate were subjected to homogeneity tests using the Statistical Analysis System 9.30 (SAS, 2010). This program was also used to do regression analysis between disease and NE reactions. In addition to calculating correlation values, a one-way Student's t-test was done in order to compare disease score averages between lines with NE sensitivity and those which lacked it to see if there was any significance.

4. RESULTS

4.1. Initial line characterization (Part A)

The averaged disease phenotype data as well as NE sensitivities recorded for the 161 lines are summarized in the appendix (Table A1). The subset of lines that were selected for continued testing were marked with asterisks in this table. The checks 6B365, 6B662, Glenlea, and Salamouni reacted to the different isolates as expected. Glenlea's average ranged from 3.5-4 between the three *P. tritici-repentis* isolates tested (Pti-2, AR CrossB10, and 331-9) while 6B365 averaged from 3.5-5 between the three isolates and showed chlorosis symptoms with race 3 (331-9) it would often show the chlorosis symptom (due to being highly sensitive to Ptr ToxC). The average reaction observed for 6B662 ranged from 2.5-3.5 among the three isolates tested while the resistant check Salamouni would range from 1-2. However, at times Salamouni did not rate as being resistant, so the line was replaced by BR34. BR34 ranged from 0.5-2 between the three *P. tritici-repentis* isolates as well as when used in *P. nodorum* isolate SN4 evaluations. The susceptible *P. nodorum* check, Grandin, averaged 4.50 during artificial infections of this isolate. Infiltration checks included Glenlea as the Ptr ToxA sensitive check, 6B662 and Grandin for SnTox3, and three lines (Grandin, Chinese Spring, and Salamouni with Chinese Spring giving the strongest reaction) which are known to be sensitive to SnTox1. Glenlea gave the expected score of 3 for being very sensitive to the NE Ptr ToxA. Both Chinese Spring and Salamouni reacted sensitively to SnTox1 (scoring a 3 and 2). Line 6B662 gave a 2 reaction to SnTox3 while neither Chinese Spring nor Salamouni reacted to this NE.

After screening the 161 lines for resistance using the individual P. tritici-repentis and P. nodorum isolates variation was observed among the lines, as expected. The distribution for average disease scores of these lines can be seen in Figure 3. The average disease scores for the 161 lines

were 3.08, 4.62, 4.75, and 3.95 for isolates Pti-2, AR CrossB10, 331-9, and SN4, respectively. In total, 38, 10, six, and 14 lines were resistant to Pti-2, AR CrossB10, 331-9, and SN4, respectively. Six different germplasm groups were involved in this screening. Table 2 summarizes the average disease score and range for each group along with the total number of lines within each group.

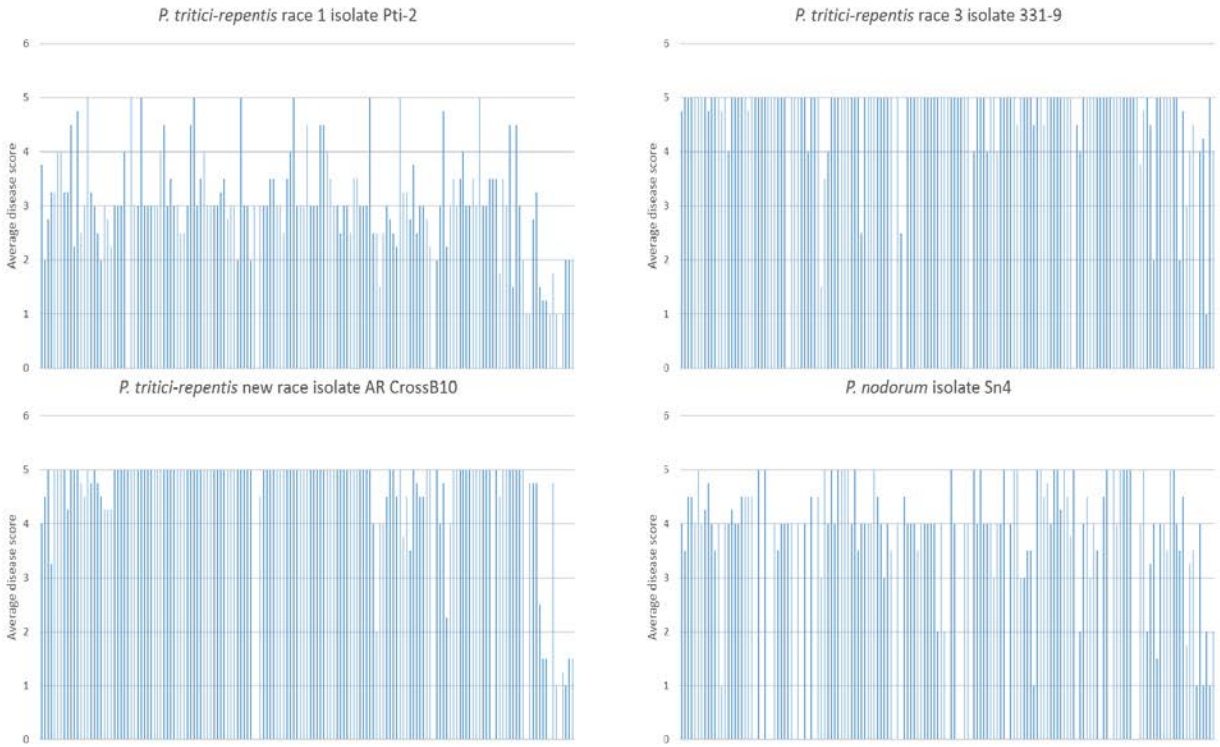

Figure 3: Average disease score distribution for all evaluated lines.

Table 2: Disease score distribution within each of the six germplasm groups.

		P. tritici-repentis						***P. nodorum***	
		R1 (Pti-2)		**AR (AR CrossB10)**		**R3 (331-9)**		**SN4**	
	Total Lines	**Range**	**Avg.**	**Range**	**Avg.**	**Range**	**Avg.**	**Range**	**Avg.**
Advanced lines	133	1.5-5	3.19	2-5	4.80	1.5-5	4.82	0-5	4.07
Current	11	2-4.5	3.30	3.25-5	4.64	4.75-5	4.95	3.5-5	4.18
Exotic	5	2.5-5	3.81	4.5-5	4.81	4-5	4.69	1-4	3.25
S. African	5	1-3.25	1.90	2.5-4.75	4.19	2-5	4.40	3.5-5	4.20
Synthetics	7	1-1.75	1.21	1-4.75	2.00	3-4.75	4.08	1-4.5	2.71
Tritipyrum	3	2	2.00	1-1.5	1.33	1-5	3.33	1-2	1.67

R1= race 1, AR= new race, and R3= race 3

Some lines were resistant to one or more of the fungal isolates. Thirty-two lines were resistant to only a single isolate while seven possessed resistance to two isolates. Six lines were

resistant to three fungal isolates, while one was identified with resistance to all four isolates. Table 3 shows which isolates the lines were resistant to and the number of lines with each specific combination. In Table 3 , *P. tritici-repentis* isolates are labeled R1, AR, and R3 (race 1 isolate Pti-2, new race isolate AR CrossB10, and race 3 isolate 331-9) while SN4 is the *P. nodorum* isolate.

Table 3: Isolate resistance combinations observed among lines.

Combination of isolates	**No of lines**
R1, AR, R3, SN4	1
R1, AR, R3	1
R1, AR, SN4	5
R1, AR	3
R1, R3	2
R1, SN4	2
R1	24
R3	2
SN4	6

Among the eleven current winter wheat cultivars currently grown in ND only Decade and WB Grainfield were resistant and only to isolate Pti-2. The best multiple isolate resistance occurred in the three Tritipyrum lines with one (159) having resistance to every isolate tested while the other two (160 and 161) possessed resistance to three isolates (Pti-2, AR CrossB10, and SN4). Six of the seven synthetic derivatives were resistant; three (lines 153, 156, and 158) possessed resistance to three isolates (Pti-2, AR CrossB10, and SN4), one (line 152) had

resistance to two isolates (Pti-2 and AR CrossB10), and the remaining two only had resistance to Pti-2. Among the winter wheat breeding lines that were tested only four lines (43, 102, 107, and 143) were resistant to a maximum of two fungal isolates. All four were resistant to *P. tritici-repentis* isolate Pti-2 while 107 was resistant to *P. nodorum* isolate SN4, lines 43 and 143 were resistant to isolate 331-9 and line 102 was resistant to AR CrossB10.

Averages pertaining to the sensitivity of the 161 lines to pathogen NEs are listed in Table A1 (in the Appendix). The NE Ptr ToxA, from *P. tritici-repentis*, was used along with SnTox3 and SnTox1 from *P. nodorum* which also produces SnToxA. Variation was evident among the 161 lines with respect to their degree of sensitivity to each NE. The data is shown in Figure 4. Lines which scored a 0 (lacking a sensitvite reaction) were considered insensitive to each infiltrated NE. Lines insensitive to multiple NEs were found from screening. Figure 5 shows the percentage of lines observed which were completely sensitive or were insensitive to one or more NE.

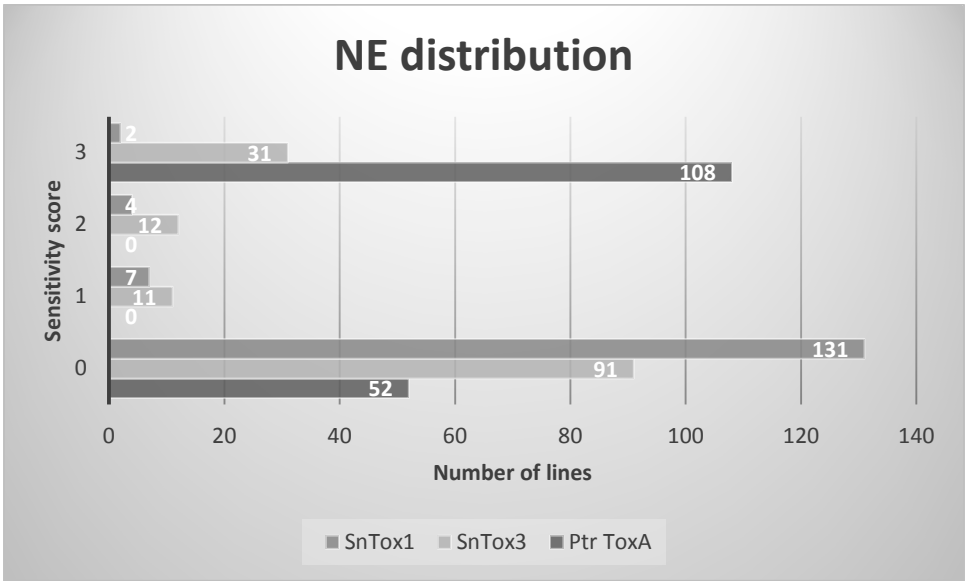

Figure 4: Sensitivity reactions to each tested necrotrophic effector.

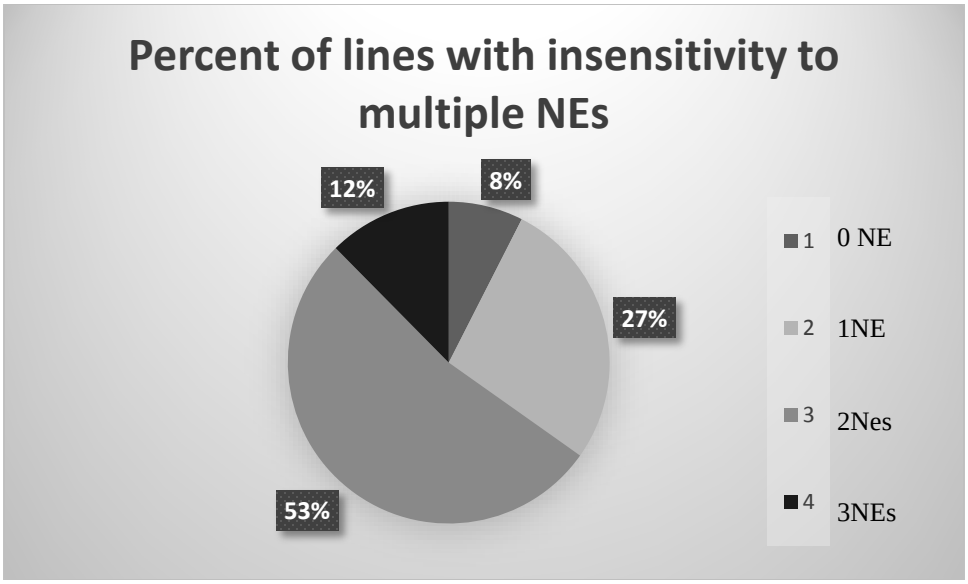

Figure 5: Lines with insensitivity to none or more than one necrotrophic effector.

Correlations between disease development scores obtained from the fungal inoculations and the sensitivity readings following NE infiltrations were studied (Table 4). The only significant correlation observed, was between SN4 induced disease susceptibility which correlated with SnTox1 sensitivity (r^2= 0.1296, negatively correlated). For the two isolates Pti-2 and SN4, disease averages were calculated and compared between lines with or without sensitivity to the NEs Ptr ToxA/SnToxA, SnTox3, and SnTox1. The Ptr ToxA insensitive lines averaged 3.12 while the sensitive lines averaged 3.06 for disease susceptibility to isolate Pti-2. However, for the SN4 and SnToxA sensitive and insensitive lines, those insensitive averaged 3.70 while the sensitive lines averaged 4.04. Between SN4 and SnTox3, insensitive lines averaged 3.91 while sensitive lines averaged 4.02. Finally between SN4 and SnTox1, the averages were 4.06 and 2.92 (insensitive and sensitive respectively). Resulting t-values were 0.42, -1.88, -0.58, and 3.89 (Pti-2 and Ptr ToxA, SN4 and SnToxA, SN4 and SnTox3, and SN4 and SnTox1, respectively). Resulting Pr>|t| values ranged from 0.0002 to 0.6741. Disease averages were found to be significantly different between SN4 and SnTox1, but the sensitive

lines expressed far less disease than insensitive lines. Then between SN4 and ToxA the two averages were not significantly different (Pr>|t| of 0.0624).

Table 4: Coefficients of determination between disease susceptibility and necrotrophic effector sensitivity.

	Ptr ToxA	SnTox3	SnTox1
Pti-2	r^2= 0.0009(-)	n/a	n/a
SN4	r^2=0.0256	r^2=0.0064	r^2=0.1296* (-)

4.2. Resistant inbred line development (Part B)

From the total 1,764 F_2 individuals screened with an inoculum mix of four *P. tritici-repentis* isolates (Dw5, Pti-2, AR CrossB10, and 331-9), ≈ 800 were selected as they either lacked susceptibility or showed intermediate reactions. Following the winter growth habit test, the selected individuals were reduced to ≈ 600 $F_{2:3}$. A comparatively small group of families (25%) were identified as being spring types. This is the result of the fact that only 25% of the parentage of the RMS multicross was contributed by spring wheat. In addition, some of the growth habit phenotypes were difficult to classify with certainty. The F_4 generation once again showed segregation and wide variation was seen among the lines when scoring them for tan spot and SNB disease symptoms (inoculum containing multiple isolates and isolate SN4, respectively). The checks used during both the F_2 and F_4 disease screenings reacted as expected each time. The ones in the very first *P. tritici-repentis* screening were not scored. During the second round of disease evaluation, Grandin averaged 4.5 while BR34 averaged 1.5 for *P. nodorum* isolate SN4. For *P. tritici-repentis*, Glenlea averaged a 4.0, 6B662 averaged 4.5, 6B365 averaged 5.0, and the resistant check Salamouni averaged 1.5. For the two fungi, scores ranged from 1.0-5.0 and 0.5-5.0 (*P. tritici-repentis* and *P. nodorum* respectively). Disease score

distributions of the 594 F_4 individuals are shown in Figure 6 while the number of lines within specific ranges can be found in Figure 7. Very few resistant lines were observed. Utilizing a 2.50 cutoff, 30 lines with *P. tritici-repentis* resistance were identified while 34 lines were found to have resistance to *P. nodorum* isolate SN4. Only two lines were found to be resistant to both fungi. The 13 (tan spot), 16 (SNB), and one (both fungi) best lines of which adequate seeds were available were chosen for the final evaluation trial. These F_4 selections included: lines 36, 63, 113, 123, 140, 189, 270, 362, 394, 457, 490, 559, and 595 with TS (tan spot, *P. tritici-repentis*) resistance; lines 35, 68, 76, 93, 94, 104, 150, 204, 279, 391, 475, 482, 501, 502, 516, 518, 524, 536, 540, and 563 with *P. nodorum* resistance; and line 428 (both fungi).

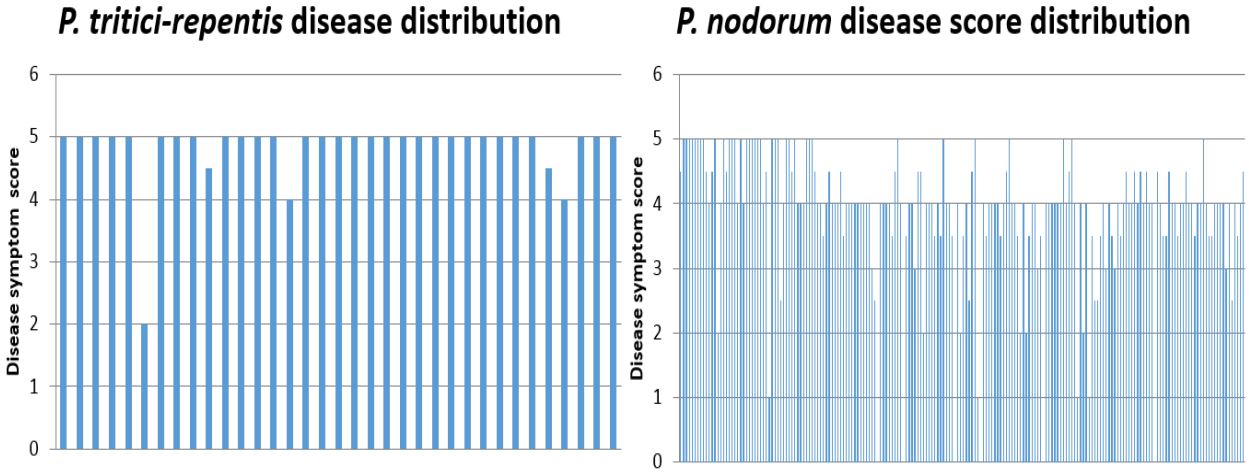

Figure 6: *P. tritci-repentis* and *P. nodorum* disease scores.

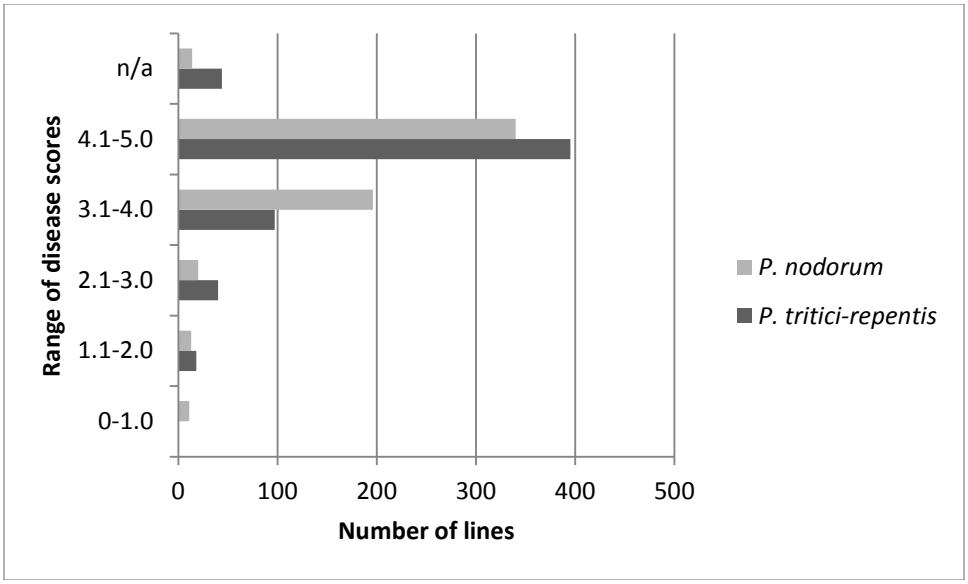

Figure 7: F_4 individuals with disease scores in a specific range. Lines with missing data are indicated as 'n/a'.

4.3. Final evaluation trial

The best 22 lines from Part A with good resistance capabilities and the 30 best $F_{4:5}$ lines from Part B (13 TS; 16 SNB and 1 (both)) were evaluated in a final evaluation trial, the results of which are summarized in the Appendix (Table A2) along with disease symptom and NE averages for the checks involved. The lines which did the best were marked with an asterisk.

Among the checks used during the artificial inoculations, BR34 averaged 1.17, 1.33, 1.00, and 0.88 to Pti-2, AR CrossB10, 331-9, and SN4. Grandin averaged 3.50 to SN4, as the susceptible check. Line 6B662 averaged 2.83, 2.50, and 2.83 to the *P. tritici-repentis* isolates Pti-2, AR CrossB10, and 331-9. Line 6B365 however averaged 4.83, 4.17, and 4.00 to these three isolates. The last check, Glenlea, averaged 4.00, 3.17, and 3.33 to Pti-2, AR CrossB10, and 331-9. The distribution of disease scores for isoltaes Pti-2, AR CrossB10, 331-9, and SN4 can be found in Figure 8. The averages of the 52 lines for Pti-2, AR CrossB10, 331-9, and SN4 were 2.32, 2.42, 2.31, and 2.40, respectively. Four lines, 27, 26, 31, and 28 were resistant to the

recently named isolates. Table 5 gives the average and range of disease scores among the lines by germplasm group, which now includes the $F_{2:5}$.

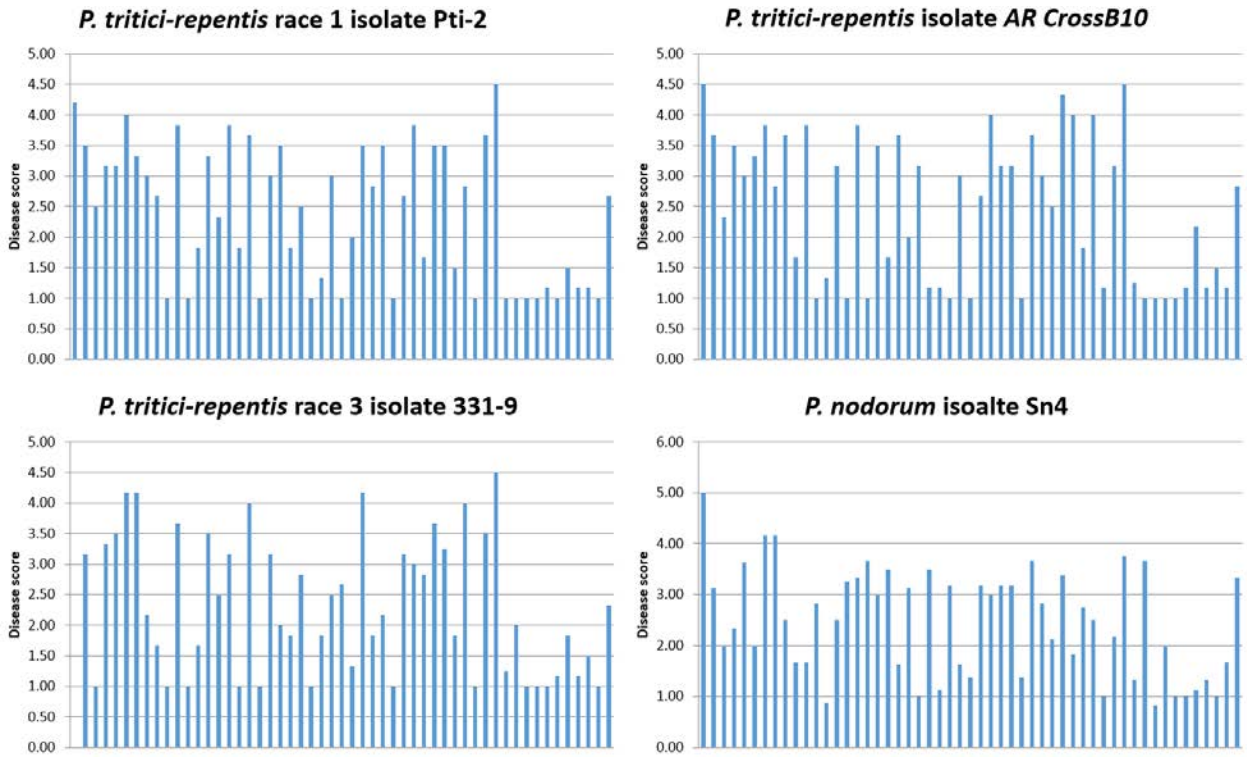

Figure 8: Disease score distributions for each isolate.

Table 5: Disease symptom scores and averages by germplasm group.

		P. tritici-repentis						*P. nodorum*	
		R1 (Pti-2)		**AR (AR CrossB10)**		**R3 (331-9)**		**SN4**	
	Total	**Range**	**Avg.**	**Range**	**Avg.**	**Range**	**Avg.**	**Range**	**Avg.**
Advanced	8	1.00-4.00	2.75	1.67-3.83	2.90	1-4.17	2.67	1.67-4.17	2.83
Current	2	2.00-3.50	2.75	2.33-3.67	3.00	1-3.17	2.09	2-3.13	2.57
Exotic	1	3.17	3.17	3.5	3.50	3.33	3.33	2.33	2.33
S. African	2	1.00-2.67	1.84	1.17-2.83	2.00	1-2.33	1.67	1.67-3.33	2.50
Synthetics	6	1-1.17	1.03	1.00-1.25	1.07	1.00-2.00	1.24	1.83-3.67	1.65
Tritipyrum	3	1.17-1.50	1.28	1.17-2.17	1.61	1.17-1.83	1.50	1-1.33	1.15
F_5	30	1-4.50	2.55	1.00-4.50	2.60	1-4.50	2.47	0.88-3.75	2.46

The numbers of lines with resistance to multiple fungal isolates are shown in Figure 9 whereas Table 6 shows the combinations of isolates involved and the number of lines possessing the specific resistance combinations. Figures 10, 11, and 12 show examples of the disease symptoms observed on leaves in the final evaluation trial.

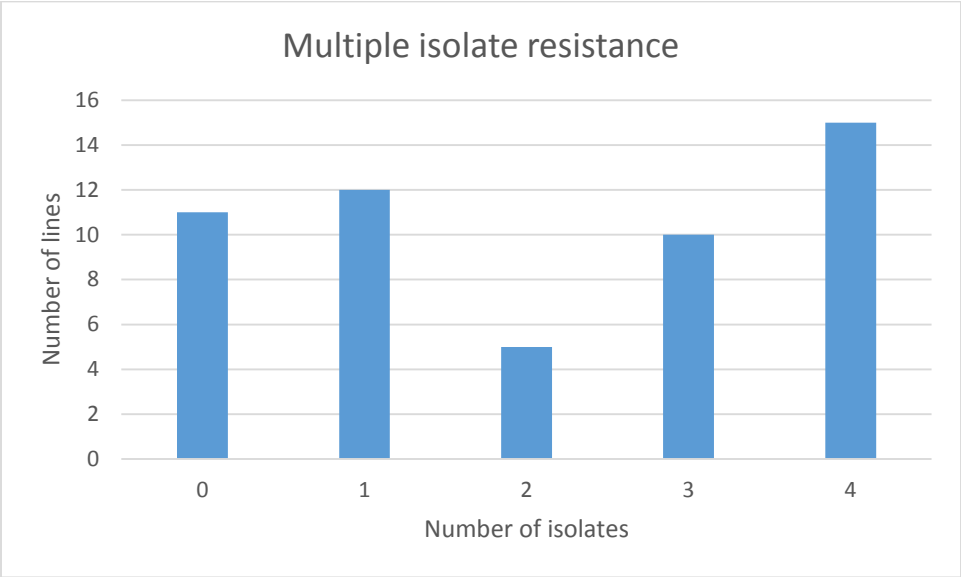

Figure 9: Lines with resistance to multiple isolates.

Table 6: Fungal isolate resistance combinations among the lines.

Combination of isolates	No. of lines
R1, AR, R3, SN4	16
R1, AR, R3	8
R1, R3, SN4	1
R1, AR, SN4	1
R1, R3	1
R3, SN4	2
AR, R3	1
R3	4
SN4	8

R1= Race 1 isolate Pti-2, AR= AR CrossB10, and R3= race 3 isolate 331-9.

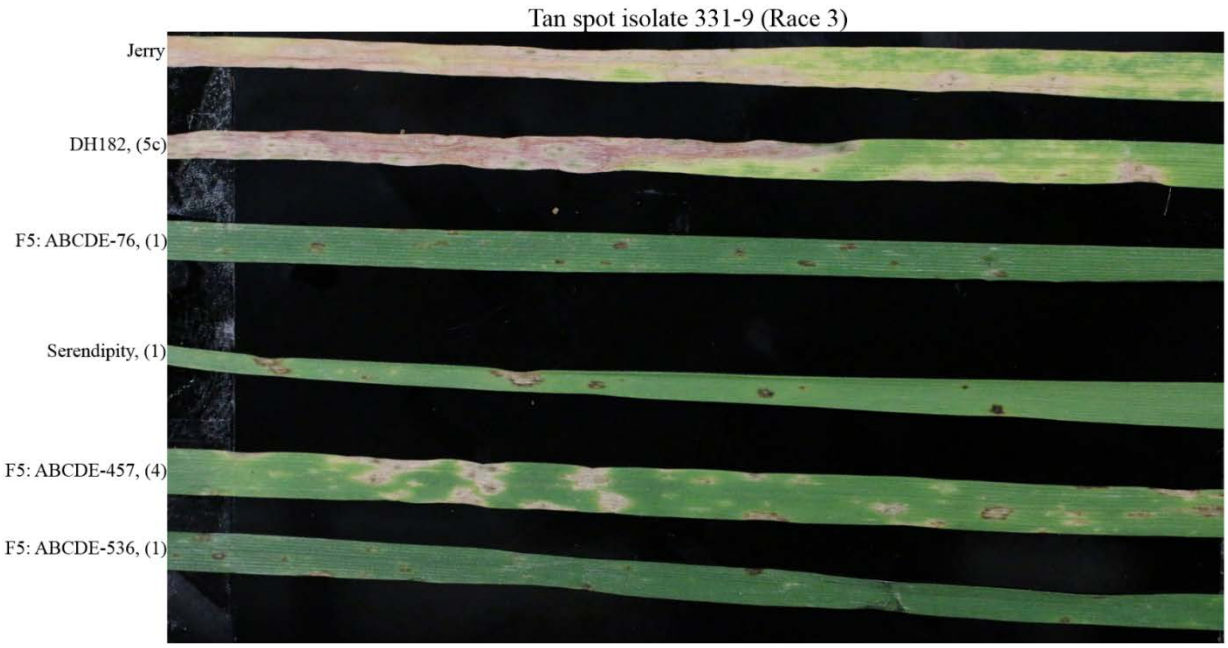

Figure 10: Observed symptoms on leaves post *P. tritici-repentis* isolate 331-9 infection, disease symptom scores from 0-2.5 and 3-5 were considered resistant and susceptible, respectively. Line names are followed by their disease score in (brackets) and those with a "c" showed chlorosis symptoms.

Figure 11: Observed symptoms on leaves after *P. nodorum* isolate Sn4 infection, disease symptom scores from 0-2.5 and 3-5 were considered resistant and susceptible, respectively. Line names are followed by their disease score in (brackets) and those with a "c" showed chlorosis symptoms.

Figure 12: Disease symptoms on leaves after P. tritci-repentis isolate AR CrossB10 infection, disease symptom scores from 0-2.5 and 3-5 were considered resistant and susceptible, respectively. Line names are followed by their disease score in (brackets) and those with a “c” showed chlorosis symptoms.

During the final evaluation; BR34, 6B662, Salamouni, and Chinese Spring did not react to Ptr ToxA while Jerry and Grandin were highly sensitive to this NE. SnTox3 caused Grandin to develop a sensitivity symptom of 2 while Salamouni, Jerry, and Chinese Spring did not react. Chinese Spring gave the strongest sensitivity average (2.5) to SnTox1.

NEs SnTox3 and SnTox1 had the highest number of lines insensitive to each and the number of lines which were insensitive or sensitive can be found in Figure 13. The advanced lines, current winter wheat cultivars, exotic winter wheat, and Tritipyrum lines were completely insensitive to SnTox3.

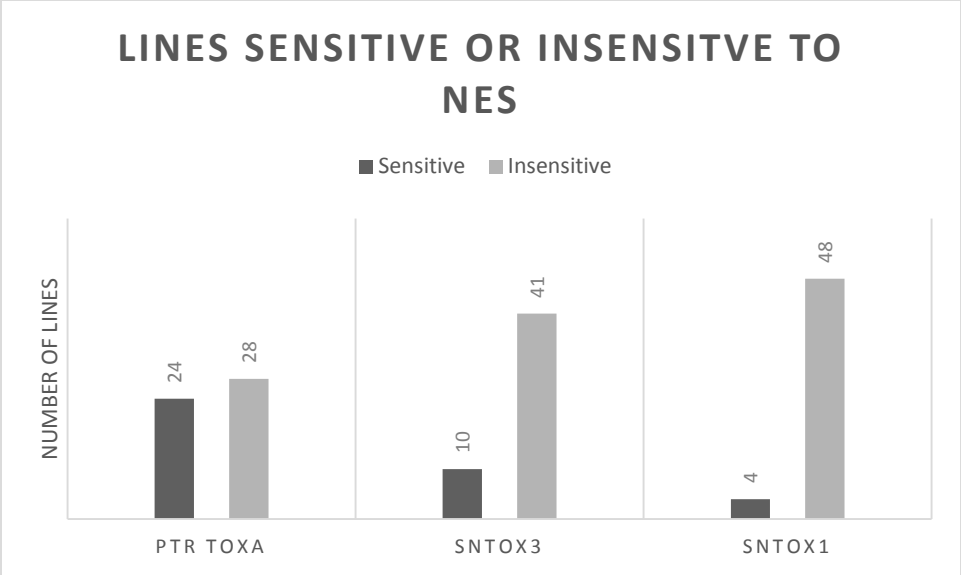

Figure 13: Number of necrotropic effector (NE) sensitive and insentive lines.

Ptr ToxA sensitivity was observed within at least one line for all the groups except for the South African and Tritipyrum lines. The two South African lines were both insensitive to the ToxA NE produced by both fungi while all three Tritipyrum lines were highly sensitive to this NE. Almost half (14) of the F_5 lines were sensitive to Ptr ToxA. Among the advanced breeding lines five were sensitive to Ptr ToxA. All of the lines in every group except for the Synthetics were completely insensitive to SnTox1. Examples of sensitive versus insensitive reactions to the NE Ptr ToxA are shown in Figure 14, lines F5: ABCDE 559, F5: ABCDE 362, and F5: ABCDE 36 (numbers 38, 51, and 52 respectively). Percentage of lines with insensitivity to one or more NEs were observed and are shown in Figure 15 (1= 0NEs, 2= 1 NE, 3= 2 NEs, and etc.).

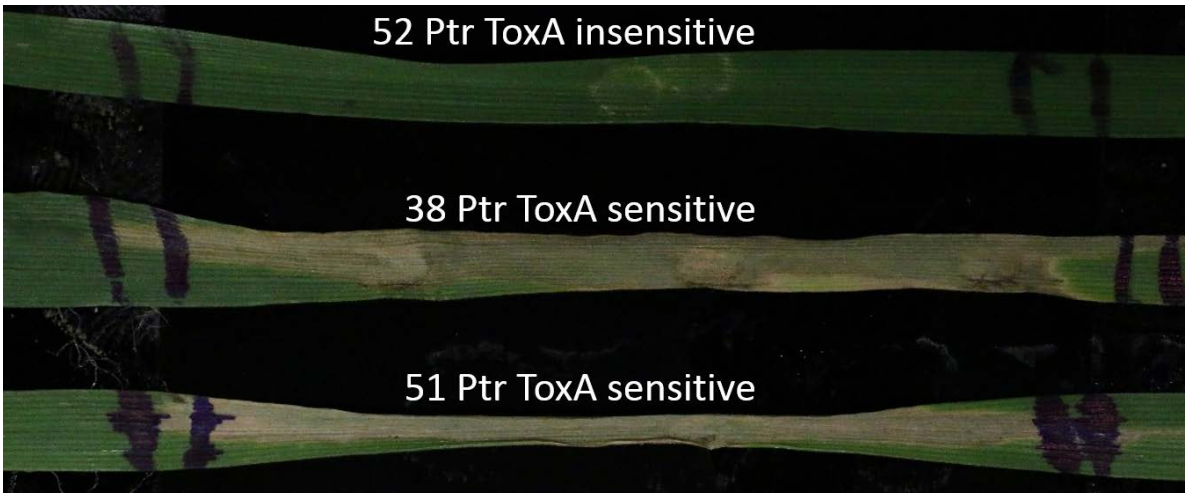

Figure 14: Picture showing sensitive and insensitive reactions to *P. tritici-repentis* NE Ptr ToxA.

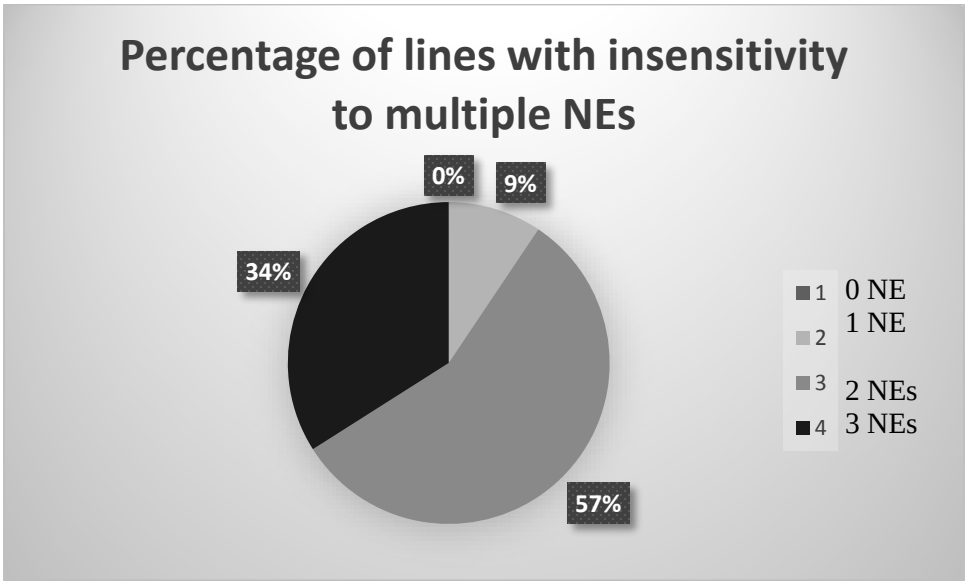

Figure 15: Percentage of lines with insensitivity to none, one, or more NEs.

Correlations between NE sensitivity and disease susceptibility were calculated and the respective coefficients of determination (r^2) are summarized in Table 7. In the final evaluation trial, sensitivity to Ptr ToxA was not significantly correlated with susceptibility to *P. tritici-repentis* isolate Pti-2. Susceptibility to *P. nodorum* isolate SN4 was significantly and positively correlated to ToxA sensitivity but negatively correlated to SnTox1 sensitivity. However, the

latter correlation is probably an artefact of the data set as virtually all of the lines in the trial were SnTox1 insensitive (the only sensitivity occurred in four of the synthetics).

Table 7: Coefficients of determination (CD) between disease susceptibility and NE sensitivity.

	CD and P-values		
	Ptr ToxA	**SnTox3**	**SnTox1**
Pti-2	$r^2 = 0.0121$	n/a	n/a
SN4	$r^2 = 0.16$*	$r^2 = 0.0324$	$r^2 = 0.1156$*(-)

Among the lines with sensitivity to Ptr ToxA, the Pti-2 disease score mean was 2.37 while insensitive lines averaged 2.29 (t = -0.26, non-significant). With respect to *P. nodorum* isolate SN4, the SnToxA sensitive lines averaged 2.83 while the insensitive lines averaged 2.04 (t = -3.10, significant). For SN4 infections, the SnTox3 sensitive lines averaged 2.74 while insensitive lines averaged 2.34 (t = -1.13, non-significant). When comparing the SN4 and SnTox1 results, it seemed that the sensitive lines averaged 1.29 while the insensitive lines averaged 2.50 (t = 2.43, significantly different).

4.4. Test for the presence/absence of Tsn1 using marker Xfcp623

Results obtained following marker screening to detect *Tsn1* are summarized in the appendix (Table 2A) while the gel pictures are shown in Figure 16. Twenty four lines tested positive for *Tsn1* and were also sensitive to Ptr ToxA. The remaining 28 lines did not have *Tsn1* and were Ptr ToxA insensitive. The results for the checks were as expected and as previously found by Faris et al. (2010). Grandin, Glenlea, and Jerry were found to possess the dominant *Tsn1* gene while Salamouni, BR34, and Chinese Spring lacked a band (they lack the dominant *Tsn1* gene).

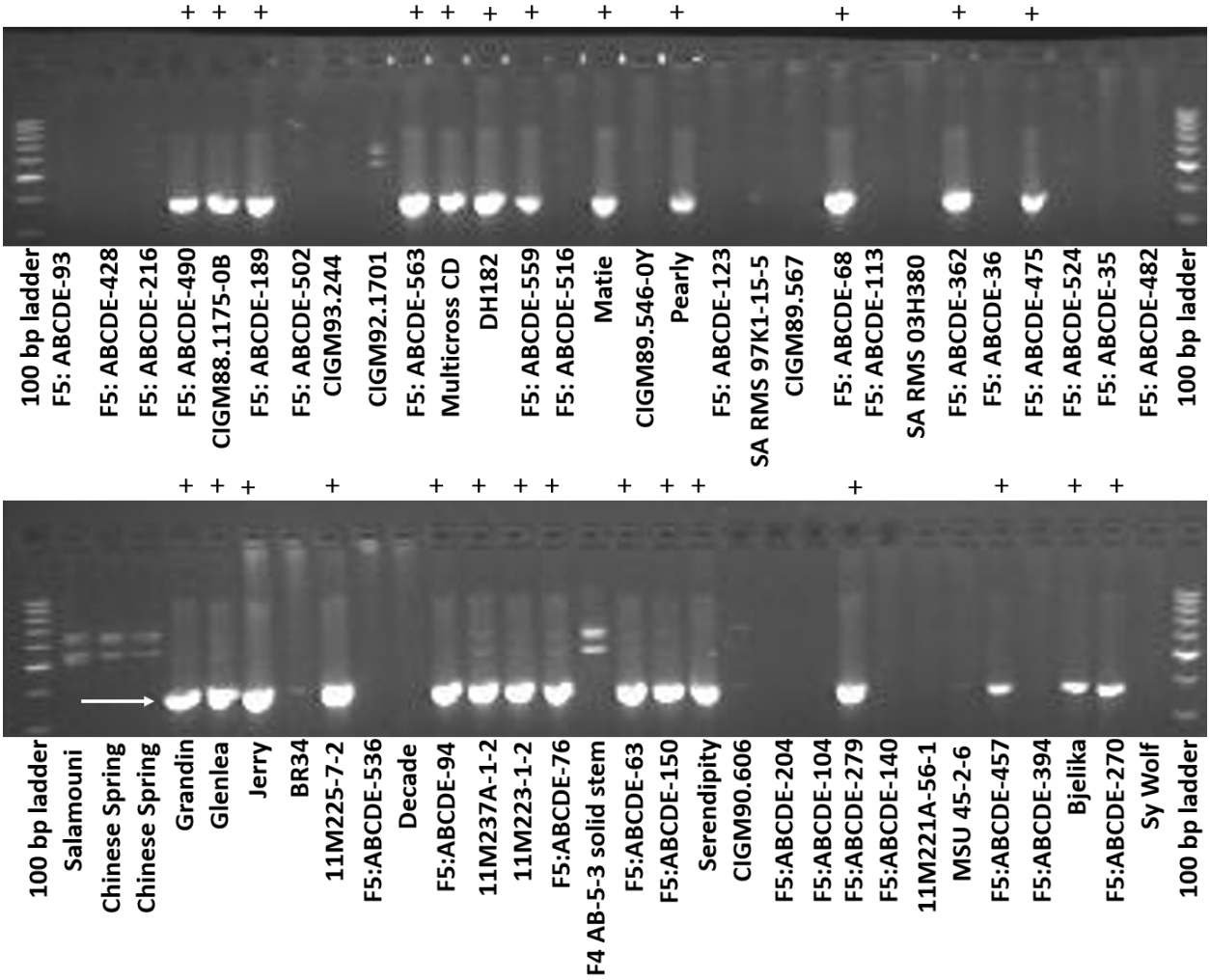

Figure 16: Confirmation of the presence/absence of *Tsn1* in the individual lines using marker *Xfcp623*. The diagnostic band (~390bp) is indicated with a white arrow.

4.5. Confirmation of a rye translocation being present/absent

Results obtained following the testing of the entries in the final trial with marker *IB-267* are summarized in the appendix (Table 2A) and can be seen in Figure 17. The checks Jerry (non-1RS), Decade (non 1RS), SY Wolf (1BL.1RS), BR34 (1BL.1RS), Glenlea (non 1RS), Grandin (non 1RS), Norstar (non 1RS), and RWG28 (1BL.1RS) yielded the expected results. Marker *IB-267* amplified the diagnostic band (≈115bp) in those checks that are known to possess the 1BL.1RS translocation. In addition to the positive checks, six lines tested positive for 1RS. The advanced line 11M237A-1-2 has RWG28 as one of its parents and could possess the 1BL.1RS

rye translocation. The remaining five lines derive from a recurrent selection base population (Multicross CD; F5:ABCDE-35; F5:ABCDE-123; SA RMS 97K1-15-5; and SA RMS 03H380) and could carry either of the 1AL.1RS or 1BL.1RS translocations, as both are known to occur in the base population. The latter five lines are also SnTox1 insensitive and therefore lack the *Snn1* allele on 1BS. However, this does not reveal whether these translocations involve chromosome 1B as virtually all of the entries, excepting four synthetics, were highly insensitive to SnTox1 suggesting that the *snn1* allele is present at a high frequency. Thus, it is also possible that the five lines with an unknown 1RS translocation could possess a 1AL.1RS translocation plus the *snn1* allele on 1BS.

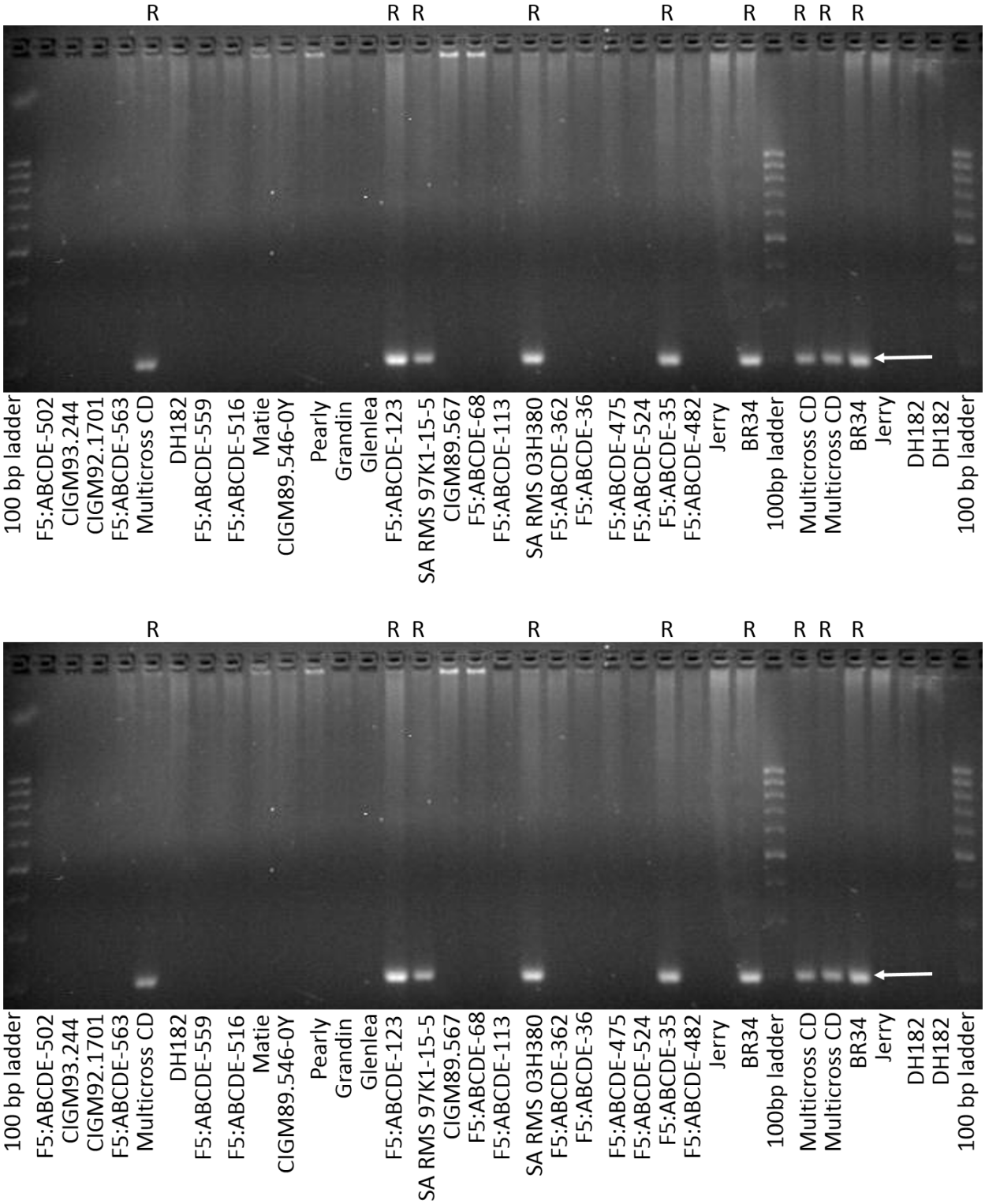

Figure 17: Detection of the 1RS.1BL rye translocation using the Sr50 marker IB-267 with bands approximately 115bp in length.

5. DISCUSSION

Both *P. tritci-repentis* and *P. nodorum* occur in North Dakota and negatively affect the yield of wheat by causing up to 50% yield loss (Chu et al., 2008). According to IPM surveys done in 2014 and 2015 (NDSU Extension Service), *P. tritici-repentis* can be very severe in some years. This is a concern for the winter wheat breeding program since no lines have previously been screened for resistance to *P. tritici-repentis* or *P. nodorum*. Host resistance to the two diseases is complex, polygenic, and could involve both direct host resistance mechanisms to combat fungal infection and insensitivity to NEs. Current winter wheat cultivars are generally lacking resistance making it crucial to find useful germplasm that can be employed in the breeding program. Even though SY Wolf and Decade were previously identified as being resistant by Liu et al. (2015), more and diverse sources of additional resistance are needed. The development of resistant varieties will benefit both conventionally farmed areas and organic farms. Fungicides can and have been useful to partially control the two fungi, but they lack the sustainable structure resistant lines can bring. In addition to this, Reimann and Deising (2005) found *P. tritici-repentis* isolates with resistance to fungicides containing triazole and strobilurin in Germany during 2000 and 2001. Jorgensen and Olsen, (2007) also found *P. tritici-repentis* isolates in Denmark that are strobilurin resistant. It is therefore possible that fungicide resistant *P. tritici-repentis* isolates may also arise is the US.

In parts A and B of this study, useful germplasm with good resistance and NE insensitivity levels was sought initially among two diverse groups. One group involved 161 wheat lines which included well established NDSU HRWW inbred lines plus more diverse germplasm that can be used as sources of additional resistance. Simultaneously, in part B of the study, useful resistance levels were searched for among a set of 1,764 F_2 individuals from a RMS

pre-breeding population that were subjected to inbreeding by modified SSD. From evaluations involving these two groups, promising individuals were identified and subjected to a final evaluation in order to identify the very best individuals for future use. In parallel with the disease and NE symptom data, an attempt was made to also validate the Ptr ToxA sensitivity reactions utilizing a PCR-based DNA marker, *Xfcp623* (Faris et al., 2010). Additionally, the individuals were screened for the presence of a rye translocation (1AL.1RS/1BL.1RS) utilizing the rye-specific marker *IB-267* because this translocation negatively affects wheat dough quality (Mago et al., 2002).

Majority of the lines evaluated in part A expressed resistance to *P. tritici-repentis* isolate Pti-2; fewer lines had resistance to *P. nodorum* isolate SN4; still fewer were resistant to the new *P. tritici-repentis* isolate AR CrossB10 originating from Arkansas; and the least resistance was found with respect to *P. tritici-repentis* isolate 331-9. The lower incidence of resistance to 331-9, compared to Pti-2, may have been the result of too strict scoring of the inoculum being too concentrated. Some of the lines were therefore rescreened with AR CrossB10 and 331-9, however the new results were in agreement with the first scores. Isolate AR CrossB10 is a newly identified isolate and neither it nor isolate 331-9 may be present in North Dakota yet, but 331-9 is present in Canada while another race 3 isolate (OH99) was found in Ohio (Faris and Friesen, 2005). Having germplasm with these resistances allows for potential lines to be developed and released prior to either isolate spreading into North Dakota. Even with the large diversity among the 161 lines, it was surprising that 131 lines insensitive to NE SnTox1 and that 12.4% of them were insensitive to all three NEs while 52.8% were insensitive to two NEs. The least insensitivity was observed for both Ptr ToxA and SnTox3. From the combined data, a total of 22 lines were selected for final evaluation in a combined trial. In part B of the study SSD inbreeding

with selection was utilized to derive potentially useful $F_{4:5}$ lines. With the many diverse parents that were used to create this highly heterogeneous RMS base population, a lot of variation would be expected. During the evaluation of the F_2 with inoculum containing a mix of *P. tritici-repentis* isolates a little more than half of the group was discarded on the basis of susceptibility. Following a second infection with the four *P. tritici-repentis* isolates and *P. nodorum* isolate SN4, 30 individuals were included in the final evaluation trial. In the final evaluation the smaller number of individuals selected in parts A and B were scored more carefully and over more replications. As a result some of the disease symptom scores differed between the initial scoring and what was observed in this final trial. Furthermore, most of the lines had been included in the trial based on having resistance to only *P. tritici-repentis* or only *P. nodorum* rather than both.

A collection of current winter wheat lines were also evaluated for resistance and NE sensitivity to the two diseases by Liu et al. (2015). A total of 120 adapted HRWW cultivars/experimental lines currently being grown in North America were included. Some of the genotypes tested by Liu et al. (2015) were also used in this study, most notable of these were the cultivars Decade and SY Wolf. SY Wolf was previously shown by Liu et al. (2015) to have good resistance to all the isolates tested for both fungi and is insensitive to every NE tested. Part of the resistance in this line may be due to the 1RS.1BL rye translocation. *Snn1* was identified and mapped to chromosome arm 1BS by Liu et al. (2004a). With the 1BL.1RS translocation having replaced chromosome arm 1BS, the dominant *Snn1* allele which confers SnTox1 sensitivity would have been lost as well. SY Wolf was found to be insensitive to the NE SnTox1 which would confirm absence of *Snn1*. In this study, resistance results for SY Wolf coincide with what was previously found (Liu et al., 2015). However, the results in this study do not match what Liu

et al. (2015) found with respect to Decade. The present study found Decade to be susceptible to all isolates tested, yet this result could have been due to a mixed seed source.

The 1RS.1BL translocation causes “sticky dough” and reduces the dough strength, which is thought to be from the presence of the *Sec-1* gene along with a substitution of both glutenins and gliadins (Graybosch et al., 1993; Martin and Stewart, 1990; and Dhaliwal and MacRitchie, 1990). Simons et al. (2012) did a study Evaluating bread making quality which included mapping responsible QTL as well as comparing lines BR34, Grandin, and some individuals in the BG (BR34 x Grandin) population. BR34 possesses the 1RS.1BL rye translocation, and it along with 24 BG individuals were found to have poor bread making performance. The poor performance was due to the translocation resulting in the loss of low molecular weight (LMW) glutenins located on chromosome arm 1BS which reduced the total glutenin quantity (Graybosch, 2001). Losing LMW glutenins was found to cause a greater negative effect in hard wheat than soft wheat (Lee et al., 1995; McKendry et al., 1996; and Johnson et al., 1999).

One of the five best breeding lines selected in the final evaluation trial is 11M237A-1-2 which showed promising resistance to *P. tritici-repentis* isolates Pti-2, AR CrossB10 and 331-9 as well as *P. nodorum* isolate Sn4 (averaging 1.75, 1.67, 1.00, and 1.67 respectively). Interestingly, this line has *Tsn1* and is sensitive to Ptr ToxA. Line 11M237A-1-2 has been named 15NORD-39 and is currently being evaluated in a regional yield trial. Unfortunately, it carries the 1BL.1RS translocation. Four lines, derived from the part B selections, are similarly promising. Lines F5: ABCDE-35, -428 and -536 lack *Tsn1,* and they are resistant to all isolates and insensitive to all of the NEs. Line F5: ABCDE-150 has *Tsn1* and is sensitive to Ptr ToxA; however, it is resistant to all of the isolates and insensitive to the remaining NEs. Of the four lines, only F5: ABCDE-35 has a 1RS translocation. The four lines have not been field tested

before and are being increased for agronomic evaluation in 2017 and will also be used extensively in the upcoming crossing blocks of the HRWW breeding program.

Line F5: ABCDE-123 (with 1RS) showed resistance to all isolates barring *P. tritici-repentis* isolate AR CrossB10 and was insensitive to all of the NEs. Five additional lines: Multicross CD (with 1RS); F5: ABCDE-76, -94, -189 and -516 had excellent resistance to the three *P. tritici-repentis* isolates but not to *P. nodorum*. Conversely, 11M221A-56-1, F4 AB-5-3 solid stem, F5: ABCDE-104, 204, -216, -482, -502, and -559 showed strong resistance to *P. nodorum* isolate SN4, but were susceptible to *P. tritici-repentis* (with the exception of lines -216 and -482 since they had some *P. tritici-repentis* resistance). Clearly, the fourteen lines could also be useful as cross parents and they will similarly be included in upcoming field trials.

The spring wheat line SA RMS 97K1-15-5 (with 1RS) showed strong resistance not only to both fungi, but also to every isolate used, yet it has sensitivity to SnTox3. Transfer of this resistance to winter wheat will be straight forward but will necessitate backcrosses to recover the winter growth habit and cold-hardiness. If multiple QTL need to be transferred, some of which could be recessive, backcrossing will be more time consuming than deriving resistance from a winter wheat source.

Also included in the final trial were six synthetic derivatives. Some of these showed useful resistance in previous evaluations in which not all of them included NE reactions (Xu et al., 2004 and Morris et al. 2010). The six synthetics were highly resistant to all three of the *P. tritici-repentis* isolates, and barring CIGM88.1175-OB (sensitive to Ptr ToxA), they were also insensitive to the *P. tritici-repentis* NEs. Five synthetics were resistant to SN4 and two and four insensitive to the *P. nodorum* NEs (SnTox1 and SnTox3 respectively). Synthetic wheat has been used in studies for finding resistance QTL and for finding new sources of resistance to *P. tritici-*

repentis and *P. nodorum.* Tadesse et al. (2006) screened 98 synthetic wheat lines to find additional sources of resistance, then at the same time mapped the newly discovered genes to specific chromosome locations. In their study, eight *P. tritici-repentis* isolates were used to create a differential set of wheat lines to distinguish each isolate, but only a single isolate (Ascb1) was used to screen the synthetic lines. They found two genotypes which were immune to *P. tritici-repentis* (score of 0) and twenty with strong resistance. Three synthetic lines (XX41, XX45, and XX110) were used for resistance QTL mapping. The same lines had also been used in a previous study by Siedler (1991) and were reported to give a strong resistance response when tested using a mixture of *P. tritici-repentis* isolates. Later studies provided additional evidence for resistance to multiple *P. tritici-repentis* isolates among synthetic wheat lines (Siedler et al., 1994; and Xu et al., 2004). Similar strong resistance responses were found among the seven synthetics included in this study (lines 152-158). Xu et al (2004) also screened synthetic hexaploid wheats for *P. nodorum* resistance along with the durum parents that were used to make them. The majority of the 155 synthetics tested were insensitive to ToxA. The durum parent, Cerceta, showed ToxA sensitivity along with average tan spot and SNB disease reactions of 3.33 and 3.67 respectively. However, one synthetic using this parent and the *Ae. tauschii* accession 895 was insensitive to ToxA and had average disease scores of 1.17 and 3.17 to the tan spot and SNB diseases. This specific synthetic is one which was also tested in this current study CIGM89.567 (line 155) and has close to the same average when screened for resistance to the *P. tritici-repentis* isolate Xu et al. (2004) used, Pti-2 (race 1). The ToxA reaction from this current study is also in agreement with the findings of Xu et al. (2004).

Six years after the study done by Xu et al. (2004) looking at resistance and NE reactions, Morris et al. (2010) conducted another study looking at the variations of resistance in synthetic

wheat accessions derived from durum wheat parents. The synthetic lines used in this current study are also present in the study by Morris et al. (2010). Differences in plant age at infection and exact isolates used vary between Morris et al. (2010), Xu et al. (2004), and this study which may impact the disease scores observed. Despite the small differences in methodology, the disease scores observed in both Part A and the final trial of this study closely match previously reported results (Morris et al., 2010; and Xu et al., 2004).

Introgression of resistance/NE insensitivity QTL from the synthetic wheats will require extensive pre-breeding yet will be easier to attain because the donor species derive from the primary gene pool of wheat. Crosses to HRWW followed by backcrosses of resistant progeny to HRWW can be employed to reduce the amount of linked, undesirable alien DNA that is currently associated with these lines and resistance QTL.

Wheat alien species derivatives were studied by Oliver et al. (2008), looking for tan spot and SNB disease resistance. In total, they evaluated 199 derivatives from the following species: *Aegilops tauschii, Leymus racemosus, Elymus rectisetus, Thinopyrum junceum, Th. ponticum, Th. intermedium, Th. elongatum, Dasypyrum villosa, Avena sativa*, and *Secale cereale*, using *P. tritici-repentis* isolate Pti-2 (race 1) and *P. nodorum* isolate Sn2000. The data presented shows the resistant check, BR34, as having an average disease score of 0.16 and 1.35 for *P. nodorum* and *P. tritici-repentis*, respectively while the susceptible check Grandin averaged 3.62 and 3.88. In this study, about the same averages were seen with BR34 for both fungi, but Grandin was only used as a *P. nodorum* check. Oliver et al. (2008) identified genotypes with resistance to *P. tritici-repentis* and *P. nodorum* from 199 wheat-alien species derivatives which included the alien species: *A. tauschii, L. racemosus, E. rectisetus, T. ponticum, T. junceum, T. intermedium, T. elongatum, Dasypyrum villosa, Avena sativa*, and *Secale cereale*. From their study it was

concluded that a number of derivatives showed significantly higher levels of resistance to *P. tritici-repentis*, *P. nodorum*, or both fungi compared to their parents. Lines derived from *Thinopyrum ponticum* greatly improved the resistance seen for both in most of the combinations. *Thinopyrum junceum* improved *P. tritici-repentis* resistance more than *P. nodorum* and *Thinopyrum intermedium* derivatives had the opposite effect (improved *P. tritici-repentis* resistance). The three Tritipyrum lines used in the present study were derived from a cross between *Triticum turgidum* ssp durum and *Thinopyrum distichum*. Tritipyrums Serendipity, Matie, and Pearly were resistant to all three *P. tritici-repentis* isolates. Matie had the best resistance with averages ranging from 1.17-1.25 for the three *P. tritici-repentis* isolates. All of the Tritipyrums also had *P. nodorum* resistance. All three entries were sensitive to Ptr ToxA but insensitive to Ptr ToxB. Despite them being sensitive to Ptr ToxA, the three Tritipyrum lines had resistance scores of 1.00-1.33 when inoculated with *P. nodorum* isolate SN4 (Part A results). In addition the three lines were insensitive to the *P. nodorum* NEs SnTox1 and SnTox3.

Gene transfer from *Thinopyrum* (tertiary gene pool species) will be difficult and time-consuming, necessitating the use of chromosome engineering methodologies. In addition, fairly big chromosome segments get transferred during the translocation of alien genes to wheat that often necessitates subsequent tailoring and size reduction. This occurred with lines derived from a cross between a Turkish common wheat line 178704 and *Thinopyrum ponticum* in a study by Oliver et al. (2008). Even though *Th. ponticum* increased resistance of the derivatives for both fungi, some derivatives had large amounts of added chromatin from the *Th. ponticum* carrying undesirable traits which caused late maturity and low yield.

In this study as well as that of Liu et al. (2015), lines were found that are insensitive to an NE but still susceptible. Also, instances were seen where a line has NE sensitivity, yet was

resistant, for example the three Ptr ToxA sensitive Tritipyrum lines. Ptr ToxA sensitivity was previously observed to be highly correlated with disease susceptibility to *P. nodorum* (Liu et al., 2015), but in this study it was weakly correlated with susceptibility to *P. nodorum* isolate SN4 (r^2 = 0.0256 (part A) and r^2 = .16 (final trial), respectively). However, the correlation between Ptr ToxA sensitivity and susceptibility to *P. tritici-repentis* isolate Pti-2 was not significant in either data set. Both SnTox3 and SnTox1 could have played a role in causing *P. nodorum* disease susceptibility, but SnTox3 has been shown to be masked by SnToxA and SnTox2 (Friesen et al., 2008; Liu et al., 2015). SnTox1 had only 13 lines (part A) and four lines (final trial) being sensitive to it. SnTox1 did not play a big role in aiding susceptibility and this probably explains the observed negative correlations (r^2 = 0.1296, part A and r^2 = 0.1156, final trial) that might simply be artefacts of the limited variability for sensitivity. Unfortunately, this study cannot pin point which other *P. nodorum* NEs may have played a role in causing disease susceptibility because isolate SN4 produces all known NEs to date and only three of them were included in this study.

5.1. Conclusion

The low incidence of resistance among the 149 winter wheat lines in part A of the study, was also observed among the initial 1,764 F_2 individuals screened in part B. Despite the large number of initial F_2 individuals, the potentially useful families were reduced to only 30. Even though the overall incidence of resistance was low there were some exceptional lines identified within the final group of 52. Five HRWW lines were resistant to all the *P. tritici-repentis* and *P. nodorum* isolates with three of these lines also being insensitive to all of the NEs. Another five lines were resistant to all three *P. tritici-repentis* isolates while nine additional lines showed resistance to *P. nodorum* isolate SN4. These selections will be employed directly in crosses to

increase resistance levels in the winter wheat breeding population. It is important to note that only two of these best selections derive from regular crosses (11M221A-56-1 and 11M237A-1-2). The remainder are F_5 that were each obtained from a different F_2 that was taken from a large and highly diverse RMS base population (109 contributing parents) and the selections are unrelated to each other genetically. The HRSW line SA RMS 97K1-15-5 also had exceptional resistance to all isolates and showed sensitivity to SnTox3. The South African lines had been selected in an area where the occurrence of tan spot and SNB disease was common and they may possibly have resistance QTL not present among North American HRWW genotypes. This line could be used in crosses with, and backcrosses to, HRWW in an attempt to acquire additional resistance QTL. However, in the RMS population, about 12.5% of the parentage derives from South African HRSW, thus some of the resistance contained among the F_5 selections could derive from this source.

Even though some of the *P. nodorum* resistance that was observed could have resulted from absence of *Snn1* through the presence of the 1BL.1RS translocation, many lines lacking this translocation were SnTox1 insensitive and therefore carry the *snn1* allele instead. Thus, when using the best lines as parents in the breeding program, selection against the 1BL.1RS translocation coupled with selection for SnTox1 insensitivity (presence of *snn1*) should avoid the "sticky dough" problem without sacrificing resistance.

Novel resistance is attainable from the primary gene pool species, *Ae. tauschii*. In this respect, CIGM89.567 will be the best starting material. Similarly, either Matie or Pearly could be used as starting material for the transfer of resistance from the tertiary gene pool species, *Th distichum*.

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APPENDIX

Table A1: Averaged disease phenotype and effector response data obtained following the evaluation of 161 wheat lines with *P. tritici-repentis* and *P. nodorum*.

				P.tritici-repentis				*P. nodorum*		
				Race			**Effector**	**Race**	**Effector**	
Entry No.	**Cross no.**	**Habit**	**Pedigree**	**R1 (Pti-2)**	**AR (AR CrossB10)**	**R3 (331-9)**	**Ptr ToxA**	**Sn4**	**SnTox3**	**SnTox1**
1*	SY Wolf	W		3.75	4.00	4.75	0	4.00		
2*	Decade	W		2.00	4.50	5.00	0	3.50	0	0
3	Norstar	W		2.75	5.00	5.00	3	4.50	0	0
4	Ideal	W		3.25	3.25	5.00	3	4.50	0	0
5	WB Matlock	W		3.25	5.00	5.00	3	4.00	0	0
6	Accipter	W		4.00	5.00	5.00	3	5.00	3	0
7	Moats	W		4.00	5.00	5.00	3	4.00	1	0
8	Flourish	W		3.25	5.00	5.00	3	4.25	3	0
9	Emerson	W		3.25	4.25	4.75	3	4.75	3	0
10	Gateway	W		4.50	5.00	5.00	0	4.00	0	0
11	WB Grainfeld	W		2.25	5.00	5.00	3	3.50	0	0
12	PI181161	W	Alba (Belgium)	4.75	5.00	5.00	0	4.00	3	0
13*	PI184197	W	Bjelika (Bosnia and Herzegovina)	2.50	4.75	4.75	3	1.00	0	0
14	WBC 990	W	D16404-19-15	3.00	4.50	5.00	3	4.00	0	0
15	WBC 991	W	D17003-1-12	5.00	5.00	4.00	0	4.00	3	3
16	MSU 44-2-2	W	MT0859/SD06070 = Nord 1405	3.25	4.75	5.00	3	4.25	0	0
17	MSU 44-2-3	W	MT0859/SD06071 = Nord 1406	3.00	5.00	5.00	0	4.00	0	0
18	MSU 45-2-4	W	Decade/Armour = Nord 1401	2.50	4.75	5.00	0	4.00	0	0
19*	MSU 45-2-6	W	Decade/Armour = Nord 1402	2.00	4.50	5.00	0	4.50	1	0
20	MSU 45-8-2	W	Decade/Armour = Nord 1403	3.00	4.25	5.00	0	4.50	0	0
21	MSU 44-2-1	W	MT0859/SD06069 = Nord 1404	2.75	4.25	4.75	3	4.50	0	0
22		W	MT0859/SD06072 = Nord 1407	2.25	4.25	5.00	0	4.50	0	0
23	11K014-3-3	W	Arapahoe/SD97W609//MT0097/NE01481	3.00	5.00	5.00	0		3	0
24	11K019-1-5	W	Arapahoe/SD97W609//Buteo	3.00	5.00	5.00	3	5.00	0	0
25	11K022-1-1	W	Arapahoe/SD97W609//Accipiter	3.00	5.00	5.00	0		3	0
26	11K022-1-3	W	Arapahoe/SD97W609//Accipiter	4.00	5.00	5.00	3	5.00	0	0

1 Derives from a recurrent mass selection program, thus, a pedigree cannot be provided.

* Indicates lines selected for the final evaluation with resistance and insensitivity to at least two isolates and effectors.

R1= race 1, AR= new race, R3= race 3

SY Wolf and Decade were kept for further confirmation since Liu et al. (2015) evaluated these lines.

Table A1. Averaged disease phenotype and effector response data obtained following the evaluation of 161 wheat lines with *P. tritici-repentis* and *P. nodorum* (continued).

				P.tritici-repentis					*P. nodorum*	
				Race		Effector	Race	Effector		
Entry No.	**Cross no.**	**Habit**	**Pedigree**	**R1 (Pti-2)**	**AR (AR CrossB10)**	**R3 (331-9)**	**Ptr ToxA**	**Sn4**	**SnTox3**	**SnTox1**
27	11K022-1-4	W	Arapahoe/SD97W609//Accipiter		5.00	5.00	3			
28	11K027-10-16	W	OK00611W/SD97W609/3/MT0423//MT0419/KS00F5-20-3	5.00	5.00	5.00	0		3	0
29	11K027-10-18	W	OK00611W/SD97W609/3/MT0423//MT0419/KS00F5-20-3	3.00	5.00	5.00	3	4.00	3	1
30	11K027-10-26	W	OK00611W/SD97W609/3/MT0423//MT0419/KS00F5-20-3	3.00	5.00	5.00	0	3.50	3	0
31	11K027-10-32	W	OK00611W/SD97W609/3/MT0423//MT0419/KS00F5-20-3	5.00	5.00	5.00	0	4.00		
32	11K027-10-30	W	OK00611W/SD97W609/3/MT0423//MT0419/KS00F5-20-3	3.00	5.00	5.00	3	4.00		
33	11K132-8-4	W	MT03176/MT0685//NE0649	3.00	5.00		0	4.00		
34	11K132-8-5	W	MT03176/MT0685//NE0649	3.00	5.00	5.00	3	4.00	0	1
35	11K132-8-6	W	MT03176/MT0685//NE0649	3.00	5.00	5.00	3			
36	11K132-8-7	W	MT03176/MT0685//NE0649	3.00	5.00	5.00	0	4.00	0	0
37	11K188-1-5	W	SD08133/Buteo	4.00	5.00	5.00	3		3	0
38	11K188-1-6	W	SD08133/Buteo	4.50	5.00	5.00	3	4.00	3	0
39	11K188-1-7	W	SD08133/Buteo	3.00	5.00	4.00	3		0	0
40	11K188-1-13	W	SD08133/Buteo	3.50	5.00	5.00	3	4.50	3	0
41	11K218-6-25	W	Ransom/Sturdy2K//RCATL33	3.00	5.00	5.00	3		3	2
42	11K218-6-26	W	Ransom/Sturdy2K//RCATL33	3.00	5.00	5.00	3	4.50	3	0
43	11K218-6-27	W	Ransom/Sturdy2K//RCATL33	2.50	5.00	1.50	3	3.00		
44	11K218-6-28	W	Ransom/Sturdy2K//RCATL33	2.50	5.00	3.50	3	5.00	3	0
45	11K229-2-1	W	MT0423//MT0419/KS00F5-20-3/3/SD07165	3.00	5.00	4.00	3	4.00	0	0
46	11K229-2-2	W	MT0423//MT0419/KS00F5-20-3/3/SD07165	4.50	5.00	5.00	3	5.00	0	0
47	11M217-6-1	W	Alsen/Norstar	5.00	5.00	5.00	3	4.00	0	0
48	11M217-6-2	W	Alsen/Norstar	3.00	5.00	5.00	3	5.00	0	0
49	11M217B-6-1	W	Alsen/Norstar	3.50	5.00	5.00	3	5.00	0	0
50	11M217-14-2	W	Alsen/Norstar	4.00	5.00	5.00	3	5.00	1	1

1 Derives from a recurrent mass selection program, thus, a pedigree cannot be provided.

* Indicates lines selected for the final evaluation with resistance and insensitivity to at least two isolates and effectors.

R1= race 1, AR= new race, R3= race 3

SY Wolf and Decade were kept for further confirmation since Liu et al. (2015) evaluated these lines.

Table A1. Averaged disease phenotype and effector response data obtained following the evaluation of 161 wheat lines with *P. tritici-repentis* and *P. nodorum* (continued).

				P.tritici-repentis				*P. nodorum*		
				Race			**Effector**	**Race**	**Effector**	
Entry No.	**Cross no.**	**Habit**	**Pedigree**	**R1 (Pti-2)**	**AR (AR CrossB10)**	**R3 (331-9)**	**Ptr ToxA**	**Sn4**	**SnTox3**	**SnTox1**
51	11M219-30-2	W	5602HR/Peregrine	3.00	5.00	5.00	3	5.00	0	0
52	11M219-42-1	W	5602HR/Peregrine	3.00	5.00	5.00	3	4.00	3	0
53	DH219-17	W	5602HR/Peregrine	3.00	5.00	5.00	3	5.00	0	0
54	11M221A-2-1	W	CM82036/Jerry	3.00	5.00	5.00	3	3.50	0	0
55	11M221A-6-1 (b)	W	CM82036/Jerry	3.25	5.00	2.50	3	4.00	1	0
56	11M221A-6-2	W	CM82036/Jerry	3.50	5.00	5.00	3	4.00	0	0
57	11M221A-8-1 (b)	W	CM82036/Jerry	2.75	5.00	5.00	3	4.00	0	0
58	11M221-8-2	W	CM82036/Jerry	3.00	5.00	5.00	3	4.00	0	0
59	11M221A-8-2	W	CM82036/Jerry	3.00	5.00	5.00	3	5.00	0	1
60	11M221-10-1	W	CM82036/Jerry	2.00	5.00	5.00	0	4.50	0	0
61	11M221-12-1	W	CM82036/Jerry	5.00	5.00	5.00	3	4.00	0	0
62	11M221-16-2	W	CM82036/Jerry	3.00	5.00	5.00	3	3.00	3	0
63	11M221-17-1	W	CM82036/Jerry	3.00	5.00	5.00	3	4.00	0	0
64	11M221-17-2	W	CM82036/Jerry	2.00	5.00	5.00	3	3.50		
65	11M221-23-1	W	CM82036/Jerry	3.00			0		0	0
66	11M221A-27-1	W	CM82036/Jerry	3.00	5.00	5.00	3	4.00	3	0
67	11M221-27-2	W	CM82036/Jerry	3.00	4.50	2.50	3		0	0
68	11M221-29	W	CM82036/Jerry	3.00	5.00		0	4.50	0	0
69	11M221-31-1	W	CM82036/Jerry	3.00	5.00	5.00	0	4.00	2	0
70	11M221A-36-1	W	CM82036/Jerry	3.50	5.00	5.00	0	4.00	0	0
71	11M221A-36-2	W	CM82036/Jerry	3.50	5.00	5.00	0	4.00	0	0
72	11M221A-37-1	W	CM82036/Jerry	3.00	5.00	5.00	3	3.50	0	0
73	11M221A-37-2	W	CM82036/Jerry	3.00	5.00	5.00	3	4.00	0	0
74	11M221A-38-1	W	CM82036/Jerry	2.50	5.00	5.00	3	4.00	0	0
75	11M221A-38-2	W	CM82036/Jerry	3.50	5.00	5.00	3	4.00	0	0
76	11M221A-39-1	W	CM82036/Jerry	4.00	5.00	5.00	0	4.00		
77	11M221A-39-2	W	CM82036/Jerry	5.00	5.00	5.00	0	4.00	2	0
78	11M221A-54-1	W	CM82036/Jerry	3.00	5.00	5.00	3	2.00	2	0
79	11M221A-54-2	W	CM82036/Jerry	3.00	5.00	5.00	3	4.00	3	0
80*	11M221A-56-1	W	CM82036/Jerry	3.00	5.00	5.00	0	2.00	0	0

1 Derives from a recurrent mass selection program, thus, a pedigree cannot be provided.

* Indicates lines selected for the final evaluation with resistance and insensitivity to at least two isolates and effectors.

R1= race 1, AR= new race, R3= race 3

SY Wolf and Decade were kept for further confirmation since Liu et al. (2015) evaluated these lines.

Table A1. Averaged disease phenotype and effector response data obtained following the evaluation of 161 wheat lines with *P. tritici-repentis* and *P. nodorum* (continued).

				P.tritici-repentis				*P. nodorum*		
				Race			Effector	Race	Effector	
Entry No.	**Cross no.**	**Habit**	**Pedigree**	**R1 (Pti-2)**	**AR (AR CrossB10)**	**R3 (331-9)**	**Ptr ToxA**	**Sn4**	**SnTox3**	**SnTox1**
81	DH151	W	CM82036/Jerry	4.50	5.00	5.00	0		2	0
82	DH153	W	CM82036/Jerry	3.00	5.00	5.00	3	5.00	0	0
83	DH159	W	CM82036/Jerry	3.00	5.00	5.00	3	4.00	0	0
84	DH160	W	CM82036/Jerry	3.00	5.00	5.00	0		3	0
85	DH162	W	CM82036/Jerry	4.50	5.00	5.00	0		2	0
86	DH163	W	CM82036/Jerry	4.50	5.00	5.00	0	4.00		
87	DH165	W	CM82036/Jerry	4.00	5.00	5.00	3	4.00	2	0
88	DH168	W	CM82036/Jerry	3.50	5.00	5.00	0	4.00	3	0
89	DH170	W	CM82036/Jerry	3.00	5.00	4.00	3	5.00	3	0
90	DH172	W	CM82036/Jerry	3.00	5.00	5.00	3	4.00	2	0
91	DH173	W	CM82036/Jerry	2.50	5.00	5.00	3	5.00		
92	DH174	W	CM82036/Jerry	3.00	5.00	5.00	0	4.00	3	0
93	DH181	W	CM82036/Jerry	3.00	5.00	4.00	3	4.00	3	0
94*	DH182	W	CM82036/Jerry	2.50	5.00	5.00	3	4.00	0	0
95	DH187	W	CM82036/Jerry	3.50	5.00	5.00	3	3.00	1	0
96	DH191	W	CM82036/Jerry	3.50	5.00	4.00	3	4.00	1	0
97	DH208	W	CM82036/Jerry	3.00	5.00	5.00	3	4.00	3	0
98	DH233	W	CM82036/Jerry	3.00	5.00	5.00	3	5.00	0	0
99	DH234	W	CM82036/Jerry	3.00	5.00	5.00	3	0.00	0	0
100	DH248	W	CM82036/Jerry	5.00	5.00	5.00	0	4.00	2	0
101	11M223-1-1	W	Frontana/Peregrine	2.50	4.00	5.00	3	5.00	0	0
102*	11M223-1-2	W	Frontana/Peregrine	2.50	2.00	4.50	3	5.00	0	0
103	11M223-3-1	W	Frontana/Peregrine	1.50	4.00	5.00	3	3.00	1	0
104	11M225-4-1	W	RWG10(*Fhb1*)/Jerry	2.50	4.00	5.00	3	3.00	0	0
105	11M225-4-2	W	RWG10(*Fhb1*)/Jerry	3.00	4.50	5.00	3	3.50	0	0
106	11M225-7-1	W	RWG10(*Fhb1*)/Jerry	2.75	5.00	5.00	3	3.50	0	0
107*	11M225-7-2	W	RWG10(*Fhb1*)/Jerry	2.50	5.00	4.50	3	1.00	0	0
108	11M225-8-1	W	RWG10(*Fhb1*)/Jerry	2.25	4.50	5.00	3	5.00	1	0
109	11M225-96-2	W	RWG10(*Fhb1*)/Jerry	5.00	5.00	5.00	3	5.00	0	0
110	11M225-97-1	W	RWG10(*Fhb1*)/Jerry	3.25	3.75	4.50	3	4.50	0	0

1 Derives from a recurrent mass selection program, thus, a pedigree cannot be provided.

* Indicates lines selected for the final evaluation with resistance and insensitivity to at least two isolates and effectors.

R1= race 1, AR= new race, R3= race 3

SY Wolf and Decade were kept for further confirmation since Liu et al. (2015) evaluated these lines.

Table A1. Averaged disease phenotype and effector response data obtained following the evaluation of 161 wheat lines with *P. tritici-repentis* and *P. nodorum* (continued).

				P.tritici-repentis				*P. nodorum*		
				Race			**Effector**	**Race**	**Effector**	
Entry No.	**Cross no.**	**Habit**	**Pedigree**	**R1 (Pti-2)**	**AR (AR CrossB10)**	**R3 (331-9)**	**Ptr ToxA**	**Sn4**	**SnTox3**	**SnTox1**
111	11M225-97-2	W	RWG10(*Fhb1*)/Jerry	3.25	4.50	5.00	3	4.75	0	0
112	11M225-99-2	W	RWG10(*Fhb1*)/Jerry	2.75	3.50	5.00	3	4.00	1	0
113	11M225-105-2	W	RWG10(*Fhb1*)/Jerry	3.75	5.00	5.00	0	5.00	0	0
114	11M225-123-1	W	RWG10(*Fhb1*)/Jerry	2.50	4.75	5.00	3	5.00	0	0
115	11M225-123-2	W	RWG10(*Fhb1*)/Jerry	3.00	4.50	5.00	3	4.25	0	0
116	11M225-126-1	W	RWG10(*Fhb1*)/Jerry	3.00	4.50	5.00	3	5.00	0	0
117	11M225-126-2	W	RWG10(*Fhb1*)/Jerry	2.75	5.00	5.00	0	4.50	0	0
118	DH312	W	RWG10(*Fhb1*)/Jerry	2.25	5.00	5.00	3	3.75	0	0
119	DH336	W	RWG10(*Fhb1*)/Jerry					5.00		
120	DH347	W	RWG10(*Fhb1*)/Jerry	2.00	5.00	4.50	0			
121	DH348	W	RWG10(*Fhb1*)/Jerry	3.00	4.00	4.00	3	2.00		
122	DH354	W	RWG10(*Fhb1*)/Jerry	4.75	4.75	5.00	0	4.00	0	0
123	DH355	W	RWG10(*Fhb1*)/Jerry	2.25	2.25	5.00	3	4.50	0	0
124	11M228-19-1	W	RWG21/Jerry	3.00	4.00	5.00	3	3.00	0	
125	11M228-22-2	W	RWG21/Jerry	3.50	5.00	5.00	3	4.00	0	0
126	11M228-25-2	W	RWG21/Jerry	3.00	5.00	5.00	3	3.50	0	0
127	11M228A-31-1	W	RWG21/Jerry	3.50	5.00	5.00	0		0	0
128	11M228A-32-2	W	RWG21/Jerry	4.00	5.00	5.00	3	4.50	0	0
129	11M228A-33-1	W	RWG21/Jerry	3.00	5.00	5.00	3	5.00	0	0
130	11M228A-38-1	W	RWG21/Jerry	3.00	5.00	5.00	3		0	0
131	11M228A-38-2	W	RWG21/Jerry	3.50	5.00	5.00	3	5.00	0	0
132	11M228A-39-1	W	RWG21/Jerry	3.00	5.00	5.00	3	4.00	0	0
133	11M228A-44-1	W	RWG21/Jerry	5.00	5.00	5.00	3	5.00	0	0
134	11M228A-44-2	W	RWG21/Jerry	3.00	5.00	5.00	3	5.00	0	0
135	11M228A-57-1	W	RWG21/Jerry	3.00	5.00	5.00	3	5.00		
136	11M228A-57-2	W	RWG21/Jerry	3.50	5.00	5.00	3	5.00	0	0

1 Derives from a recurrent mass selection program, thus, a pedigree cannot be provided.

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R1= race 1, AR= new race, R3= race 3

SY Wolf and Decade were kept for further confirmation since Liu et al. (2015) evaluated these lines.

Table A1. Averaged disease phenotype and effector response data obtained following the evaluation of 161 wheat lines with *P. tritici-repentis* and *P. nodorum* (continued).

				P.tritici-repentis				*P. nodorum*		
				Race			**Effector**	**Race**	**Effector**	
Entry No.	**Cross no.**	**Habit**	**Pedigree**	**R1 (Pti-2)**	**AR (AR CrossB10)**	**R3 (331-9)**	**Ptr ToxA**	**Sn4**	**SnTox3**	**SnTox1**
137	DH10	W	ND2710/Norstar	3.50		5.00	3		1	0
138	DH21	W	ND2710/Norstar	3.50	5.00	5.00	0			
139*	11M237A-1-2	W	RWG28(*Fhb1*)/Norstar	1.75	4.5	3.75	3	4.00	0	0
140	11M237C-3-1	W	RWG28(*Fhb1*)/Norstar	3.50	5.00	4.75	3	5.00	0	0
141	F4 AB 5-3 solid stem	W	n/a[1]	3.00	5.00	5.00	0	2.00	0	0
142	F4 AB-5-2 solid stem	W	n/a[1]	4.50	5.00	4.50	0	3.25	2	0
143*	Multicross CD	W	n/a[1]	1.50	5.00	2.00	3	4.00	0	0
144*	F4 AB-5-3 solid stem	W	n/a[1]	4.50	5.00	5.00	0	1.50	2	1
145	F3 solid stem AB cross (1)	W	n/a[1]	3.00	5.00	5.00	3	4.00	3	0
146	F3 solid stem AB cross (2)	W	n/a[1]	2.00	5.00	5.00	0	4.00	3	0
147	SA RMS 07US39	S	n/a[1]	1.00		5.00	0	3.50	1	1
148*	SA RMS 03H380	S	n/a[1]	1.00	4.75	5.00	0	5.00	3	0
149	SA RMS 03H254	S	n/a[1]	2.75	4.75	5.00	0	5.00	2	0
150	SA RMS 07US66	S	n/a[1]	3.25	4.75	5.00	3	4.00	2	0
151*	SA RMS 97K1-15-5	S	n/a[1]	1.50	2.50	2.00	0	3.50	0	0
152*	CIGM88.1175-0B	S	DOY1/*AE.SQ.* (188)	1.25	1.50	4.75	3	4.50	3	0
153*	CIGM89.546-0Y	S	CROC_1/*AE.SQ.* (826)	1.25	1.50	3.00	0	1.75	0	2
154*	CIGM90.606	S	RABI//GS/CRA/3/*AE.SQ.* (914)	1.00		4.00	0	3.25	0	2
155*	CIGM89.567	S	CETA/*AE.SQ.* (895)	1.75	4.75	4.50	0	3.50	0	0
156*	CIGM92.1701	S	RASCON/*AE.SQ.* (385)	1.00	1.00		0	1.00	0	2
157	CIGM93.229	S	DOY1/*AE.SQ.* (372)			4.00	3	4.00	3	0
158*	CIGM93.244	S	CROC_1/*AE.SQ.* (444)	1.00	1.25	4.25	0	1.00	0	3
159*	Serendipity	S	Tritipyrum	2.00	1.00	1.00	3	2.00	0	0
160*	Matie	S	Tritipyrum	2.00	1.50	5.00	3	1.00	0	1
161*	Pearly	S	Tritipyrum	2.00	1.50	4.00	3	2.00	0	0
162	Glenlea	W	Control				3		0	0
163	6B662	S	Control				0		2	0
164	Chinese Spring	S	Control				0		0	3
165	Salamouni	S	Control				0		0	2

1 Derives from a recurrent mass selection program, thus, a pedigree cannot be provided.

* Indicates lines selected for the final evaluation with resistance and insensitivity to at least two isolates and effectors.

R1= race 1, AR= new race, R3= race 3

SY Wolf and Decade were kept for further confirmation since Liu et al. (2015) evaluated these lines.

Table A2: Observed disease and NE reactions of the included 52 lines in the final evaluation.

			Tan spot (*P. tritici-repentis*)					SNB (*P. nodorum*)			
Name/ Cross #	**Pedigree/Type**	**Habit**	**R1 (Pti-2)**	**AR CrossB10**	**R3 (331-9)**	**Ptr ToxA**	***Xfcp623***	**Sn4**	**SnTox3**	**SnTox1**	**1RS.1BL**
Jerry	Variety	W	4.20[1]	4.50[1]	4.20[1]	3	P	5.00	0	0	A
Decade	Variety	W	3.5	3.67	3.17	0	A	3.13	0	0	A
SYWolf*	Variety	W	2.50	2.33	1.00	0	A	2.00	0	0	P
Bjelika	PI184197 ex Bosnia/ Herzegovina	W	3.17	3.50	3.33	3	P	2.33	0	0	A
MSU 45-2-6	Decade/Armour = Nord 1402	W	3.17	3.00	3.50	0	A	3.63	0	0	A
11M221A-56-1	CM82036(*Fhb1, 5A*)/Jerry	W	4.00	3.33	4.17	0	A	2.00	0	0	A
DH182	CM82036(*Fhb1, 5A*)/Jerry	W	3.33	3.83	4.17	3	P	4.17	0	0	A
11M223-1-2	Frontana/Peregrine	W	3.00	2.83	2.17	3	P	4.17	0	0	A
11M225-7-2	RWG10(*Fhb1*)/Jerry	W	2.67	3.67	1.67	3	P	2.50	0	0	A
11M237A-1-2*	RWG28(*Fhb1*)/Norstar	W	1.00	1.67	1.00	3	P	1.67	0	0	P
F4 AB-5-3 solid stem	Recurrent selection line	W	3.83	3.83	3.67	0	A	1.67	0	0	A
Multicross CD*	Recurrent selection line	W	1.00	1.00	1.00	3	P	2.83	0	0	P
F5:ABCDE-35*	Recurrent selection line	W	1.83	1.33	1.67	0	A	0.88	0	0	P
F5:ABCDE-36	Recurrent selection line	W	3.33	3.17	3.50	0	A	2.50	0	0	A
F5:ABCDE-63*	Recurrent selection line	W	2.33	1.00	2.50	3	P	3.25	0	0	A
F5:ABCDE-68	Recurrent selection line	W	3.83	3.83	3.17	3	P	3.33	3	0	A
F5:ABCDE-76*	Recurrent selection line	W	1.83	1.00	1.00	3	P	3.67	0	0	A
F5:ABCDE-93	Recurrent selection line	W	3.67	3.50	4.00	0	A	3.00	0	0	A
F5:ABCDE-94*	Recurrent selection line	W	1.00	1.67	1.00	3	P	3.50	0	0	A
F5:ABCDE-104	Recurrent selection line	W	3.00	3.67	3.17	0	A	1.63		0	A
F5:ABCDE-113	Recurrent selection line	W	3.50	2.00	2.00	0	A	3.13	0	0	A
F5:ABCDE-123*	Recurrent selection line	W	1.83	3.17	1.83	0	A	1.00	0	0	P
F5:ABCDE-140*	Recurrent selection line	W	2.50	1.17	2.83	0	A	3.50	0	0	A
F5:ABCDE-150*	Recurrent selection line	W	1.00	1.17	1.00	1.5	P	1.13	0	0	A
F5:ABCDE-189*	Recurrent selection line	W	1.33	1.00	1.83	1.5	P	3.17	0	0	A
F5:ABCDE-204	Recurrent selection line	W	3.00	3.00	2.50	0	A	1.63	3	0	A
F5:ABCDE-216*	Recurrent selection line	W	1.00	1.00	2.67	0	A	1.38	0	0	A
F5:ABCDE-270	Recurrent selection line	W	2.00	2.67	1.33	3	P	3.17	2	0	A
F5:ABCDE-279	Recurrent selection line	W	3.50	4.00	4.17	3	P	3.00	0	0	A
F5:ABCDE-362	Recurrent selection line	W	2.83	3.17	1.83	3	P	3.17	0	0	A
F5:ABCDE-394	Recurrent selection line	W	3.50	3.17	2.17	0	A	3.17	3	0	A
F5:ABCDE-428*	Recurrent selection line	W	1.00	1.00	1.00	0	A	1.38	0	0	A
F5:ABCDE-457	Recurrent selection line	W	2.67	3.67	3.17	3	P	3.67	1.5	0	A

[1] Indicates data obtained by Liu et al. (2015).

* Lines selected with resistance to four or three isolates and insensitive to two, three, or four effectors.

R1= race 1, AR= new race, and R3= race 3

Table A2: Observed disease and NE reactions of the included 52 lines in the final evaluation (continued).

			Tan spot (*P. tritici-repentis*)					SNB (*P. nodorum*)			
Name/ Cross #	**Pedigree/Type**	**Habit**	**R1 (Pti-2)**	**AR CrossB10**	**R3 (331-9)**	**Ptr ToxA**	***Xfcp623***	**Sn4**	**SnTox3**	**SnTox1**	**1RS.1BL**
F5:ABCDE-475	Recurrent selection line	W	3.83	3.00	3.00	3	P	2.83	0	0	A
F5:ABCDE-482*	Recurrent selection line	W	1.67	2.50	2.83	0	A	2.13	0	0	A
F5:ABCDE-490	Recurrent selection line	W	3.50	4.33	3.67	3	P	3.38	0	0	A
F5:ABCDE-502	Recurrent selection line	W	3.50	4.00	3.25	0	A	1.83	0	0	A
F5:ABCDE-516*	Recurrent selection line	W	1.50	1.83	1.83	0	A	2.75	0.25	0	A
F5:ABCDE-524	Recurrent selection line	W	2.83	4.00	4.00	0	A	2.50	0	0	A
F5:ABCDE-536*	Recurrent selection line	W	1.00	1.17	1.00	0	A	1.00	0	0	A
F5:ABCDE-559	Recurrent selection line	W	3.67	3.17	3.50	3	P	2.17	0	0	A
F5:ABCDE-563	Recurrent selection line	W	4.50	4.50	4.50	3	P	3.75	0	0	A
CIGM90.606*	RABI//GS/CRA/3/AE.SQ. (914)	S	1.00	1.25	1.25	0	A	1.33	0	2	A
CIGM88.1175-0B*	DOY1/AE.SQ. (188)	S	1.00	1.00	2.00	1.5	P	3.67	3	0	A
CIGM93.244*	CROC_1/AE.SQ. (444)	S	1.00	1.00	1.00	0	A	0.83	0	1.5	A
CIGM92.1701*	RASCON/AE.SQ. (385)	S	1.00	1.00	1.00	0	A	2.00	0	1	A
CIGM89.546-0Y*	CROC_1/AE.SQ. (826)	S	1.17	1.00	1.00	0	A	1.00	0.5	2	A
CIGM89.567*	CETA/AE.SQ. (895)	S	1.00	1.17	1.17	0	A	1.00	0	0	A
Serendipity*	Tritipyrum	S	1.50	2.17	1.83	3	P	1.13	0	0	A
Matie*	Tritipyrum	S	1.17	1.17	1.17	3	P	1.33	0	0	A
Pearly*	Tritipyrum	S	1.17	1.50	1.50	3	P	1.00	0	0	A
SA RMS 97K1-15-5*	Recurrent selection line	S	1.00	1.17	1.00	0	A	1.67	2.5	0	P
SA RMS 03H380*	Recurrent selection line	S	2.67	2.83	2.33	0	A	3.33	1	0	P
Br34	Control	S	1.17	1.33	1.00	0	A	0.88	0	0.5	P
Grandin	Control	S	0.00	0.00	0.00	3	P	3.50	2	0	A
6B662	Control	S	2.83	2.50	2.83	0		0.00	0	0	
6B365	Control	W	4.83	4.17	4.00			0.00			
Glenlea	Control	S	4.00	3.17	3.33		P	0.00			A
Salamouni	Control	S				0	A		0	1.5	
Chinese Spring	Control	S				0	A		0	2.5	

[1] Indicates data obtained by Liu et al. (2015).

* Lines selected with resistance to four or three isolates and insensitive to two, three, or four effectors.

R1= race 1, AR= new race, and R3= race 3